

**INTERACTION OF CHEMOTHERAPY  
AND THE IMMUNE RESPONSE  
IN EXPERIMENTAL MALARIA INFECTIONS**

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ABSTRACTINTERACTION OF CHEMOTHERAPY AND THE IMMUNE RESPONSEIN EXPERIMENTAL MALARIA INFECTIONS

by

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Plasmodium chabaudi malaria infections in CBA mice showed a characteristic and consistent pattern. Primary parasitaemias reached a peak of 30-50% but resolved within 3 weeks. Small recrudescences occurred at 4 and at 8 weeks and parasites were demonstrable up to 9 weeks by subinoculation of blood and other tissues. Infections in T-lymphocyte deprived mice were not fatal but the mice remained parasitaemic for at least 120 days. By contrast, P. berghei killed both intact and deprived mice; the survival time of the T-cell deprived mice was significantly longer.

Treatment of P. chabaudi infections with chloroquine, quinine or pyrimethamine was generally more effective in intact than in T-cell deprived mice. Chloroquine and quinine treatment of P. berghei infections, however, was more effective in the deprived mice. None of the recrudescences of either P. berghei or P. chabaudi infections following treatment showed evidence of increased resistance to the drugs.

Mice treated with cortisone acetate died of P. chabaudi infections and were not as effectively treated with quinine or chloroquine as infected controls. Induced protein energy malnutrition prolonged P. chabaudi parasitaemias but these were as readily treated as infections in controls on balanced diet.

Suppression of P. chabaudi infections by subcurative chemotherapy gave rise to a strong immunity. Curative treatment given early in infections resulted in only partial immunity to challenge whereas treatment late in the infection gave a high degree of resistance to infection.

Generalized immunodepression during P. chabaudi infection was related to the degree of parasitaemia. There was some increase in the rate of catabolism of immunoglobulins as a result of the infection.

P. chabaudi infections were significantly suppressed in mice which had mature Schistosoma mansoni infections but acute or chronic infections did not influence development of the schistosomiasis.

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## I. GENERAL INTRODUCTION

This study is concerned with the interactions of chemotherapy and the immune response in malarial infections. Two different types of parasite-host systems were used to determine the mechanisms, one benign (Plasmodium chabaudi) the other virulent (P.berghei), in mice.

The initial experiments were designed to establish basic data on the parasite-host relationship and the nature and the intensity of its immunodepressive effect. The immunodepressive effects of the antimalarial drugs to be used were also examined.

The best method of treatment in immunologically intact mice to obtain a maximal immunity was also determined. A large proportion of my experiments dealt with the chemotherapy of immunologically (T-cell) deprived mice as well as intact control mice to demonstrate the importance of immune mechanisms in drug treatment. This included studies on the possibility that drug resistant strains might arise as a result of chemotherapy of T-cell deprived mice.

I then studied the effectiveness of antimalarial chemotherapy in mice suffering from protein-energy malnutrition, and after treatment with a commonly used immunodepressive drug (corticosteroid).

A detailed study was carried out on the interactions of malaria and schistosomiasis (Schistosoma mansoni) in mice.

Since I dealt with four major subjects - parasitology, immunology, chemotherapy and protein-energy malnutrition, I had to do a wide literature review. However, I have tried to review only the background information for each section rather than go into great detail. The first part of my literature research deals with speciation, infection and immunity to malaria. Later, protein-energy deficiency and the effects on immunity to infection are reviewed and then concurrent infections of malaria and other parasites are also studied.

It is important to try to understand the mode of actions of the drugs used and mechanisms of resistance to them, so a section is included on this. In the field of immunity and chemotherapy, not much work has been done on the interactions of chemotherapy and immunity with parasite models, so I have extended my review a little further to include other diseases.

## II LITERATURE REVIEW

## A. Rodent malarias

1. Isolation and speciation

The malaria parasites infecting rodents constitute part of the subgenus Vinckeia (Garnham, 1964) from the genus Plasmodium.

The first rodent malaria found, P.berghei was isolated by Vincke and Lips in 1948 from thicket rats, Grammomys surdaster, in the highlands of Katanga Province (Zaire). Four more murine malaria parasites resembling P.berghei were isolated in lowland areas from Thamnomys rutilans, and were specified as subspecies of P.berghei. P.berghei yoelii from the Central African Republic (Landau and Killick-Kendrick, 1966), P.berghei killicki from Brazzaville (Landau, Micheal and Adam, 1968) and P.berghei nigeriensis from Nigeria (Killick-Kendrick, 1973).

Another species of Plasmodium, which was morphologically and biologically different from P.berghei was isolated from Anopheles dureni millecampsi mosquitoes caught in the same forests of Katanga and was named Plasmodium vinckei (Rodhain, 1952). Though A. dureni feeds exclusively on rodents the natural vertebrate host of the parasite is still unknown.

Three subspecies of P.vinckei have been isolated up to now. P.vinckei bruce-chwatti was originally found by Bruce-Chwatt and Gidson (1955) from wild rodents, Praomys tullergi in West Africa and later by Bafort (1970) and by

Killick-Kendrick (1975) in T.rutilans from the same area. Another subspecies P.vinckei lentum was isolated from T.surdaster from the Democratic Republic of Congo (Adam et al., 1966) and was named by Landau et al. (1970). Carter and Walliker derived a number of lines of malaria parasites from the rodents, T.surdaster, in the Central African Republic. For one of these lines they proposed the name P.vinckei petteri (Carter and Walliker, 1975).

Landau (1965) detected another new malaria parasite, morphologically similar to P.vinckei, in T.surdaster, from the tropical rain forests of the Central African Republic and named it P.chabaudi. Bafort (1968) reduced it to a subspecies P.vinckei chabaudi, but later workers accepted the parasite as a species distinct from P.vinckei (Ott, 1969; Peters, 1967a; Carter and Walliker, 1975; and Rosario, 1976).

The validity of the speciation and subspeciation was repeatedly questioned by Bafort. He said that some of the strains were overlapping (Bafort, 1970) and postulated that the murine malaria parasites were simply one polymorphic species with P.vinckei at one pole and P.berghei on the other (Bafort, 1971).

However, Killick-Kendrick later raised P.berghei yoelii from a subspecies to a species, P.yoelii, with subspecies killicki and nigeriensis transferred from the species P.berghei to P.yoelii (Killick-Kendrick, 1974a; 1974b).

P.berghei reverted to a monotypic species when Killick-Kendrick used the distribution (Vincke and Lips, 1948, Killick-Kendrick, 1973; Landau, Michel and Adam, 1968), morphology (Garnham, 1966; Rodhain, 1952) and biology of all stages of life cycles (Landau and Killick-Kendrick, 1966; Garnham et al., 1967; Wery, 1968) as the main criteria. The speciation was in agreement with the work of Carter (1973), who showed that the enzymes GPI & 6PGD were never the same between P.berghei and the other three isolates. Chance and Warhurst (1973) also showed by DNA hybridization that P.berghei is widely separated from P.yoelii nigeriensis. Innate resistance to chloroquine in P.yoelii (Warhurst and Killick-Kendrick, 1967) the sensitivity of P.berghei (Peters, 1965b; Warhurst and Hockley, 1967) might also favour this view.

Carter and Walliker (1976), after studying the parasites derived from naturally infected thicket rats, T.rutilans, from the Congo (Brazzaville), concluded that a third species was present together with the former two species, P.y.killicki and P.v.lentum. According to them this is a subspecies of P.chabaudi, which prefers mature red cells, which they named P.c.adami. They said that the two subspecies of P.chabaudi are morphologically identical but P.c.adami is less virulent and is different in enzyme patterns from P.c.chabaudi. Thus the authors claim that there are two subspecies of P.chabaudi, P.c.chabaudi and P.c.adami, and four subspecies of P.vinckei, P.v.lentum, P.v.petteri, P.v. bruce-chwatti and P.v.vinckei.

## 2. Infections in laboratory animals

### 1. Plasmodium chabaudi

P.chabaudi occurs naturally in wild-thicket rats, Thamnomys rutilans (Landau, 1965) in the Central African Republic. In the laboratory, Swiss TO mice (Garnham, 1966), CF mice (Ott, 1969), C<sub>57</sub> black mice (Walliker et al., 1975) and multimammate rats (Garnham, 1966) are susceptible both to inoculation of blood and to sporozoite stages. Though hamsters and rats are refractory to the parasite, it can be adapted to rats by infecting them after splenectomy (Musoke and Cox, 1977) which subsequently grows well even in intact rats. There is a single record of a successful infection of a guinea pig (Garnham, 1966).

There is no difference between infection in conventional and in specific pathogen free mice, but factors such as route of inoculation, size of inoculum and preparation procedures influence the course of the infection (Ott, 1969). Associated E.cocoides infections and low PABA content in the diets (Peters, 1967a) can inhibit the P.chabaudi infection.

While mice infected with P.chabaudi usually recover from the infection (Cox and Voller, 1966) there may sometimes be some deaths (Cox, 1970). The parasitaemia can be enhanced by rapid blood passage (Cox and Voller, 1966; Garnham, 1966). Different strains of mice may have different patterns of infection (Peters, 1967a).

The maximal peak parasitaemias are also variable. Peters (1967a) recorded 35%, Cox (1970) 40%, and Ott and

Stauber (1967) and Carter and Walliker (1975) recorded 80% as the peak parasitaemia. Probably the strains of parasites, strains of mice (Peters, 1967a), different laboratory conditions, E.coccoides infections (Ott and Stauber, 1967), the preparations and dosage of inoculum (Ott, 1969) and PABA contents of the diets (Peters, 1970a) need to be considered as factors which determine these variations in their results.

P.chabaudi preferentially invades the mature erythrocytes and gives rise to a synchronous infection in mice and thicket-rats, with a periodicity of 24 hours (Carter and Walliker, 1975). The ring trophozoites are similar to those of P.vinckei (Garnham, 1966). Infected cells containing single parasites predominate at first but multiple infections appear in the late stage (Carter and Walliker, 1975). Gametocytes became more frequent after the peak of parasitaemia (Carter and Walliker, 1975).

According to Cox (1970) the parasites appeared in blood on the second day of the blood induced P.chabaudi infection in Swiss TO mice and reached a peak of 10% on the seventh day. Thereafter, parasitaemia declined and no parasites could be seen after day 14. During the fourth or fifth week after infection a recrudescence occurred in certain mice, the level of parasitaemia never reaching that of the first peak.

#### 11. P.berghei

The natural vertebrate hosts of P.berghei are the thicket-rats, Grammomys surdaster, (Vincke and Lips, 1948), Praomys jacksoni and Lagada bella (Vincke, 1954). In the

laboratory, many species of rodents, rabbits, bats and some species of monkeys are susceptible to blood-induced infections (Wellde et al., 1966; Cox, 1967); most strains of mice (Most et al., 1966; Vincke and Bafort, 1968), white rats and hamsters (Yoelli and Most, 1960; 1965b) are susceptible to infection by blood stages or sporozoites.

Blood stage infections are usually given by intraperitoneal, intravenous or subcutaneous injections of infected red blood cells, of which the intraperitoneal route is the easiest and is 100% successful. Mice and rats are the main laboratory animals used for P.berghei infections. The early infections of blood stages in white rats and mice occur in normocytes, later there is a predilection for immature erythrocytes, polyparasitism often causing hypertrophy of the host cell and ring forms occasionally have twin nuclei. Synchrony produce 6-10 merozoites in mice and 16 in rats and hamsters (Garnham, 1966). The asexual cycle takes 22-25 hours (Thurston, 1952). Gametocyte production declines and disappears after several passages (Vincke, 1963).

P.berghei infections are invariably fatal in most strains of mice (Greenberg et al., 1953) golden hamsters (Wright, 1968) and in young rats, though the adult rats have only 7% mortality (Sergent and Poncet, 1955). The mortality depends on several factors. The survival time is much longer in some strains of mice, female mice survive longer than the male mice and the survival time also increases with age (Greenberg et al., 1953). P.berghei infections in rats

show that as the age of the host increases, the mortality decreases, the day of malarial crises occurs earlier and the parasite burden decreases. The causes of this age immunity, according to Smalley (1975), did not appear to involve non-antibody serum factors and he proposed that it was due to the differences in the effectiveness of acquired protective responses. However Tosta and Filho (1976) showed that normal adult rat serum prolonged the mean survival time to seven-day old rats and concluded that a factor (or factors) in normal adult rat serum, not related to malarial antibodies, decreased the infectivity of P.berghei.

The course of P.berghei infection depends on the size of inoculum (Wellde et al., 1966) and so if the size of inoculum was increased by a factor of 10 the latent period decreased by one day (Warhurst and Polwell, 1968). In P.berghei infections in Swiss TO mice there was no correlation between the log inoculum and the number of deaths (Sengers et al., 1971b); the survival time was inversely proportional to the size of inoculum (Schindler & Mehlitz, 1965; Sadun, 1966; and Sengers et al., 1971b).

The mean rate of increase in parasitaemia was not different when parasites were injected from different phases of the infection of the donor mice (Sengers and Elteren, 1971). In rats, by increasing the body temperature by injecting TAB vaccine (0.02 ml) or by lowering the body temperature to 15-20°C, the parasitaemia was very depressed (Basu et al., 1962) and the liver schizonts also decreased in size and number (Yoeli et al., 1975) when the body temperature was lowered.

Dietary factors play a major role in P.berghei infections. Maegraith et al.(1952) observed that P.berghei infections were less severe in mice maintained on a milk diet than on the normal one. Adler (1958) also found that mice kept on a meat diet were relatively resistant to P.vinckei infections. Hawking (1953) demonstrated that PABA was the essential factor for the growth of P.berghei which was lacking in a milk diet. Kretschmar (1965) showed the correlation of the PABA content of the diet and the mortality of the mice infected with P.berghei.

P.berghei preferentially invades immature erythrocytes (Corradetti and Verulini, 1951; and Singer,1954a), so the conditions which evoke reticulocytosis, like repeated bleedings (Viens et al.,1971), phenylhydrazine treatments (Ott,1968; Fabriani et al.,1952; Viens et al.,1971) and anaemia provoked by eperythrozoon infections (Ott et al.,1967) increase the parasitaemia. On the other hand, transfusion of blood (Fabiani et al.,1952; Ladd and Lalli,1966), corticosteroid treatments (Cox,1974) and cyclophosphamide treatments (Spina et al.,1972) will decrease the number of reticulocytes in the blood and with this the P.berghei parasitaemia and so increase the survival time.

In P.berghei infections in mice, the animals become progressively more anaemic as the infection progresses, as shown by total erythrocyte counts and haematocrit values, with an increase in the number of circulating reticulocytes. Erythrocyte destruction exceeds the rate of erythropoiesis, though erythropoietin production is not impaired (Renricca et al.,1974).

### 3. Immunity

#### a. Mechanism of acquired immunity in malaria

Innate immunity can operate against all the stages of the life cycle of malaria infection (Garnham,1966) but specific acquired immunity of the host is directed primarily against the asexual blood stages (Targett,1973). Sporozoites remain extracellular only a matter of hours at most (Farley, 1947; Nusserzweig et al.,1972). There is evidence that a strong immunity to sporozoites can be induced by vaccination with attenuated or viable sporozoites (Nussenzweig et al.,1969; Nusserzweig et al.,1972; Vehave,1975) but the occurrence of an immune response to sporozoites in natural infections has not yet been shown (WHO,1975). There is no evidence for synthesis of specific antibody against the exoerythrocytic stage nor is it known at present if repeated natural infections induce immunity against this stage of infection (Cohen and Butcher, 1971).

The first indication of the immune reaction by the host is a slowing in the rate of parasite increase in the peripheral blood (Brown,1975).

#### i. Humoral immunity

Humoral immunity plays a major role in acquired resistance to malaria infection (WHO,1975). Humoral immunity involves the production of antibody by the plasma cells (B-cells). For many antigens co-operation of T cells and a third cell referred to variously as an accessory or adherent cell, which adheres to glass or plastic surfaces in cultures and is

found in populations rich in macrophages, are required for antibody formation (Fitch, 1975).

In malaria infections, both human and experimental, there is a marked increase in concentration of immunoglobulins (Cohen and McGregor, 1963). Repeated infection with malaria in man is associated with considerable elevation of IgG and IgM (Rowe et al., 1968). In vitro and in vivo tests have been carried out with antisera to determine the effects of different types of immunoglobulins. Serum transfer experiments have been carried out in rodents (Fabiani and Fulchiron, 1953; Bruce-Chwatt and Gibson, 1955; Isfan, 1966; Briggs et al., 1968; Diggs and Osler, 1969; 1975; and Roberts and Tracey-Patte, 1969) in monkeys (Coggeshall and Kumm, 1937) and in human infections (Cohen et al., 1961; and Cohen and McGregor, 1963).

Antisera lengthen the prepatent period in rats and mice (Roberts and Tracey-Patte, 1969; Briggs et al., 1968; and Jayawardena, 1975) or can produce complete neutralisation (Golenser et al., 1975). The protective effect of serum is known to increase with the number of immunizing injections in both monkey (Coggeshall and Kumm, 1938) and rodent malaria (Briggs et al., 1966). Antisera may also have suppressive effects on pre-existing parasitaemias (Fabiani and Fulchiron, 1953; Cohen and McGregor, 1963; Diggs and Osler, 1969; and Zuckerman et al., 1969) and is dose dependent in rats (Diggs and Osler, 1975; and Golenser et al., 1975). The effectiveness of the antisera also depends on the time it was taken after infection (Phillip and Jones, 1972). Cohen et al. (1961) also showed the antiparasitic action of immune IgG in humans. In vitro assays of protective antibodies in immune serum

have shown that IgG is mainly responsible though some activity has also been shown in IgM fractions as well (Cohen and Butcher, 1970; and WHO, 1975). A purified IgG fraction had been shown to have a lower protective effect than the whole immune serum in monkeys (Butcher et al., 1970). Freeman et al. (1970) were able to show that less than 5% of the IgG was parasite specific by inoculating  $^{131}\text{I}$  labeled homologous immune IgG and  $^{125}\text{I}$  labeled normal serum into P.knowlesi infected monkeys. Similarly, actual Ig which could be specifically absorbed from immune sera by various parasite preparations was shown to be 2.6% of total IgG in immune New Guinea serum, 5.4% in immune Gambian serum and 0.9% in immune rhesus monkey serum (Cohen and Butcher, 1969; and Butcher et al., 1970).

Cohen and Butcher (1970), by incorporation of  $^3\text{H}$  leucine into protein in vitro culture, were able to demonstrate that the growth of P.knowlesi within the erythrocytes was unaffected by the immune serum but the invasion of new red cells by the merozoites and the subsequent parasite multiplication were inhibited. The malaria antibody seemed to directly attack the surface coat of the merozoites (Miller et al., 1975) in the culture medium, caused them to agglutinate, and reduced the ability to invade new red cells (Butcher and Cohen, 1970; and Miller et al., 1975). The inhibitory effect of immune serum is species specific as shown by the failure of serum from a monkey immune to P.cynomolgi bastianellii to inhibit P.knowlesi (Cohen and Butcher, 1971). This is consistent with that of malaria immunity generally which also seems to be species and strain specific (Cox and Voller, 1966; and Targett, 1968).

The protective action of immune serum was shown to be complement independent being unaffected by heating to 56°C for 3 hours or by the addition of fresh normal guinea pig serum (Cohen and Butcher, 1971). Cobra venom factor (CVF) also had no effect on the parasitaemia (Atkinson et al., 1975) but Ree (1976) observed a low level of  $C_3$  in the acute infection and a return to normal level after treatment of malaria in Gambian children and suggested  $C_3$  and parasitaemia were correlated.

Jerusalem and Bruchhausen (1967) reported that there are antibodies in the serum of the acute stage of rodent malaria infection involved in protecting against the toxic products of the malaria.

Brown and Hills (1974), after studying chronic P.knowlesi in rhesus monkeys, reported that there are two types of variant-specific antibody in the serum. The first antibody was detectable by a schizont infected cell agglutination (SICA) test (Brown, K.N. and Brown, 1965), specific for the infecting intrastrain variant (Brown et al., 1970a and b) and is not protective. The other variant specific antibody is parasitocidal, opsonizing antibody, and can be detected by an opsonization test (Brown, K.N. et al., 1970b). Although opsonizing antibodies have not been found without SICA antibodies being present, SICA antibodies may be found when opsonizing antibodies are not detectable (Brown and Hill, 1974). After the end of the crisis of malaria infection damaged intracellular parasites of all stages of maturity are always seen (WHO, 1975) which was supposed to be due to

direct cytotoxic action of antibody on the parasitised red cells (Taliaferro, 1949). This effect has not been shown in vitro by incubation of parasitised red cells in immune serum or with immune lymphocytes (WHO, 1975). Clark and his colleagues (1976) were also able to demonstrate intra-erythrocytic death of P. vinckei parasites in mice infected one month after intravenous inoculation of  $2 \times 10^7$  BCG. Clark et al. (1977) showed that formalin killed Corynebacterium parvum given intravenously or intraperitoneally in mice gave similar results. According to them there was no evidence that antibody to the parasites was involved in the resistance and in discussing other possible mechanisms, the authors concluded that protection was most likely afforded by non-specific soluble mediators.

Although serum antibody appears to play a primary role in acquired malarial immunity, its clinical effectiveness may be dependent upon a synergistic action with the macrophage system (Cohen and Butcher, 1971). The fact that immune serum is more effective in intact than in splenectomised animals (Mulligan, <sup>et al.</sup> 1940; Zuckerman et al. 1973; Brown and Phillips, 1974) and that the histological appearances of the livers and spleen of the malaria infected animals shows considerable phagocytosis (WHO, 1968) reveals that phagocytosis is an important mechanism of acquired immunity. The role of specific antibody in promoting macrophage ingestion of parasites has been demonstrated in vitro. Brown and Hills (1974) produced some more evidence for production of such antibodies using an experimental system involving serum and P. knowlesi from infected monkeys and murine macrophages.

Criswell et al. (1971) demonstrated the enhanced killing of parasites by macrophages in association with humoral factors by implanting millipore chambers containing macrophages and parasitised red cells into the peritoneal cavities of normal mice and of others with chronic infections of P.berghei. In vitro study of specific antibody that induced phagocytosis of P.berghei infected erythrocytes has been shown in rat macrophages by Chow and Kreier (1972) but surprisingly of only the damaged RBC.

#### ii. Cell-mediated immunity

The specific effector cells of cell-mediated immune responses arise directly from the antigen-driven proliferative response of progenitors that are members of a circulation pool of T-cells (Thymus dependent lymphocytes) (WHO,1974). T-cells initiate delayed hypersensitivity reactions, the specific recognition and rejection of allografts and some tumors, non-specific activation of macrophages and co-operation with B-cells during induction of antibody synthesis. (Cohen,1975).

In malaria infection there is as yet no direct evidence of the part played by T-cells though there is indirect evidence (WHO,1975). Many people have tried to suppress the cell-mediated immune response by procedures that deplete the recirculating pool of T-cells e.g. neonatal thymectomy (Miller and Osba,1967), antilymphocyte serum (ALS) treatment (Medewar,1969), or adult thymectomy followed by lethal irradiation and bone marrow reconstitution (Parrot et al., 1970). But according to Doenhoff (1972) even thymectomy

followed by irradiation does not totally deplete the animals of their T-cell pool for 10% is left behind.

The ability of rats to control a P.berghei infection is thymus dependent. P.berghei produces a resolving type of infection in adult rats. Brown et al. (1968), Stechschulte et al. (1969) and Hanson & Chapman (1971) showed that anaemia, parasitaemia and mortality were higher in neonatally thymectomised rats than in the non-deprived controls. Spira et al. (1970) also demonstrated similar results in ALS treated rats infected with P.berghei. Antibody titres measured by agglutination and fluorescent antibody techniques were not lowered in the neonatally thymectomised rats (Stechschulte et al., 1969) or in ALS treated rats (Spira et al., 1970).

The ability of mice to control P.yoelii infections was also shown to be thymus dependent.

In nude mice (Clark and Allison, 1974) or in thymectomised irradiated and bone marrow reconstituted mice (Jayawardena et al., 1975) the P.yoelii infection is fulminant and fatal, though in intact mice it is self resolving. Jayawardena et al. (1975) showed by chromosome marking of T-cells, that P.yoelii infections induce massive T-cell activation. P.berghei infections in normal mice are fatal but T-cell deprivation either by treatment with ALS or by adult thymectomy, irradiation and bone marrow reconstitution prolonged survival (Sheagren and Monaco, 1969; Jayawardena et al., 1975). The same is true with fatal P.berghei infections in hamsters, which could be prolonged by either neonatal thymectomy or ALS

treatment (Wright, 1968; Wright et al., 1971). The fatal P.berghei infections in mice, in contrast to resolving P.yoelii infections, elicit a weak T-cell response with generalised failure of T-cell activity (Jayawardena et al., 1975). In mice infected with P.berghei the T-cell numbers detected by cytotoxic tests with anti-theta antiserum and B-cell populations (Rosette test) were lowered while there was an increase in the "null" cell population (Krettli and Nussenzweig, 1974). Patients infected with P.falciparum, especially children, also showed a reduction in T-cells (E rosette forming cells) no change in B-cell numbers (EAC rosettes) and an increase in null cells. T-cell reduction was most marked in patients with splenomegaly and it was suggested that there is sequestration of T lymphocytes during infection (Wyler, 1976). Histological studies showed that there was marked thymus involution with P.falciparum infections in children and in mice infected with P.berghei though there were none with mild P.yoelii infections (WHO, 1975). Phillips (1970) demonstrated the possible role of cell-mediated immunity by showing the protective effects of adoptive cell transfer from the immune rats to non-immune rats infected with P.berghei. The cells were either from the spleen, lymph nodes, thymus, peritoneal exudate or bone marrow. Also spleen cell transfer from the immune to non-immune rats has been shown to give some protection from P.berghei (Steckschulte et al., 1969; Roberts, 1971; Phillips and Jones, 1972; Cabrera et al., 1973; Brown, 1974). The degree of protection was shown to depend upon the time the spleen cells were taken and the dosage of the of the cells given (Roberts, 1971; Cabrera et al., 1973; Kasper

and Alger,1973). Brown (1971) demonstrated the importance of T-cells by showing recrudescence of a chronic infection in rats treated with ALS. Spleen cells from ALS treated rats also failed to confer immunity in adoptive transfer experiments. Later, Zuckerman and Jacobson (1976) tried to prove the same fact by showing that spleen cells from cyclophosphamide treated P.berghei immune rats could still transfer immunity to recipient animals. The authors suggested that the cyclophosphamide treated rats have spleens depleted of B lymphocytes with a higher proportion of T-cells. Thus, according to them, T-cells are involved in acquired immunity to malaria. On the other hand Jayawardena et al. (1975) demonstrated that anti-theta treatment in vitro of cells from mice which had recovered from infection did not impair their ability to transfer immunity to either intact or T-cell deprived recipients.

Since passive transfer of serum is relatively ineffective (Phillips,1970; Phillips and Jones,1972) and since the development of protection is dependent on the viable T-cells (Cabrera,and Alger,1973), Brown (1971) suggested that the key to strain-specific immunity resided in sensitization of the T-cell population. He postulated that protective antibody in malaria is variant-specific and that protection is mediated through a succession of variant-specific protective antibody responses. He added that T-cells responded to the antigenic determinant common to all variants of a strain and that these T-cells act as 'helper' cells in synthesis of variant specific protective antibody by direct bone marrow derived B-cells (review by Brown,1975).

Some in vitro studies also indicate an important role for cell-mediated immunity in malaria infections. Wyler and Oppenheim (1974) showed that lymphocytes taken from the circulation of P.falciparum infected patients proliferated in the presence of malaria antigen more than cells from uninfected controls. Zuckerman (1974) showed that spleen cells from rats infected with P.berghei have enhanced lymphocyte transformation and macrophage migration inhibition responses. Coleman et al. (1976) also reported an in vitro correlate of cell-mediated immunity, the macrophage migration inhibition test, is positive in mice infected with P.berghei. They demonstrated the inhibition of peritoneal macrophages by splenic lymphocytes from P.berghei infected mice in the presence of parasite antigen. Attempts to show cell-mediated cytotoxicity in vitro in malaria infections have not yet been successful. Phillips et al. (1970) tried to show a cytotoxic effect on P.knowlesi infected erythrocytes with spleen cells from immunized monkeys and Ade-serrano and Osunkoya (1975) attempted the same with cells from chronically infected rhesus monkeys without success.(cited by WHO, 1975). Coleman et al. (1975) did obtain indications of a cytotoxic effect in P.berghei in mice which they considered involved both lymphocyte and antibody. Isfan and Dinculescu (1971) extracted a factor from P.berghei parasites and from the infected liver and spleen, which had a cytotoxic effect on chicken embryo cells in vitro and could be neutralised by immune serum. According to them the cytotoxic factor was present in the plasma.

Though people try to separate the functions of T and B cells or humoral and cell-mediated immune responses, there is in fact no natural demarcation line between them at all. The continuous changing life cycle, the unending process of cellular reorganization causing antigenic plasticity and a massive and everchanging antigenic challenge of malaria infection causes the complex host responses (Brown, 1975).

b. Survival of malaria parasites in the immune host.

The persistence of malaria parasites for long periods in the host is due to survival of the parasites as either exoerythrocytic (EE) or erythrocytic stages. Though Yoeli and Most (1965a) suggested that the persistence of an exoerythrocytic stage is due to antigenic variation, because of difficulty in the experimental procedures we know little about the EE stages. The intracellular location is probably of considerable importance in protecting them from immune attack (Garnham, 1966).

The ways in which the blood stages, mainly the asexual stages, might evade the immune mechanisms have been reviewed in detail (CIBA, 1974; WHO, 1975; and Ogilvie and Wilson, 1976).

1. Antigenic variation

The occurrence of a wide spectrum of intra-strain antigenic variants during the course of the asexual cycle has been clearly demonstrated in P.knowlesi malaria in rhesus monkeys by Brown and his colleagues (Brown,

1974) with the schizont infected cell agglutination (SICA) test. The SICA test shows no correlation with protective immunity and, according to Brown, antigenic variation is antibody induced and not an immunoselective process. So the growth of P.knowlesi parasites in the presence of serum containing variant specific SICA antibodies triggers a change of serotype without detectable destruction of parasites (Brown,1974). The appearance of intra-strain antigenic variants has been shown in other ways in P.cynomolgi in monkeys, P.berghei in mice, some avian malaras and with less certainty in the human malaria parasite P.falciparum (WHO,1975). The schizont agglutinins have been shown to be present in P.gallinaceum infections (Todorovic et al.,1968) but not in many other malaria infections. Though a capacity to express different serotypes in P.knowlesi malaria infection in rhesus monkeys is an important phenomena for the survival of the parasite, the importance of it in other plasmodium species remains uncertain.

#### 11. Antigenic tolerance

Soluble malaria antigens have been described in monkeys infected with P.knowlesi, ducks infected with P.lophurae, chickens infected with P.gallinaceum, rats infected with P.berghei and in humans infected with P.falciparum ( Cited by Boonpucknavig et al.,1976),Wilson et al.(1969;1973) by using the agar gel double diffusion technique of Ouchterlony to indicate soluble plasma antigens and antigens extracted from infected red cells in Gambian children, thought that both antigens were associated with P.falciparum infections. The antigens were classified on the basis of

their heat stability as S, L and R antigens. From in vitro studies on uptake of amino acids, Wilson (1974) suggested that S antigens which are most common in plasma during acute infections are of host origin (Wilson et al., 1975a, 1975b). After chemotherapy the antigens never circulate for more than a week, their disappearance frequently being associated with the appearance of homologous antibody (Wilson, 1974). Houba and Williams (1972) have shown that when an antigen is in excess, the serum antigens may circulate as complexes with IgM.

Dooris and McGhee (1975) have shown that immunological tolerance to serum antigens in neonatal chickens is present by infecting them intravenously with serum obtained from a P.gallinaceum infected chicken. They were able to demonstrate that there were no antibody responses in the chicken previously injected with the infected serum if the chickens were rechallenged with P.gallinaceum after the 11th day though control birds did respond. Wilson (1974) discussed some ways in which such antigens might affect the immune response to the parasite and prolong its survival by blocking or neutralizing the effective immune response through for example tolerance, antigenic competition or immune deviation.

### iii. Immunodepression

Malaria infection is associated with immunodepression and the extent of this parallels the degree of parasitaemia. It is short-lived in P.yoelii infections but long lasting in chronic experimental malarias (Wedderburn, 1974).

From epidemiological data there is some evidence that malaria diminishes the incidence of auto-immune diseases in man (Greenwood, 1968). The incidence of auto-immune haemolytic anaemia and renal disease in strains of mice which develop these disorders spontaneously is also reduced by P.berghei infections (Greenwood, Herrick and Voller, 1970). Antibody production to some antigens is also reduced in mice during P.berghei infections; these include sheep red blood cells (Salaman, Wedderburn and Bruce-Chwatt, 1969), human immunoglobulins (Greenwood, Playfair and Torrigiani, 1971a) and Salmonella typhimurium (Kaye et al., 1965). The incidence of lymphoma following inoculation of Maloney virus is increased in mice infected with P.berghei (Wedderburn, 1970). This might support the view of association of Burkitt's lymphoma and malaria in Africa (Salaman et al., 1969).

But the antibody response to Keyhole limpet haemocyanin, and bacteriophage is not significantly impaired and also the allograft skin rejection and contact sensitivity to picryl chloride or oxazolone was not depressed in mice infected with P.yoelii (Greenwood et al., 1971a). Greenwood (1974) showed that children with malaria, and their controls, responded similarly to dinitrochlorobenzene (DNP) and to skin testing with purified protein derivative, candida and streptococcal antigens though they responded significantly less frequently to tetanus toxoid and O antigen of S.typhi. He suggested that in malaria, the cell-mediated immune response is not affected and the immunosuppressive effect is due to its effect on bursa derived lymphocytes, on macrophages or on cell co-operation.

In contrast Sengers et al. (1971a) showed that Swiss mice infected with P.berghei failed to reject both syngeneic and allogeneic skin grafts. Also Abdel-Wahab et al. (1974) showed the impairment of cell-mediated immune responses by showing a highly significant reduction in size of the granuloma around schistosome eggs in sensitized malaria infected mice, in comparison with uninfected controls. Whitmore (1974) also demonstrated delayed hypersensitivity reactions to methylated human serum albumin and SRBC in P.berghei infected mice. According to him, there could be a marked suppressive effect on the cell mediated immune response in P.berghei infections but that the timing of infection was a critical factor in the demonstration of this suppression.

Greenwood (1974) pointed out that in malaria, massive amounts of immunoglobulins are produced because of the production of a mitogen effective against B lymphocytes. He suggested that immunoglobulin synthesis is not impaired during acute malaria infection and he also found that malaria has no suppressive effect on an established humoral immune response in man. Steward and Voller (1973) reported that though mice infected with P.yoelii developed normal levels of antibody to human serum transferrin, the affinity of it was lower than that of the uninfected control.

Barker (1971) suggested degradation of SRBC antigens, particulate overloading of the RES and a depletion of the population of SRBC sensitive lymphoid cells as three possible mechanisms which could operate during a malaria infection. But Greenwood et al. (1971b) found no evidence of reticuloendothelial blockade in P.yoelii infected mice. On

the contrary, at the time of maximal parasitaemia and maximal immunosuppression the clearance of carbon or  $^{51}\text{Cr}$ -labelled SRBC from the peripheral blood was enhanced, an observation of the evidence for RES hyperactivity in infected mice.

Loose et al. (1972) showed that peritoneal macrophages obtained from malaria infected mice had a normal ability to ingest SRBC in vitro; such SRBC exposed macrophages induced significantly fewer plaque forming cells than mice not infected with malaria. Loose et al. (1971) have shown that macrophages from malaria-infected mice are very ineffective in detoxifying endotoxin. This defect in handling and processing of antigen by macrophages was proposed to play a major role in immunosuppression of malaria infection (Loose et al., 1972, Greenwood, 1974).

Salaman et al. (1969) considered the phenomenon of antigenic competition to be a possible 'mechanism' in malarial immunosuppression. But Greenwood et al. (1970) found that infection of rats with P.yoelii one week before, or at the time of, an injection of adjuvant reduced the incidence and severity of the ensuing adjuvant arthritis. According to them antigenic competition was not responsible for the suppression of adjuvant arthritis.

Hudson et al. (1976) suggested a possible link between high IgM levels of Trypanosoma brucei brucei infection in mice and immunodepression. According to them, the increased background count of IgM in the infected unimmunized mice and the enhanced responsiveness of both IgM and IgG PFC to SRBC in the early part of the infection in T.brucei

infected mice were suggested to be due to polyclonal activation. The polyclonal activation of B-cells in the presence of a continuous trypanosome infection is likely to result in a progressive depletion of antigen reactive B lymphocytes as these are activated to change into secretor cells, resulting in the depletion of B-cells capable of recognising the introduced antigen. Polyclonal B-cell activation has previously been suggested to occur in malaria (Greenwood, 1974) and in trypanosomiasis (Freeman, 1975).

The exact mechanism of immunodepression is still obscure. It is a complex phenomenon and probably T-cells, B-cells and macrophages are all involved, either directly or in co-operation and antigenic competition either at the macrophage level or to B or T cells may be present as well.

## B. Protein-energy deficiency and immunity to infection

The interaction of infection and malnutrition may be considered to be cyclic insofar as one condition is capable of accentuating the other (WHO, 1971). "Protein and energy deficiencies affect the ability of the organism to make new cells and to synthesize and secrete proteins and so the reactive capacity of the organism to injury, be it microbial, toxic or genetic is severely restricted" (Ramalingaswami, 1975).

### 1. Humoral immune response

Contradictory results have been reported for immunoglobulin levels in protein and energy deficient children. Protein-energy deficient children are reported to have high (Keet and Thom, 1969; El-Gholmy et al., 1970; and Neumann et al., 1975), normal (McFarlane, 1970; Rosen et al., 1971; and Neuman et al. (1975) or Low (Brown and Katz, 1966; and Aref et al., 1970) levels of Ig. Watson and Freeman (1970) suggested that high Ig levels in Kwashiorkor may be in response to infections. This suggestion is in agreement with the results of Purtilo et al. (1976), who showed that the increase in Ig (IgA, IgM, IgG and IgE) were related significantly to the intensity of the intestinal nematode infestation rather than to the state of the nutrition of the children. According to Lechtig et al. (1970) Ig levels in nourished and malnourished persons living in the same areas were similar apart from raised IgA in protein energy malnutrition (PEM). Price and Bell (1976) demonstrated in mice that the antibody response in PEM to T-cell independent antigens, polyvinyl pyrrolidone (PVP) and Brucella abortus, are not affected.

But responses to T-cell dependent antigens like sheep red blood cells and tetanus toxoid are either normal, elevated or suppressed depending on the dose, and in the case of tetanus toxoid, the age of the host. According to Bell et al. (1976a) though there is a reduction of lymphoid tissue in PEM mice, antibody production is not reduced because the amount of antibody produced per unit of lymphoid tissue of the animal is increased. Sirisinha et al. (1973) have reported a normal level of C<sub>4</sub> and low levels of C<sub>1</sub>, C<sub>3</sub>, C<sub>5</sub>, C<sub>6</sub> and C<sub>9</sub> in malnourished South-East Asians. Also Olusi et al. (1976) reported that apart from C<sub>4</sub> and C<sub>5</sub>, C<sub>1</sub> to C<sub>9</sub> were all depressed in PEM children.

## 2. Cell mediated immune response

Children with PEM and measles sustain a high mortality and morbidity and often lack a rash, which is supposed to be due to T-cells. The children sometimes die of giant cell pneumonia (Faulk et al., 1975). This implies a depression of CMI in PEM. Also Smythe et al. (1971) have shown that PEM children have impaired responses to contact sensitivity to 2-4 dinitrochlorobenzene, the lymphocytes from them show less PHA response, and the tonsils are smaller. Chandra (1972) confirmed these results in the malnourished children. He showed reduced delayed hypersensitivity responses of skin to BCG. Watts (1969) also showed that children who died of protein and energy malnutrition had a reduced thymus/bodyweight ratio and marked atrophy of the thymus. Several investigators have shown decreased T-cell rosettes in malnutrition (Bang and Bang, 1973; Chandra, 1974; Ferguson et al., 1974). Bell et al. (1976a) showed that mice maintained

from weaning on a 4% albumin diet have only 10-20% of the numbers of lymphoid cells found in normally-fed mice.

The anaemia produced by PEM is well recognised and may be reproduced experimentally by feeding animals diets deficient in protein or a variety of other nutritional factors. Under these conditions all the cellular constituents of blood fall below the levels of normally fed controls (Bell et al., 1976b).

The interactions of nutrition and infection have been reviewed in detail by Scrimshaw et al. (1959). There were a lot of controversial results but almost all of the bacterial infections and malnutrition give a synergistic effect with an increase in the infection. Deficiency of proteins permits greater development of Ascaris, Trichinella, Nippostrongylus, Hymenolepis and Ancylostoma caninum in various animal hosts.

However meat diets suppress P.berghei malaria infections in mice (Adler, 1958), and milk diet suppresses P.berghei infections in mice (Maegraith et al., 1952), P.knowlesi in monkeys (Maegraith, 1953), and plasmodium infections in man (Chauduri and Dutta, 1955). These effects are due to lack of para-aminobenzoic acid (PABA) rather than due to protein (Adler, 1958; Jacobs, 1964).

Rats starved for 5-10 days were able to suppress the parasitaemia of a P.berghei infection (Ramachrisnam 1953). Similarly P.knowlesi infection in monkeys was suppressed in animals starved for 24-48 hours (McKee and Geiman, 1948).

Seeler and Ott (1945) have shown that pure protein

deficiency caused the enhancement of P. loohuræ infection in birds. Also Suntharasamai and Marsden (1972) showed that protein deficiency in mice with P. yoelii infection gave rise to more anaemia and prolonged parasitaemia with less splenomegaly.

### C. Concomitant infections of malaria and other infections

Malaria has been shown to be immunosuppressive, the intensity of the depression being related to the parasitaemia. The virulent forms like P.berghei in mice and P.falciparum in man produce marked involution of the thymus but this does not occur with a milder infection like P.yoelii in mice (Jayawardena et al.,1975). It is also a common observation that generalised immunodepression may occur during certain bacterial, viral and parasitic infections (Schwab,1975). Consequently many people are interested in the interactions of malaria and other infections, viruses, bacteria and other parasites.

Eperythrozoan coccoides infections in mice produce anaemia and reticulocytosis (Peters,1965a;Ott et al.,1967). P.chabaudi infections were less severe with 100% survival in the Eperythrozoan infected mice in an otherwise fatal infection, but P.berghei was slower in developing in Eperythrozoan infected mice, and was accompanied by a delay in time to death (Ott et al.,1967; Ott and Stauber, 1967). Since P.berghei favours the reticulocytes and P.chabaudi the mature erythrocytes, the authors drew the conclusion that the effects of E.coccoides on reticulocytosis must be the basis for the explanation. Ott et al. (1967) also found out that P.berghei and P.chabaudi infections caused the lengthening of the prepatent period of E.coccoides.

Virus infections induced the production of interferon in mice (Merigan,1970) as do the erythrocytic infections of P.berghei (Huang et al.,1968). Schultz and Huang (1968)

demonstrated that Newcastle disease virus prolonged the survival times and decreased mortality in P.berghei-infected mice. The authors proposed that it was due to the effect of interferon. Jahiel et al.(1970) proved the fact by exogenously administered serum containing interferon. This protected the mice against P.berghei infection, the level of protection depending on the dosage used. There are reports that malaria infection reduced the antibody response to tetanus toxoid in man (McGregor and Barr1962) and to pertussis vaccine in mice (Viens et al.,1974). Wedderburn (1974) has demonstrated the enhancement of lymphomagenesis together with depressed levels of total virus neutralizing antibody in mice infected with Moloney lymphomagenic virus (MLV) and P.yoelii infections. The mild P.yoelii infection also became severe in doubly infected mice. Also,P.chabaudi or P.yoelii infections combined with Rowson-Parr virus (RPV) lead to exacerbation of the plasmodium infection with reduction in antiparasitic antibody levels (Salaman,1970; Cox,1974b ). Burkitt's lymphoma (Burkitt,1959) occurs mainly in definite geographical areas, and a causal connection between this lymphoma and holoendemic malaria has been suggested (Dalldorff et al.,1964; Burkitt,1969).

Bacterial endotoxins, particularly from Escherichia coli, prolonged the life of mice infected with P. berghei (Martin et al.- 1967 ). Intravenous injection of killed Corynebacterium parvum in mice was shown to have some protection for the mice against P.berghei (Nussenzweig,1967), P.chabaudi and P.vinckei infections (Clark et al.,1977). Nussenzweig(1967) showed that the stimulation of reticuloendothelial system (RES) and

increasing phagocytosis was responsible for protection. Bazaz .- Malik (1973) also demonstrated that monkeys infected with Mycobacterium tuberculosis of simian origin had lower parasitaemias when infected with P.inui or P.cynomolgi than the control. The authors suggested that the initial effect was due to the non-specific stimulation of RES and that subsequent effects were due to the adjuvant nature of the tubercular infection. Intravenous infection of BCG into mice was shown to protect them from P.vinckei and P.yoelii infections (Clark et al., 1976). The authors proposed that the protective effect was due to the non-specific soluble mediators like interferon or lymphokines which were increased by intravenous injection of BCG or C.parvum (Clark et al., 1977).

Mice which had recovered from Babesia microti infections were nearly immune to P.vinckei or P.chabaudi infections and the reverse was also true as well (Cox, 1968).

Strickland et al. (1972) have shown that Toxoplasma gondii infection in mice led to an increase in anaemia, parasitaemia, diminished immune response and mortality if the mice were infected with P.berghei. If a P.yoelii infection was given one week before the usually mild Trypanosoma musculi infection in mice, the T.musculi infection turned into a severe form (Cox, 1975). Also Jackson (1959) showed that the mild infections of T.lewisi and P.berghei in rats became severe when given together with a mortality of 68%. P.yoelii infection before, together with or after T.b.rhodesiense caused a shortening of the period of survival of the mice and the T.b.rhodesiense

enhanced the normally mild P.yoelii infection (Dallas,1976). Leishmania enrietti inoculated intradermally into hamsters produced a granulomatous lesion. When P.yoelii infection was given to hamsters together with or shortly after L.enrietti infection, the granuloma size was larger than with the L.enrietti infection alone, but if it was given two weeks after P.yoelii infection there was a reduction in granuloma size compared with the control (Belehu et al., 1976). According to the authors, the size of the granuloma depended on the relative timing of the two infections. They got similar results when they did the same experiment with chronic malaria produced by the infection of P.berghei in P.yoelii immune hamsters. But Ebert (1970) demonstrated that, though P.vinckei was invariably fatal in white mice, when given together with Leishmania donovani intraperitoneally 15 of the 35 mice recovered and 29 mice had an increased survival time. The numbers of leishmania parasites present in both infections were reduced compared with the leishmania infection alone. Cross-immunity between the malaria parasites has been shown (Yoeli et al.,1966; Cox and Voller, 1966). Complete cross-resistance was demonstrated between P.chabaudi and P.vinckei, mice which had recovered from infections of either of these species being resistant to challenge with the other. But previous infection with P.berghei in rats protected completely against P.yoelii, however the latter only induced partial protection against P.berghei. P.berghei did not protect against either P.chabaudi or P.vinckei nor did P.vinckei and P.chabaudi protect against P.berghei (Cox and Voller, 1966). This, according to the authors, was due to the differences in antigenic characteristics of P.yoelii

and of P.berghei on the one hand, and P.vinckei and P.chabaudi on the other.

Concomitant infections of malaria and schistosomiasis have frequently been observed in areas where both diseases are endemic (Yoeli, 1956). According to Nelson (1958) spleen rate increases in younger children (below 10) where malaria and schistosomiasis are both prevalent as in the West Nile region of Kenya. Schistosomiasis is also known to produce immunosuppression of humoral responses to tetanus toxoid if the toxoid was given 6 weeks after infection (Brito et al., 1976). A marked diminution of the response of mice infected with Schistosoma mansoni to injection of sheep red blood cells has been shown (Mota-Santos et al., 1976; 1977) and to lipopolysaccharide from Escherichia coli (Mota-Santos et al., 1977). According to these authors the immunosuppression in S.mansoni was induced by adult worms and not by eggs and the antigens involved in immunosuppression originated from the surface membrane of schistosomes. Araujo et al. (1977) showed that there was depression of cell-mediated immunity also by delayed rejection of skin grafts in mice after 60 days of schistosomiasis infection. Pelley et al. (1976) have shown that there was profound unresponsiveness to Concanavalin A and phytohaemagglutinin after 12 weeks of schistosomiasis infection in mice. They suggested the possibility of suppressor T-cell activity being the explanation. Kloetzel et al. (1973) studied the concomitant infections of Trypanosoma cruzi and S.mansoni in albino mice. They showed that if the T.cruzi infection preceded that of S.mansoni, the number of trypanosomes in the blood was about double that of the

controls and the worm recovery was sometimes reduced, the worms tending to be stunted. When S.mansoni infection preceded that of T.cruzi, the trypanosomes in the blood were many times more numerous and they persisted longer. But with malaria infections Yoeli (1956) demonstrated that if a P.berghei infection was given shortly or 1-2 weeks after exposure to S.mansoni in the field vole a high proportion developed an infection which ran a chronic course. But if malaria was given 4-7 weeks after the S.mansoni infection, the parasitaemia was of a low mild course without any mortality, though there was a 21.7% death rate after infection with P.berghei alone. According to the author the latter effect was due to the nonspecific stimulation of reticuloendothelial system by the S.mansoni infection with a marked increase in phagocytosis causing the control of concomitant plasmodial infection. Lewinsohn (1975) also attempted to find out the combined effect of S.mansoni and P.yoelii infections in mice. According to her there were no significant differences between the single and double infections in the haemoglobin levels, reticulocyte counts and splenomegaly. There was marked suppression of the malaria parasitaemia level in the group of mice infected with P.yoelii 5 weeks after exposure to cercariae compared with that of the control, whereas the group of mice infected with P.yoelii 3 weeks after cercarial infection did not show any significant difference in parasitaemia compared with that of the malaria control. The author suggested that this might be due to the variation in response of the mice.

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Abdel-Wahab et al. (1974) demonstrated a marked reduction in size of granulomas in lungs of mice injected intravenously with S.mansoni eggs 16 days after P.berghei infection. This effect was supposed to be due to the immunodepressive effects of malaria on the granuloma formation.

## D. Chemotherapy of malaria infection

1. Mode of action of antimalarials

The established pharmacological actions of the antimalarial drugs are discussed in most of the standard textbooks of pharmacology and tropical medicine. I intend here to consider specifically the drugs that were used in my experiments. The antimalarials are grouped, in accordance with their modes of action, as 8-aminoquinolines, antimetabolites and schizonticidal groups of antimalarials (Warhurst, 1973).

a. 8-aminoquinoline antimalarials

The 8-aminoquinoline group of antimalarials consists of primaquine, pamaquine, quinocide and others, some of lesser importance. This group of drugs has a unique antimalarial spectrum (Pinder, 1971) and is effective against all stages of the life cycle of plasmodia (Warhurst, 1973). Tissue stages of malaria are more sensitive to the 8-aminoquinolines than the erythrocytic schizonts; Warhurst and Killick-Kendrick (1967), Gregory and Peters (1970) and King et al. (1972) showed with P. yoelii and P. berghei infections in mice that exoerythrocytic stages were susceptible to pamaquine and primaquine though the erythrocytic stages were not.

The mechanism of the mode of action of the 8-aminoquinolines is not yet known (Warhurst, 1973) but what is known has been reviewed by Goodwin and Rollo (1955), Peters (1970a, 1974a) and Warhurst (1973). Greenberg et al. (1951) showed that pamaquine was inactive in vitro against blood

stages of P.gallinaceum but metabolites produced by the chickens given the drug were active. The metabolism of the 8-aminoquinolines to form the active form in vivo has been discussed by Warhurst (1973) (referring to Tarlov et al., 1962 and Smith, 1956). The metabolism of pamaquine and of primaquine and pentaquine to the form active against P.gallinaceum blood stages is by demethylation of the 6-methoxy group to the 6-hydroxy form, and oxidation in the 5 position to give the 5-6 dihydroxy derivative. This is in oxidation-reduction equilibrium with a reduced form, the 5-6 quinoline diquinone.

Skelton et al. (1968, 1970) have shown that P.lophurae, P.knowlesi, P.cynomolgi and P.berghei synthesize ubiquinone 8 (Coenzyme Q8). The vertebrate blood itself contains either ubiquinone 9 or 10 only (Warhurst, 1973). Skelton et al. (1968) showed that primaquine, chloroquine and several other antimalarials acted by affecting the enzyme systems involving mitochondria and biosynthesis of ubiquinones in P.lophurae. But the 8-aminoquinolines were shown also to bind nucleic acid in vitro (Morris et al., 1970). Whichard et al. (1972) showed that the 8-aminoquinolines and two hydroxylated derivatives inhibited certain bacterial DNA polymerases. Conklin and Chou (1970) working with Tetrahymena pyriformis said that primaquine acted by blocking the amino acid uptake.

The major drawback to the clinical use of the 8-aminoquinolines is the toxic effects, even at the therapeutic dosages (Rollo, 1970). They effect the central nervous system causing anorexia, cyanosis, pain in abdomen and

vomiting, and effects on haemopoietic tissues causing leukaemia, anaemia, methaemoglobinemia, and suppression of myeloid activity (Hill, 1963). The haemolytic side effects occur in relation to a glucose 6-phosphate dehydrogenase (G6PD) deficiency (Rollo, 1970; Peters 1974a).

Only primaquine now has widespread therapeutic use, though quinocide is also used in Eastern Europe and the USSR (WHO, 1961) for the radical cure of P.vivax and P.malariae infections (Hill, 1963). It has been recommended that the 8-aminoquinolines should always be given with chloroquine to reduce the likelihood of drug resistance developing (WHO, 1961; Arnold et al, 1961).

Thus the mode of action of the 8-aminoquinolines can be summarized as follows:

1. They must be metabolised in vivo to form active compounds
2. They affect coenzyme synthesis of parasites
3. They also have an effect on DNA but the exact mechanism is not yet known
4. They are effective against all stages of parasites.

#### b. Antimetabolite antimalarials

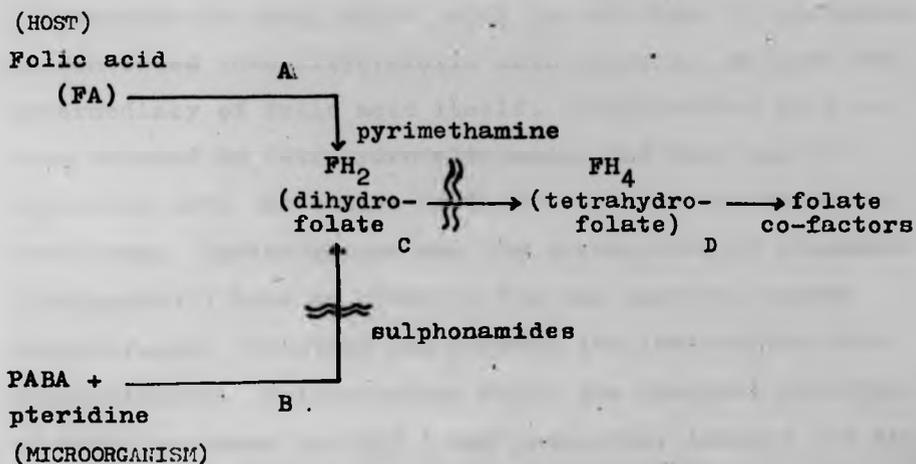
This group of antimalarials consists of pyrimethamine, sulphadiazine, dapsone, proguanil and cycloguanil. This is the only group of antimalarials whose mode of action is well known, as the name implies.

Gregory and Peters (1970) and Vincke (1970) have shown that pyrimethamine is effective against the exo-erythrocyte

stages of P.berghei. Gerberg (1971) also showed the susceptibility of sporozoite stages of P.gallinaceum to pyrimethamine and cycloguanil in Aedes aegypti. Terzian (1970) also showed pronounced oocysticidal effects of pyrimethamine and cycloguanil against P.cynomolgi in Anopheles stephensi, and that proguanil, cycloguanil and pyrimethamine interrupted growth of the oocysts in P.vivax-infected Anopheles quadrimaculatus and A.stephensi. In fact antimetabolite antimalarials are effective against all stages of the life cycle of plasmodia, apart from the gametocytic stage (Warhurst,1973; Peters,1974a).

Pyrimethamine was claimed to be inactive in vitro against the asexual blood stages of P.gallinaceum by Taylor et al (1952). Gutteridge and Trigg (1971, 1972 ) found that pyrimethamine inhibited DNA and RNA synthesis of P.knowlesi in vitro and in vivo, mainly during the schizont stage, that is after most of the synthesis of DNA for multiplication of the parasite had already taken place. They concluded that pyrimethamine inhibits parasite segmentation and schizogony, not by inhibiting DNA synthesis but by inhibiting some metabolic process like dehydrofolate reductase function for synthesis of tetrahydrofolate co-factor. Ferone and Hitchings (1966) have shown that P.berghei could not use folic acid directly from the host due to the lack of folate reductase but could synthesize its own folate co-factors. Many authors ( Ferone and Hitchings,1966; Peters,1970; Warhurst,1973) have discussed the role of antimetabolite-antimalarials in synthesis and use of folate co-factors. According to them malaria parasites, like some pathogenic bacteria, couple a pterin

Fig 1. Folate cofactor biosynthesis pathway



A folate reductase

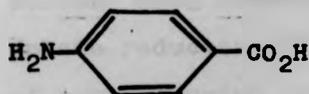
B PABA + pteridine condensing system

C dihydrofolate reductase

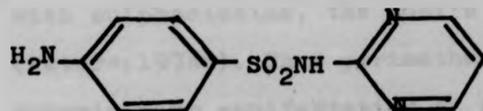
D system for adding 1 carbon unit to  $FH_4$ 

Wavy lines indicate the loci of inhibitors.

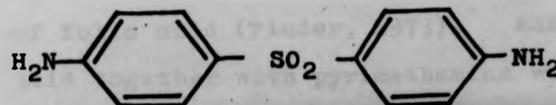
(Ferone and Hitchings, 1966)



P-aminobenzoic acid



Sulphadiazine



Dapsone

derived from guanosine monophosphate with PABA to give dihydropteroid acid, which, with the addition of glutamate is converted into dihydrofolic acid directly, without the intermediacy of folic acid itself. Dihydrofolic acid is then reduced to tetrahydrofolic acid, and this can be converted into the active co-factors by further enzymatic reactions. Pyrimethamine and the metabolites of proguanil (Cycloguanil) have an affinity for the parasite enzyme dihydrofolate reductase and prevent its interaction with dihydrofolate. Sulphonamides mimic the chemical structure of PABA (as shown in fig 1) and completely inhibit 7,8 dihydropteroate synthetase that acts on the folate pathway, and it is likely that dapsone acts the same way (Peters, 1971) and prevents tetrahydrofolate formation. Thus the combination of sulphonamide and pyrimethamine has potentiating effects on the parasites (Hitchings, 1960). The basis of the chemotherapeutic specificity of pyrimethamine lies in the extremely high affinity of the parasite enzyme for the drug compared with the mammalian enzyme dihydrofolate reductase (Warhurst, 1973). If a sufficient amount of pyrimethamine is administered either alone or together with sulphadiazine, the host's enzyme will be affected (Peters, 1974a). Thus pyrimethamine can cause macrocytic anaemia with manifestation of folic acid deficiency which can be reversed by cessation of treatment or by administration of folic acid (Pinder, 1973). Administration of folic acid together with pyrimethamine will not reduce the effect (Tong et al., 1970).

### c. Schizonticidal drugs

These consist of the most commonly used drugs for clinical treatment of malaria: chloroquine, quinine, amodiaquine and mepacrine.

#### Chloroquine

The early clinical work on chloroquine is summarised by Findlay (1951) and Pinder (1973). It is effective against the asexual blood forms in all species of human plasmodia but inactive against exoerythrocytic stages and sporozoites. Chloroquine is also gametocidal in infections with P.malariae, P.vivax and P.ovale but not active against mature gametocytes of P.falciparum. Many theories have been put forward to explain the mode of action of chloroquine against malaria infections. The following sequence of changes was seen after 80 minutes exposure to chloroquine in a concentration  $10^{-4}$ M (Ladda and Arnold, 1966):

"Increased formation of ribosomal nucleoprotein (RNP)

The single food vacuole was replaced by multiple vesicles.

Signs of focal cytoplasmic necrosis were seen.

The double-membraned vacuole showed signs of hypertrophy and infoldings of its membrane.

The nucleus developed a RNP-like 'nucleus' - stellate clumping of the RNP appeared in the cytoplasm, then the nuclear blebs were formed."

Ladda (1966) again studied the effects of antimalarial agents on blood stages of P.berghei under the electron microscope and concluded that chloroquine had a major

primary effect on nuclear metabolism with both primary and secondary cytoplasmic effects. Most studies on the biochemical effects of chloroquine action have focused on nucleic acid metabolism (Newton, 1970). The observation that chloroquine interacts with DNA and RNA polymerase reactions led to the hypothesis that the primary action of chloroquine was associated with inhibition of DNA replication (Cohen and Yielding, 1965, O'Brien et al., 1966). Gutteridge et al. (1972) showed that a chloroquine concentration of  $10^{-6}$  M had a marked effect on the growth and morphology in vitro of P.knowlesi and also on the incorporation of  $^3\text{H}$  adenosine into DNA and RNA,  $^{14}\text{C}$  isoleucine into protein, and respiration. According to them, binding to DNA is not the only action of chloroquine. Drug concentrations less than  $10^{-6}$  M had little effect in vitro. Van Dyke and Szustkiewicz (1969) had also shown that chloroquine inhibits the uptake of  $^3\text{H}$  adenosine by trophozoites in short-term culture. Theakston et al. (1972) studied the effects of chloroquine treatment on the uptake of  $^3\text{H}$  labelled adenosine and methionine by the erythrocytic stages of P.berghei using combined electron microscopy and autoradiographic techniques, and found that the uptake of both was reduced in the chloroquine treated infected blood. They thought that chloroquine interacts with the membranes of infected red cells altering their permeability in such a manner as to make them less permeable to certain small molecular weight substances and thus depriving the rapidly growing parasites of essential nutrients derived from the plasma of the host.

Trager (1967) has shown that chloroquine, pyrimethamine,

sulphadiazine and quinoline had inhibitory effects on P. lophurae maintained in vitro in duck red cell suspensions, but none of the drugs at the same concentrations inhibited the development of extracellular parasites. He suggested that it might be due to the lack of concentration of drug in the freed parasites. It has been well documented that chloroquine, amodiaquine and mepacrine are concentrated in plasmodium-parasitised erythrocytes (Peters, 1970a). Macomber et al. (1966) followed the uptake of  $^{14}\text{C}$  labelled chloroquine following a single intraperitoneal injection of  $40 \text{ mg kg}^{-1}$  into P. berghei infected mice and showed that there was a 25 times greater uptake of chloroquine by the parasitised erythrocytes compared with that by the erythrocytes of uninfected controls, the level in the infected cells presented a 100 fold concentration from the plasma. Polet and Barr (1968) found a greater concentration (300-600 fold) in erythrocytes infected with a chloroquine sensitive strain of P. berghei. The mechanism of concentration of drugs by parasitised erythrocytes remains obscure (Peters, 1970a). Warhurst et al. (1972) considered that quinine and chloroquine bound to the same receptor site in the intraerythrocytic parasite from their studies in vitro on P. berghei infected erythrocytes.

Warhurst (1973) has reviewed in detail the actions of chloroquine on formation of autophagic vacuoles in plasmodium. According to him the digestive vacuoles incorporate high affinity sites for chloroquine since it is the place where the first changes take place after drug treatment. From that site, he thought that the drug is

released into the parasite cytoplasm in high concentration. Interaction of the concentrated drug with the cellular synthetic machinery is supposed then to take place.

At present the exact mechanism of the antimalarial action of chloroquine is not yet known. But we now know that: 1. it concentrates in the erythrocytic membrane 2. it is effective against the intraerythrocytic stages of Plasmodium 3. it does not act on freed parasites in vitro, exoerythrocytic stages or on sporozoites. It is a drug of choice as a suppressive in both the acute clinical attack and for the prevention of symptoms; its toxicity is very low.

#### Quinine

Some twenty alkaloids have been isolated from the cinchona bark. The few most important are quinine, quinidine, cinchonidine and cinchanine (Pinder, 1973). Their chemistry (Turner and Woodward, 1953) and pharmacology (Findlay, 1951; Schmidt and Coatney, 1955; Hill, 1963; Peters, 1974a) have been reviewed. Yorke (1925) had shown that quinine did not destroy sporozoites since injection of quinine (10g) starting 5 days before and continuing until 7 days after infected mosquito bites did not prevent the P.vivax infection in man. Quinine controls the blood induced infections of P.cynomolgi in monkeys (Genther et al., 1948), P.berghei infection in mice (Peters, 1970a) and P.falciparum and P.vivax infections in man (Findlay, 1950). Mackerras and Eccle (1949) observed that quinine and mepacrine acted on the stages of P.vivax infection where

active digestion of haemoglobin was taking place. They also found that quinine had no immediate effect on pigment but the highly motile amoeboid trophozoites in the red cells had ceased to move 6 hours after administration of the drug. Yorke (1925), referring to Muhlens and Kirchbaum (1924), found that a mixture of equal parts of defibrinated P.vivax-infected blood and a 1:5000 solution of quinine was still infective after incubation for 12 hours at 37°C and, if the strength of the quinine solution were doubled, infection was still possible after 5 hours. This according to them was definite evidence that quinine, in concentrations considerably greater than that occurring in the blood stream, does not destroy the malaria parasites in vitro. Clarke (1952) showed that quinine, at a concentration of  $10^{-5}M$ , stopped incorporation of  $^{32}P$  into DNA in erythrocytes parasitised with P.gallinaceum in vitro, but 4 times the concentration had no effect on the incorporation by parasites freed from erythrocytes. According to Warhurst (1973) quinine competed with chloroquine for high affinity binding sites in P.berghei. He also stated that quinine concentrated in erythrocytic parasites of P.knowlesi three hours after treatment. Ceithaml and Evans (1946) confirmed this concentration of drug in plasmodium infected erythrocytes by quantitative methods.

Conklin and Chou (1970) observed that in Tetrahymena pyriformis quinine and other antimalarials block amino acid uptake.

So the actions of quinine can be summed up as:

1. concentration in the parasite
2. cessation of movement
3. it has no action in vitro especially on erythrocyte freed parasites
4. it prevents the incorporation of  $^{32}\text{P}$  into DNA and
5. quinine acts mainly on asexual blood stages of parasites.

## 2. Mechanism of drug resistance

The mechanism of development of drug resistance in malaria is not yet clear. It may be due to the selection of resistant mutants under drug pressure, to the spread of naturally resistant forms in the parasite populations (Peters, 1970b), the transfer of genetic information from resistant to sensitive strains (Yoeli et al., 1969) or to adaptation to the drug by previously sensitive parasites. Under experimental conditions drug resistance seems to depend on the immune mechanism.

The host immunity plays an important role in the successful activity of anti-protozoal drugs, and so any measure aimed at a reduction of the host's immune response will facilitate the emergence of resistance (Peters, 1970a). Under laboratory conditions, drug resistant strains are easily produced by suppressing or avoiding the immune response of the host. Warhurst (1965) and Hawking (1966) used a single injection of  $500 \text{ mg kg}^{-1}$  of ethyl palmitate into mice to produce 'clinical splenectomy' and then used these mice in experiments to produce resistance to chloroquine. Another effective way of avoiding the influence of immunity is to transfer parasites to a new host before the previous one has had time to produce an effective immune response to their presence, this is called the short passage method.

(Peters, 1970a). Two methods have been generally used in malaria infections. The first is the 'short passage method' by which parasites are passaged after exposure to the drug but before it has produced any obvious effect. The second method is the 2% technique originally described by Warhurst (1966) for use in bioassays and later modified by Peters (1968.) for development of drug resistance in P.berghei. WHO (1973) reported that a person's immunity should be taken into account before testing the drug sensitivity of their malaria parasites.

The problem of drug resistance in malaria is a major subject and it is impossible and inappropriate to discuss it comprehensively in this thesis. The subject has been extensively reviewed by Peters (1970a, 1974a). In this chapter it is intended to review only the mechanism of drug resistance relevant to chloroquine, quinine and pyrimethamine.

#### 1. Chloroquine and related schizonticidal drugs

At the present time the resistance to the 4-amino-quinolines has been confirmed with P.falciparum infections in man and has been recognised to be present in South-East Asia and parts of South and Central America (WHO, 1973). Chloroquine resistance is both increasing in virulence and spreading throughout the malarious areas of the world (Pinder, 1973).

There are at present two main theories for the development of chloroquine resistance in malaria. The first is based on the decreased uptake of chloroquine by

resistant parasitised red cells. Macomber et al. (1966) followed the uptake of  $^{14}\text{C}$  labelled chloroquine and showed that this was 2-3 times less by a chloroquine resistant strain than by parasitized cells of a drug sensitive strain.

Fitch (1970) showed that, in erythrocytes of mice infected with P.berghei and exposed to  $10^{-8}\text{M}$  chloroquine at  $27^{\circ}\text{C}$  and a pH of 7.2 to 7.4, the steady state internal to external distribution of the drug was approximately 600:1 for chloroquine sensitive parasites, 100:1 for resistant and 14:1 for uninfected cells. Homewood et al. (1972) found that an increase in the extracellular pH increased the intracellular pH of the erythrocytes and also increased the amount of clumping of pigment in the chloroquine treated parasitised erythrocytes. They proposed that resistant parasites may have less acid food vacuoles than sensitive parasites and hence attract into them less chloroquine.

However, Williams and Panimo (1975) showed that the mean intracellular pH values of erythrocytes parasitized with either chloroquine sensitive or resistant strains of P.berghei were very similar and were not significantly different from that of the extracellular pH. Fitch (1969) showed that the ability of chloroquine sensitive parasites to concentrate the drug was due to the availability of high affinity sites, but in the drug resistant strains, there was either a decrease in number or in the accessibility of chloroquine receptor sites on a constituent of the malaria parasite. Kramer and Matusik (1971) tried to

localise the different sites in lysates of P.berghei and found that the high affinity sites were associated with parasite membranes and the sites of lower affinity were cytoplasmic. According to Warhurst and Thomas (1973) the binding site was possibly a phosphate or a carboxyl group associated with lipid. So far the exact mechanism of concentrating the drug by the parasitized red cells is not known (Peters,1974) but drug-resistant parasites take up less chloroquine than the sensitive ones.

Another theory, put forward by Howells et al.(1970), was that chloroquine resistance was due to the ability of certain parasites, under chloroquine pressure, to switch prematurely from an anaerobic to an aerobic pathway involving succinate oxidation. They supported their theory by showing the presence of succinate dehydrogenase in the chloroquine resistant line which was absent in the sensitive parent line. The trophozoites of chloroquine-sensitive P.berghei lack a Krebs cycle (Theakston et al.,1970) though sporogonic stages utilize this pathway (Howells et al.,1970) It is postulated that chloroquine resistance in the trophozoites is expressed by a switch to Krebs cycle activity and the synthesis of amino-acids by transamination of intermediates of this cycle. This theory was reinforced by Palecek et al. (1967) and Cho and Aviado (1968) who showed that chloroquine resistant strains of P.berghei take up twice as much oxygen as do the normal parasites. Ali and Fletcher (1971) have also shown that there was an increased uptake of oxygen by the chloroquine resistant parasites. But, avian malaras utilize the Krebs cycle, synthesise amino acids by transamination, and

still produce haemozoin and are sensitive to chloroquine.

Long term investigations by Peters (1970b) suggested that the mechanism underlying chloroquine resistance requires a genetic mutation that is expressed when drug selection pressure is exerted. He drew the inference from the changes in response to chloroquine in P.berghei as it gained or lost resistance to the drug. This concept is strongly supported by the variation in the rate of development of resistance in different species and in its relative stability. Chloroquine resistance in P.falciparum is stable through repeated blood passages and mosquito transmissions in man, gibbons and chimpanzees, but for P.vivax, because of its low mutation rate, chloroquine resistance has not yet appeared and, moreover, the number of P.vivax parasites that are ever exposed at one time in an acute infection is much lower than those of P.falciparum (Pinder, 1973). It is also true in P.berghei infections that there are no natural variations in response to the drug treatment amongst different geographical isolates (Peters, 1970a).

Chloroquine resistance in rodent malarial parasites seems to take different forms. Spontaneous resistance to chloroquine was shown in the wild population of the rodent malaria P.yoelii by Warhurst and Killick-Kendrick (1967). P.berghei, P.chabaudi and P.vinckei are sensitive to chloroquine. Stable chloroquine resistance has been produced in P.vinckei (Powers et al, 1969) and in P.chabaudi (Rosario, 1976) by drug selection in the laboratory but resistance to P.berghei developed in a similar way is usually unstable in the

absence of drug pressure.

#### 11. Antifolic antimalarials

Pyrimethamine and proguanil have extremely flat dose response curves, they have significant antimalarial activity at smaller doses ( $\mu$ g) but are not 100% effective in curing apparently susceptible infections even at multi-milligram levels because both are intrinsically slow in action, allowing at least one full development of the cycle before the infection is controlled (Pinder, 1973). Hence the rapid emergence of resistance to these drugs in the field (Peters, 1974a).

Single or multiple drug resistance may be transferred from one strain of bacteria to another during conjugation by means of a 'package' of DNA or episome that carries the necessary code to the recipient (Peters, 1967b). Yoeli et al. (1969) proposed the term 'synpholia' for the transfer of drug resistance in their experiments. They injected a pyrimethamine resistant strain of P. vinckei and a sensitive strain of P. berghei simultaneously into CF<sub>1</sub> mice. Selection of strains of P. berghei was achieved by subsequently passing the mixed infection into hamsters, in which host P. vinckei does not grow. This biological filter allowed the isolation of P. berghei which became resistant to pyrimethamine. According to them it was due to the transfer of an unidentified factor from drug resistant P. vinckei to drug sensitive P. berghei at a time during the height of the mixed infection when the chances of trophozoites of both species invading the same individual mouse erythrocyte would be quite likely. Ferone et al.

(1970) supported the theory of 'synpholia' by showing that the character of the dihydrofolate reductase extracted from the resistant P.berghei was intermediate between that of the drug resistant P.vinckei and the drug sensitive P.berghei. They suggested that P.berghei acquired resistance from P.vinckei through direct transfer of genetic material for mutant enzymes. However, other workers have been unable to repeat the work on 'synpholia' (Peters, 1970a; Walliker et al., 1971). Walliker et al. (1973) proved that genetic exchange takes place during cross-fertilization of gametes in the mosquito between a pyrimethamine sensitive and a pyrimethamine resistant strain of P.yoelii and dismissed the possibility of 'synpholia'.

Ferone et al. (1970) and Ferone (1973) have shown that resistance to pyrimethamine is due to a decreased binding affinity of the enzyme for this compound, associated with an increased enzyme production by the resistant parasites. This is true with pyrimethamine, cycloguanil and trimethoprim. Diggins et al. (1970) also showed that dihydrofolate reductase from the pyrimethamine resistant line of P.berghei showed a reduced affinity for substrate, a decreased sensitivity to pyrimethamine, and was present at a higher level in the parasites when compared with the parent strain enzyme.

Resistance to pyrimethamine can follow a single exposure of a population of malaria parasites to this drug. According to Jacobs (1964) the pyrimethamine resistant P.berghei needs more PABA than the sensitive one. Diggins and Gregory (1970) showed that the exoerythrocytic stages

of a pyrimethamine resistant line of P.berghei were resistant to cycloguanil and proguanil, but sensitive to primaquine and sulphadiazine. So most strains resistant to pyrimethamine can be treated with sulphonamides, especially if combined with higher than normal levels of pyrimethamine (Peters,1970a).

## E. Immunity and chemotherapy

Ehrlich (1909) suggested that "the drug kills some of the parasites and releases antigens which result in a strong 'ictus immunosatorius'" (Cited by Taliaferro and Taliaferro, 1949). The resulting antibodies were then supposed to kill the remainder of the parasites. Since that time, the co-operative effects of immunity and chemotherapy have been the subject of much study and speculation. However, since immunology at that time was a new subject most of the studies were only superficial. Here I will review interactions of immunity and chemotherapy in some bacterial, helminth and haemoprotozoan infections. Also I will include some studies on cancer since most advances in immunotherapy have been made in this field.

1. Bacterial infections

Much has been published on the effect of the immune responses of the host on antibiotic treatment. Fleming (1938a) had shown that sulphonamide (T693) alone inhibited bacterial growth and production of toxins, while patient's serum alone killed the cocci of pneumococci and streptococci bacteria in vitro within two days. Fleming (1938b) showed the synergistic effect of passive immunization with specific antiserum and sulphonamide (T693) treatment in pneumococcal infection. From these experiments he suggested that patients should be immunized actively or passively before treatment. Branham (1940) demonstrated that combination of immune serum and sulphanilamide gave a far better result than either agent alone in the treatment of meningococcal infections and streptococcal infections of

mice (Lowenthal, 1939; Boyer, 1960) or staphylococcal infections in mice (De and Basu, 1938). Weil and Gall (1941) showed that Salmonella typhi infection caused a generalised infection in 10 day old chick embryos and, when treated with sulphanilamide and antibody, a cumulative effect was obtained. They also proposed clinical possibilities of combined chemotherapy and immunotherapy. Boyer (1960) prepared hyperimmune serum in rabbits by injecting them repeatedly with killed streptococcal A and C. The immune serum administered in low doses by itself gave low protection in mice; but when given with sulphanilamide or penicillin the curative action was increased. Treffers and Muschel (1954) used turbidimetric growth assays for in vitro measurements of the inhibitory actions of chloramphenicol and of specific antibody and complement; additive effects were seen when the two were combined.

Bringhurst and Marcus (1961) demonstrated in vitro that human gammaglobulin and normal serum has an antibacterial effect. When gammaglobulin was added in the same concentration as that of normal serum the antibacterial activity of penicillin was increased 35 times against three different strains of Staphylococcus aureus. Normal pooled serum acted in the same manner. Fisher (1957) proved the same fact in vivo by showing that commercial gammaglobulin (poliomyelitis immune globulin, human, Parke Davis and Co.) as well as pooled human gammaglobulin had a synergistic effect with chloramphenicol in mice infected with species of staphylococci, streptococci, pseudomonas and proteus. Also, samples of commercial gammaglobulin given alone without antibiotic were therapeutically active

against staphylococcus and streptococcus infections. In a staphylococcus infection as little as 0.02 ml of gammaglobulin solution per kg of mouse proved synergistic with chloramphenicol. The author suggested using either gammaglobulin alone or combined with antibiotic in known cases of gammaglobulin deficiency. Waisbren (1957) used a combination of antibiotics and gammaglobulin for treatment of 46 patients suffering from different bacterial infections. The combined treatment was far superior to antibiotic alone. Gammaglobulin alone was tried only in one case of endocarditis due to staphylococcus and two cases of pyelonephritis but the results were not satisfactory.

Though gammaglobulin acted very well either alone or with antibiotics (Fisher, 1957; Bringham and Marcus, 1961; and Waisbren, 1957) in vitro as well as in vivo, no-one attempted to explain the phenomenon. It might be due to the presence of specific antibody in the gammaglobulin or due to the presence of a substance or substances which will activate the alternative complement pathway and cause the lysis of the bacteria.

Combination of antitoxin or antiserum with drug has been tried in the hope of neutralizing the toxins produced by some bacteria. Gordon and McLeod (1941) experimented with infections of Clostridium septicum in mice and Cl.welchi, Cl.septicum or Cl.morgi in guinea pigs. They showed that multiple injections of serum into the muscles of animals infected with gas gangrene were beneficial but sulphonamides were not effective as prophylactics. However,

Henderson and Gorer (1940) showed a striking synergy of antitoxin and sulphonamide both for prophylaxis and cure of Cl.welchii and Cl.senticum but not Cl.oedaematiens infections in the guinea-pig. Recent practice has been to treat tetanus (Cl.tetani), gas-gangrene (Cl.welchii) and diptheria (Corynebacterium diptheriae) by giving high doses of antitoxin and antibiotic as a routine procedure (Davies et al., 1967). There have been some attempts at active immunization and drug treatment as well. Cokkinis and McElligott (1938) found that active immunization of patients with gonorrhoea before treatment with sulphonamide gave better prospects of cure. Maclean et al. (1939) proved the value of active immunization experimentally by showing the increased survival rate of pneumococcal infected mice and rabbits treated with a single dose of vaccine at the time of sulphonamide therapy.

Marcus et al. (1955) tried to prove the importance of host immune mechanisms during drug treatment of Klebsiella pneumoniae infection by treating intact and X-irradiated (350 rads) mice with antibiotics. According to them streptomycin and chlortetracycline treated irradiated mice showed a significantly higher mortality than the intact control animals. Chloramphenicol treatment did not give a significant difference between the two. Marston et al. (1953) showed that streptomycin treatment was more effective in treatment of staphylococcus, proteus and streptococcus infections in mice exposed to 250 rads than 475 rads of total body irradiation. Depression of resistance in mice by cortisone treatment impaired the antibiotic action of streptomycin and aureomycin against K.pneumoniae infection.

(Jawetz and Merrill, 1953). Skinsnes and Wool ridge (1948) made rats abnormally susceptible to pneumococci by feeding them a low protein diet (1.8%). Despite massive antibiotic therapy the viable microbes persisted in the blood and tissues of the protein depleted host significantly longer than in control animals given similar treatment.

According to Smith and Wood (1956a) the timing of the antibiotic treatment was most important in treatment of pneumococci infected mice. When penicillin therapy was begun 9 hours after inoculation, the pneumococci were cleared from the lesions with equal rapidity in the irradiated (650 rads) and the intact control mice. They thought that the pneumococci were multiplying rapidly in both groups and therefore were promptly killed by the direct action of the penicillin. Neither showed 'cellular exudates' (leucocytes). When the start of the treatment was delayed until 24 hours after inoculation, the bacteria in both sets of lesions had already reached their maximum counts and at this stage would be more resistant to the bactericidal effect of the antibiotic. Under such circumstances the destruction of the bacteria was found to be significantly slower in the acellular lesions (irradiated mice) than in those with a normal 'cellular exudate' (intact control). The authors concluded that in established pneumococcal infections in mice the curative effect of penicillin was due, not to the bactericidal action of the antibiotic alone, but rather to the combined effect of the drug and the cellular defences of the host. Smith and Wood (1956b) also showed that suppurative lesions caused by pneumococci were resistant to penicillin treatment. This

was because the pus provided a poor medium, the pneumococci remained metabolically sluggish and, therefore, were not killed rapidly by penicillin; so it was the cellular defences of the host which played a major role in eliminating bacteria. Wood and Irons(1946) studied the action of sulphonamide chemotherapy upon the experimental pneumococcal pneumonia infections in rats and showed that phagocytosis played an important role in bringing about the final destruction of the organisms in the lungs of treated animals. But the phagocytosis which occurred in the lungs of rats receiving sulphonamide was due neither to the action of the sulphonamide nor to type specific antibody (Wood et al.,1946 ). Eagle et al.(1953) studied penicillin treatment in actively immunized and non-immunized mice infected with Streptococcus pyogenes. According to them, immunization had no effect on the direct bactericidal action; this proceeded at the same rate in the immunized animals as in the control mice. The difference in the two groups lay in the course of infection after penicillin had fallen to an ineffective level. In non-immune mice, the surviving bacteria regularly grew out to cause a fatal infection; in the immunised ones the bacteria gradually disappeared in the 24-48 hours after penicillin had ceased to be operative, and the animals survived. They concluded that the host defenses and the bactericidal action of penicillin proceeded independently of each other, but were mutually supplementary in effecting a cure in the partially immunized animals.

## 2. Helminth infections

### a. Nematodes

There have been some interesting studies on the interaction of drugs and immunity in helminth infections. Bell (1973) treated a sample of a population infected with Ascaris lumbricoides with a single dose of pyrantel pamoate. The patients showed negative stools until the end of the 3rd post-treatment month, but the mean ova count then rose progressively, and at the end of 12 months was 230.9% higher than the mean pretreatment count. It was suggested that the clearance of adult and juvenile intestinal forms of A.lumbricoides might disturb the immunity of the host and allow a greater worm burden to develop if re-infestation occurred.

Scottish hill sheep increased their strongyle egg output during spring, 'the spring rise' (Soulsby, 1962). The treatment of animals with an antihelminthic prior to the spring rise not only fails to eliminate the spring rise but was often followed by a higher level of egg output and a weaker curative mechanism at the end of the spring rise than if the antihelminthic treatment had not been given. This was probably due to the disturbance of the immunity of the host as in the Ascaris infection.

When microfilariae of Litomosoides carinii were freshly infused into a normal rat, diethylcarbamazine (DEC) did not exert its usual microfilaricidal action (Kobayashi et al, 1969; Rao et al, 1977). Kobayashi et al. (1969) also showed that though DEC (200 mg kg<sup>-1</sup>) had no effect

when given 3, 5 or 40 days after microfilarial infection, it was effective when given 12 weeks after. They also showed that when adult worms were transplanted into the abdominal cavity of rats, DEC was effective only after day 15. DEC also had an immediate but transient reducing effect on microfilarial numbers if passive immunization with serum followed after DEC treatment. The authors concluded that the microfilaricidal effect of DEC was closely associated with the presence of antibodies. Fujita and Kobayashi (1969) had already shown that haemagglutinating antibody became detectable 10 days after worm implantation. Hashigushi et al. (1972) reported that prednisolone had a slight antagonistic action to DEC in cotton rat filariasis. Takaoka et al. (1974) demonstrated that DEC was effective against the inoculated microfilariae of Litomosoides in the presence of spleen cells from the other infected cotton rats. Tanaka et al. (1977) studied the effect of DEC on ATS or ALS treated cotton rats implanted with microfilaria of Litomosoides; ATS treatment was found to suppress the action of DEC. Also, when the effectiveness of DEC was tested in cotton rats treated with ATS and inoculated with immune serum (1 ml for 5 days), humoral antibody did not influence the inhibitory effect of ATS on DEC. The authors concluded that cell-mediated immunity might play a role in the action of DEC. According to Zahner et al. (1977) if microfilaria were injected intravenously into Mastomys natalensis and treated with DEC at different intervals, the effect when the drug was given after one hour was comparable with that of DEC given 1, 3 or 14 days after the start of the infection. Thus

DEC did not need any specific antibody for its action. However, they also showed that when immature microfilariae from the peritoneal cavity of a cotton rat were used, the DEC given after 1 hour was less effective than that given after 11 days.

b. Trematodes

McMahon (1967) showed that the suppressive treatment of Schistosoma mansoni infection in baboons with astiban produced a better immunity to rechallenge infection than the untreated control. He demonstrated this by the low level of egg excretion and relative absence of mature female worms in the treated baboons following the challenge infection. Warren et al. (1977) demonstrated that curative treatment of chronic S.mansoni in mice with niridazole caused the persistence of both immunity to reinfection and suppression of immunopathology.

Doenhoff and Bain (personal communication) are working on the involvement of immune mechanisms in the drug treatment of schistosomiasis in CBA mice. They have shown that when S.mansoni infected T-cell deprived mice are treated with potassium antimony tartrate the reduction in size of their worm burden is less than in similarly treated immunologically intact animals. The defect in deprived mice could be partially restored by administration of serum obtained from S.mansoni infected normal mice and given simultaneously with the drug but by a different route. A serum component obtained from rabbits injected with an extract from S.mansoni adult worms was also found to act synergistically with the drug in S.mansoni infected

intact mice.

From the above studies one can see that chemotherapy of helminth infections clearly involves either humoral or cell-mediated immune responses of the host.

### 3. Protozoa

#### a. Flagellates

Little is known of the interactions of drug, parasite and host immunity when drugs are employed against the amastigotes of either Trypanosoma cruzi or Leishmania species (Peters, 1974b). Convit et al. (1972) and Convit and Pinardi (1974) classified the American cutaneous leishmaniases and recognised a spectrum in relation to clinical aspects. The malignant pole is represented by diffuse cutaneous leishmaniasis, characterized by massive invasion of the skin and mucous tissue of the nasopharynx and certain lymph nodes, a negative Montenegro test and little or no improvement with treatment. At the other pole, there is the localised form of the disease, with few parasites, a positive Montenegro test and a clear tendency towards complete involution after treatment. The intermediate forms of the disease show cutaneous or mucous relapses with varying numbers of parasites in the lesions, the Montenegro test is positive and the response to treatment is slower than the benign localised forms. Convit et al. (1972) took parasites from diffuse cutaneous lesions and inoculated into human volunteers or into patients with lepromatous leprosy and showed that localised cutaneous lesions which disappeared without leaving any sequelae were obtained instead of the diffuse type. They concluded that this diffuse form of the disease was due to an

immunological defect in the host and not to a special strain of parasite. The unresponsiveness to drug treatment in diffuse cutaneous leishmaniasis is thus not due to the parasite but to the impaired immune response of the individual. Bryceson (1970 a,b,c) has observed that "immune conversion" of the skin test from negative to positive during prolonged chemotherapy is accompanied by a change in the histological picture of the lesion. Walton (1970) showed that the FA test was a useful method for monitoring the effect of chemotherapeutic field trials in American leishmaniasis. According to him, a persistent detectable FA titre was interpreted as treatment failure even when clinical healing was attained; only conversion from antibody positive to antibody negative was interpreted as a radical cure. This, he said, was the only way to detect an occult infection. Beveridge and Neal (1967) transferred L.donovani from kala-azar patients, who did not respond to treatment with pentavalent antimony compounds, to hamsters and tested them for drug sensitivity. The strains were found to be drug sensitive. The authors claimed that this effect might be due to the difference in the metabolism of the drug in the two hosts. Since the parasite is drug sensitive in hamsters, the ineffectiveness of the drug in the patient might probably be due to a defect of the patient's immune response.

Attempts have been made to use suppressive or curative chemotherapy to induce development of immunity in the

host and so resistance to further infection. Bevan (1936) showed that when cattle suffering from trypanosomiasis were treated by intravenous injection of potassium antimony tartrate and returned to tsetse fly infested areas, they remained in good health in spite of re-infection. Wilson et al. (1969) infected a group of cattle with naturally infected tsetse flies (T.vivax and T.congolense were found in the area) which were caught wild. They then treated with berenil. After 6 separate challenges followed by berenil treatment, some cattle developed clinical resistance to the disease, though pathogenic trypanosomes were still circulating in their blood, and some even produced sterile immunity.

Wilson et al. (1976) maintained three groups of Boran cattle under different trypanocidal drug regimes for 29 months in an area of "median" tsetse challenge. Cattle treated with berenil on the appearance of clinical disease developed a partial immunity to trypanosomiasis after 2 years. Cattle treated as a group with berenil on the development of patent parasitaemia in any one animal of the group did not develop immunity though with another drug, samorin, they developed immunity.

Smith (1958) treated 6 cattle at two monthly intervals with 7 inoculations of T.congolense parasites and quinapyramine chemotherapy. The animals were resistant to re-infection when they were rechallenged with 16 ED<sub>50</sub> of

the same strain of trypanosomes 6 months after the last immunizing treatment. 2 of the 6 animals developed drug resistant infections. Soltys (1955) carried out a detailed study of resistance to T.congolense developed in cattle exposed to natural and artificial infections. He showed that when animals were kept continuously in enzootic areas and received prophylactic injections of antrycide every two months for 46 months, all the animals survived without giving a trypanosome positive blood or gland smear. When the prophylactic treatment only lasted for 28 months, 3 out of 6 animals died within 3 months of the last dose of treatment. If the animals received treatment for 28 months while in the enzootic area and were then transferred to an area free from tsetse flies for 10 months, 4 out of 6 survived and resisted re-infection when returned to the enzootic area. When the surviving animals from the three experiments were infected artificially with T.congolense, they were all resistant. Though the control untreated animals revealed complement fixing neutralizing antibodies when they were infected, only low titres of complement fixing antibody were detected in the drug treated group. The author suggested that the results proved the fact that resistance to T.congolense infection can be acquired by cattle prophylactically treated with antrycide pro-salt and exposed during and after treatment to T.congolense infection in an enzootic area. Once resistance to T.congolense has been acquired it can last for a considerable time even in animals no longer exposed to constant infection. Whiteside (1962) treated naturally occurring trypanosome infections (T.vivax and T.congolense) in three

groups of cattle with berenil, quinapyramine sulphate or ethidium bromide, and found that the duration of the protection was much longer than the protective effect of the individual drug. This according to the author was due to the immune response of the host. The protection period increased with the use of the long protective drug. The author assumed that the prophylactic period consisted of two parts, one due to the drug and independent of trypanosome challenge, the other added to it and was due to the immune response of the host, which may be antigenically stimulated.

Soltys (1957, 1959) was able to show the importance of immunity during the drug treatment of trypanosomiasis. Strains of T. brucei, when passaged through rabbits, after 4 weeks of infection, became more and more virulent. Sera from infected rabbits failed to neutralise either the homologous or heterologous variants, but were able to neutralise the parent strain. He claimed that he identified an "antibody resistant" strain. Soltys (1959) demonstrated that the "antibody resistant" strain of T. brucei was less sensitive to suramin and antrycide than the sensitive strain. When both strains were exposed to suramin and antrycide in vitro, "antibody resistant" strains needed 50 times more drug than the sensitive one in order to make them non-infectious to mice. Also, the rabbits treated prophylactically with suramin or antrycide resisted challenge with antibody-sensitive strains for a much longer period than challenge with the other stock. In therapeutic experiments in mice, the antibody resistant strain required three times as much drug (0.3 mg/20g) as the

antibody sensitive parasite. Against this, a drug-resistant strain of T.brucei produced by subcurative treatment, was as sensitive to the antibody as the parent strain. This, according to the author, confirmed the existence of combined mechanisms of drug and the immune defenses of the host.

Jancso and Jancso (1934) treated splenectomised and intact control mice infected with T.brucei by using a trypanocidal styryl-quinoline compound (2-p-acetyl-amino-styryl-6 dimethyl aminoquinoline methosulphate). Treatment of splenectomised mice both with border line doses and with doses which would normally effect a permanent cure, invariably led to an early re-appearance of the trypanosomes. The authors suggested that the parasites which managed to escape the immediate deleterious action of the drugs were killed by the host immune responses in intact animals, but relapses occurred in the splenectomised mice. However, according to Bauer (1958), berenil treatment of T.congolense infections was equally effective in intact and splenectomised mice.

Schnitzer et al. (1946) demonstrated that drug resistance to T.equiperdum could be obtained within 5-15 days in splenectomised mice though it took 100 days in intact mice. But, if immune serum was given, together with the drug, to the infected splenectomised mice, there was no development of resistant strains. In their experiment, one could see that the combined effects of drug and antiserum on T.equiperdum infection in splenectomised mice were far superior (cured finally) to those of either drug or serum

alone. Though the authors did not bring out this fact, the antibody seemed to supplement the effect of the drug.

Muntiu et al. (1961) have clearly shown the important role of immune mechanisms in the drug treatment of trypanosomiasis. They demonstrated that when rats were lethally irradiated (death occurring in 8-17 days) this did not influence the course of infection with a virulent strain of T.brucei. Hyperplasia of the spleen which normally occurred in the course of the infection was, however, inhibited. A dose level of neoarsphenamine which cleared the normal rats infected with this strain of T.brucei was without effect in rats which had previously been irradiated.

Another aspect of immunity in relation to chemotherapy is the combination of drug and antiserum in the treatment of American trypanosomiasis. Seneca and Peer (1963a,b) demonstrated that, by the use of hyperimmune rabbit serum in combination with furaltadone, there was 100% survival of mice infected with a lethal strain of T.cruzi. Animals treated with furaltadone alone had a survival rate of 86.7%. Though the results are not significantly different, there is some indication as to the superiority of the combined treatment.

#### b. Sporozoa

There has been a great deal of work on chemotherapy and on immunology of malaria, but very little on the interactions of the two (Peters, 1970a). There is however some published work on the development of immunity after suppressive chemotherapy. Afridi and Rahim (1959)

followed the effects of mass chemotherapy with a single dose of pyrimethamine (25 mg) to adults, before the onset of the malaria season, in 3 villages in Pakistan. Spleen and parasite rates in the 3 treated villages fell and remained low for another two years. But later, Afridi and Rahim (1962) used twice as much drug (50 mg) in a similar study, with the hope of eliminating the small amount of transmission persisting, but instead of this a major breakthrough of malaria occurred in the test villages. Perhaps the higher dose of drug removed all the parasites and as a result immunity in the population began to fall. Desowitz et al. (1966) also studied the effect of DDT spraying and chemotherapy in New Guinea. In Maprik, twice yearly spraying and mass drug administration (chloroquine 125 mg/pyrimethamine 25 mg) was used. After 4 years the control measures had resulted in a marked decrease in malaria rates associated with a higher than average antibody titre. In the Trobriand Islands, with meso to hyperendemic malaria, DDT was sprayed twice a year and chloroquine/pyrimethamine was given monthly for 5 months followed by camoquine/primaquine at 3 monthly intervals. This caused a reduction of transmission of malaria and the virtual disappearance of detectable antibody. The authors concluded that the campaign employing intensive and conservative therapeutic regimes caused the entire population in the Trobriand Islands to become immunologically sterile within four years or less.

Sergent and Poncet (1956) showed that P. berghei infected rats treated with nivaquine (chloroquine) during the first attack or later in the infection developed resistance to

reinfection. However, when mice infected with P.berghei were treated with nivaquine the mice survived the primary attack but relapsed and all died. Carrescia and Arcoleo (1957) planned to promote the development of immunity against P.berghei by treating infected mice with small doses of chloroquine ( $5\text{mg kg}^{-1}$  body weight) administered subcutaneously during the primary attack and at each relapse, so as to prolong the infection. Although some of the mice were susceptible on challenging they managed to show a certain degree of immunity, long latent period, spontaneous recovery after infection, low grade parasitaemia and a great number of negative cases in the experimental group.

These suppressive chemotherapy experiments of malaria infections show that aggressive mass suppressive chemotherapy will reduce the communal immunity of the people (Afridi and Rahim, 1959, 1962, and Desowitz et al., 1966). In laboratory experiments, the situation depends on the host parasite system. In a self-resolving infection system (rats infected with P.berghei) treatment given in the late or early stage of infection will give immunity (Sergent and Poncet, 1956), but in fatal infections (mice infected with P.berghei) prolonged suppressive chemotherapy is necessary for survival and development of immunity (Carrescia and Arcoleo, 1957 and Cox, 1958). If suppressive chemotherapy is given just to suppress the primary attack, the animal will relapse and die (Sergent and Poncet, 1956 and Cox, 1958) irrespective of the day of treatment.

There are also some experiments on the development of immunity after curative treatment of malaria. Gingrich (1948) demonstrated the persistence of immunity to P.cathemerium in canaries in which the infection had been eradicated by treatment with curative drugs. This immunity, which persisted for as long as 6 months, was demonstrated by the lower parasitaemia in challenged birds as compared to that of the controls, since the infection is a self-resolving one. Coggeshall (1938) and Meier and Coggeshall (1944) demonstrated that immunity persisted for as long as a year in monkeys in which P.knowlesi infection had been cured by treatment with sulphonamide. Treatment started before 5 days of the infection was not sufficient to protect the monkeys from death after a challenge.

Brown and Hills (1974) demonstrated two types of antibody in chronic infections of P.knowlesi in monkeys, produced by drug treatment. The two antibodies include a non-protective type capable of inducing antigenic variation, which correlates with schizont infected cell agglutination (SICA) antibodies and a parasitocidal opsonizing type of antibody. According to Brown and Hills (1974) opsonizing antibodies are always found with SICA antibodies, but SICA antibodies may be found when opsonizing antibodies are not detectable, for example in monkeys cured by drugs of a primary parasitaemia or sensitized with freeze-thawed schizont-infected cells in Freund's incomplete adjuvant. In vitro studies show the inhibiting antibody was supposed to act against merozoites, whereas SICA antibody was directed against the altered surface membranes of erythrocytes containing schizonts (Butcher and

Cohen, 1972). But Miller et al. (1977) tried to correlate the in vitro inhibition of merozoites and the resistance to reinfection in vivo in 3 rhesus monkeys cured of P.knowlesi infection. No correlation was obtained between the in vitro SICA antibody titre and the in vivo immunity or the merozoite agglutination titre and the resistance to reinfection.

Gilbertson et al. (1970) treated 10 P.berghei infected mice with intramuscular injections of  $20 \text{ mg kg}^{-1}$  chloroquine for 5 days starting from day 3 of the infection. 4 animals recrudesced and died. The remainder were challenged 20 days after the parasites had disappeared from the blood. All developed characteristic infections and died within 14 days. But prolonged suppression of infection (90 days) with milk diet resulted in a certain amount of immunity. So the authors concluded that a short high parasitaemia was not enough to promote measurable resistance and they thought that the duration of the first infection was important as well as the density.

Waki (1976) repeatedly immunized and cured white mice infected with P.berghei with 4 consecutive doses of sulphamonomethoxine of  $20 \text{ mg kg}^{-1}$  per day starting from day 3 of the infection. According to the author, protective immunity depends on the repeated infections and chemotherapy but does not attain very high levels after a single infection and treatment. However, Eling and Jerusalem (1977) showed that mice infected with P.berghei and treated with sulphathiazole developed immunity. The degree of immunity was related to the number of surviving parasites in the host which was inversely proportional to

the amount of drug administered. Too much drug diminished the development of immunity. Multiple infections during drug treatment promoted better immunity. Cox and Turner (1970) have shown by various serological methods that though P.vinckei infection in mice was terminated after 5 days with chloroquine, the IgG and IgM concentrations in serum increased rapidly in the first 8-10 days after infection, then IgM decreased and IgG continued to rise. Also Waki and Suzuki (1974), by using the indirect fluorescent antibody technique, showed that IgM and IgG antibody titres reached a peak in 7-9 days and then declined after P.berghei infection in mice. But when the infection was terminated by chemotherapy on days 3-6, IgM antibody levels peaked at day 6 and then declined to undetectable levels by day 27, whilst specific IgG levels reached a maximum on day 13 and were still detectable on day 342.

Waki and Suzuki (1974) also showed that when mice treated with sulphamethoxine were challenged with either  $10^3$ ,  $10^4$ ,  $10^6$ , or  $10^7$  parasitized red cells 12 days after the last dose of drug therapy, none of the mice survived the challenge.

Box and Gingrich (1958) also demonstrated immunity in P.berghei infection in mice after curative treatment with primaquine diphosphate starting on day 6 of the infection. 14 out of 20 mice survived upon rechallenge, though none survived in the challenged controls. The duration of immunity was found to be at least 3 months. Mice which

had been splenectomised after being cured, on the other hand, were not able to survive the challenge infection though they survived longer than the challenged controls.

Corradetti (1936) treated 15 patients suffering from general paralysis of the insane (syphilis) and infected with P.vivax followed by treatment with quinine in three different ways. Five were treated after the first attack, five others after the fifth attack and the last five after the 10th attack of fever. He followed the blood films for a period of 12 months. He showed that treatment after the first attack resulted in more frequent relapses than in the other groups. He argued that if immunity was to be maintained the treatment should be delayed, though from the point of view of the patient the treatment should start as early as possible.

The resistance to reinfection after the curative drug treatment in malaria seems to depend on the timing of treatment after infection, the later the treatment the better is the immunity (Corradetti, 1936; Coggeshall, 1938; Meier, 1944 and Box and Gingrich, 1958) and also on the immune system remaining intact (Box and Gingrich, 1958).

Some experiments in the past show the effect of treating malaria infections of hosts in different immunological states. Yorke and MacFie (1924) treated 105 cases of general paralysis of the insane infected with P.vivax with quinine plus salvisan or quinine alone. According to these authors the infections were cured easier

than in their previous experience of treating P.vivax infection alone during the war. However they did not consider the possibility of different strains of parasite. They suggested that "quinine given to a patient whose blood contains numerous malaria parasites invariably destroys directly, or more probably indirectly, large numbers, but not all, of the parasites, thus setting free a considerable quantity of soluble antigen. The antigen provokes by stimulation of the host's tissues the formation of 'immune-body'. The 'immune-body', if present in sufficient amount, destroys the remaining parasites, thus resulting in sterilization of the infection and in cure of the patient". Taliaferro (1948), Taliaferro and Kelsey (1948) and Taliaferro and Taliaferro (1949) did a series of studies on the effect of quinine on P.gallinaceum in intact and splenectomised chickens. Though the quinine blood level was increased and prolonged in the splenectomised chickens, the parasitaemia level and the mortalities were higher than in the intact treated controls. According to these authors quinine and the spleen act independently in suppressing the infection. They also showed that a greater suppression of malaria occurred with the same dose of quinine in animals previously immunized with P.lonhurae (which gives a slight cross immunity with P.gallinaceum). Acquired immunity was not necessary for the efficient action of quinine, but quinine predominantly inhibited parasite reproduction whereas acquired immunity killed the parasites. Acquired immunity was thus necessary to prevent relapse when the drug was stopped. The same authors found no evidence that immunity makes the drug more effective nor that the

drug stimulates antibody production or phagocytosis.

Elko and Cantrell (1970) studied the colloidal carbon clearance time in amodiaquine or chloroquine treated rats infected or uninfected with P.berghei together with the untreated controls. They drew the same conclusion as others that chemotherapy and phagocytosis are independent factors in suppression of P.berghei malaria.

#### 4. Cancer

The most significant developments in the study of co-operation between immune mechanisms and chemotherapy have come from cancer research. Some results which influenced the planning of my experiments, or which seem to be particularly related to control of protozoal diseases are described here.

Carter et al. (1973) have shown that the successful treatment of Gardner lymphosarcoma of mice with L-aspariginase was dependent on the host's immune response. In immunologically intact animals, solid cutaneous grafts of the tumours disappeared completely following a single intraperitoneal injection of L-aspariginase. However, the same treatment in T-cell deprived mice caused the tumour to regress initially but leaving a residue of surviving tumour cells, which killed the animals with local and disseminated lymphosarcoma later. The recurrent tumours were invariable aspariginase-resistant. The authors beleived that L-aspariginase destroyed the sensitive tumour cells while the remaining aspariginase-resistant cells were destroyed in the intact host by the immune response.

O'Neill et al. (1975) have shown in vitro that when C3H mouse thymocytes were incubated with anti- C3H serum with chlorambucil or with antiserum plus chlorambucil, the combination of antiserum and drug was more toxic than the drug alone; antiserum itself being non-toxic. Davies and O'Neill (1973) linked chlorambucil to antibody and using the mouse theta antigen system in vitro, they could show both antibody specificity and drug activity in the complex. When such drug plus tumour-specific antibodies were tested in vivo the effect on the tumours was much greater than using either drug or antibody alone. However this effect was not due to the antibodies carrying the drug to the tumour target since the best effect was obtained when antibody was given after the drug. They thought that antibody will kill cells previously affected by chlorambucil but that antibody-affected cells are not more sensitive to the drug. In contrast, Rubens (1974) believed that antibodies directed against cell surface antigens rendered those cells more susceptible to destruction by certain cytotoxic agents. Rowland et al. (1976) suggested that antibodies can act as specific carriers for cytotoxic agents provided they are covalently bound; physically adsorbed antibody/drug mixtures rapidly dissociate under in vivo conditions. Ghose et al. (1972) have also shown that chlorambucil bound to antibody was much more effective against a mouse lymphoma and a case of human malignant melanoma than the drug given alone. According to the authors the sensitization of tumour cells to ionizing radiation and cytotoxic drugs brought about by surface localizing antibodies or antibody-mediated selective concentration of cell-damaging agents in tumour tissue, or

both, was the probable explanation. Davies (1974) and Davies et al. (1974) have shown that, when treatment with drug was combined with antibody against mouse lymphoma, much greater protection was obtained than with drug or antibody alone. This was applied to drugs like melphalan, nitrogen mustards, cytosine arabinoside, chlorambucil and cyclophosphamide. Davies and O'Neill (1974) tested the effectiveness of a covalently linked complex of an alkylating agent and tumour specific antibody (DRAC) in protecting mice against the growth of EL4 lymphoma. According to these authors, DRAC (drug-antibody complex) behaved in vivo like a 'poisoned arrow', i.e. the antibody carried the drug to the tumour cells which were then killed. The DRAC effect was better than that of either drug or antibody given alone. The authors said that the drug antibody complex was more effective than drug and antibody given together without being linked. Following the in vitro and animal experiments, attempts were made to treat cancer patients with combined drug and antibody regimes. Newman and Ford (1977) treated 32 patients suffering from bronchial carcinoma with a drug/antibody combination (not covalently linked); and another 32 patients with drugs alone. All the patients had previously undergone surgery and the antiserum was raised in goats by immunizing with removed tumour cells. Although the differences were not statistically significant there have been 22 recurrences including 15 deaths after drug alone and 8 recurrences including 6 deaths in the combined group. Everall et al. (1977) treated 4 patients with widespread metastatic melanoma with chlorambucil and xenogeneic anti-melanoma immunoglobulin. Though there

were not drug and immunoglobulin alone treated controls, the authors suggested that all the 4 patients lived beyond the expected median survival-time for their particular combination of metastasis.

Gregoriadis (1977) in his review article on "Targeting of drugs", pointed out the difficulties associated with the antibody carrier approach. According to him, the difficulties will be the isolation of target specific antigens for the production of antibodies and the purification from these of immunoglobulin molecules specific for the antigen. In addition, drugs attached to immunoglobulins may mask the antigen recognition site of the protein or alter its tertiary structure and render it prone to rapid removal by the reticuloendothelial system. The question of whether the immunoglobulin-drug complex will allow the active moiety (drug) to perform its task must be solved. Severance of the bond between carrier and agent may be necessary. Other difficulties include inhibition of immunoglobulin-antigen (target) interaction by circulating antibodies raised by the host against the antigen and also the possibility of allergic reactions to the carrier itself.

## III MATERIAL AND METHODS

## A. General

1. Laboratory animalsa. Mice

Female inbred CBA/lac mice were used for most of the experiments. The mice were bred at the Winches Farm Field Station of the London School of Hygiene and Tropical Medicine and were used for experiments when 6-8 weeks old (18-20g). One batch of CBA/lac mice was obtained from the Chester Beatty Cancer Research Institute. Male TO Swiss Albino mice were used for the drug sensitivity testing. They were obtained from commercial breeders and were 6-8 weeks old (20-25g) when used.

The mice were housed either in small cages (groups of 5-6 mice) or in larger cages (8-10 mice/group). They were all kept in the same centrally heated room with the temperature ranging from 17°C to 22°C and humidity 42% to 61%. They were fed on Dixon 86 diet and given water ad lib.

b. Rabbits and guinea-pigs

Albino rabbits weighing 2-3 kg and guinea-pigs of 150-200 g were used. They were obtained from commercial breeders. They were caged singly and fed on Dixon RGP diet and given water ad lib.

## 2. Strains of Plasmodium

### a. Plasmodium chabaudi

Two C57Bl mice infected with P.chabaudi 35AS2 (stabilate 314) were kindly supplied by Dr David Walliker of the Protozoan Genetics Unit, University of Edinburgh. This is a clone and is sensitive to pyrimethamine and to chloroquine. The donor mice were bled and the parasites stored in capillaries under liquid nitrogen as stabilate LUMP 1156 (Lumsden et al., 1973) in the WHO cryopreservation bank of the Department of Medical Protozoology, London School of Hygiene and Tropical Medicine.

### b. Plasmodium berghei

P.berghei NK65 was also supplied from Edinburgh (stabilate 293). It is also sensitive to pyrimethamine and to chloroquine but is not a clone. It was stabilized as LUMP 1150.

## 3. Cloning and maintenance of parasites

### a. Cloning techniques

Clones of P.berghei NK65 were prepared according to the method used by Carter and Walliker (1975). Five CBA mice were infected from two capillaries of LUMP 1150. When the parasitaemia was still below 2% one donor mouse was chosen in which at least 90% of the infected red cells contained single parasites. The blood was diluted in solution A (Lumsden et al., 1973) until there was an average of 5-7 parasites

per ml. of diluent. 0.1 ml aliquots were then injected intravenously into 10 CBA mice. The mice were examined daily until some of them became parasitaemic. The dilution and inoculation process was repeated and blood was then stabilised in a large number of capillaries (150).

b. Cryopreservation

CBA mice with parasitaemia levels of 10-20% were bled by cardiac puncture into a 2 ml sterile syringe containing 5 units of heparin. The blood was then mixed with glycerol to a final concentration of 7.5% and was distributed with a 1 ml syringe into sterile capillaries. Both ends of each capillary were flame sealed, and the tubes were placed in a labelled holder and kept overnight in a deep freeze at  $-70^{\circ}\text{C}$ . Next morning the stabilate was transferred to a liquid nitrogen container for permanent storage.

4. Infection and monitoring of parasitaemia

a. Preparation and staining of blood-films

Two thin films of tail blood were made on each 76 x 25mm microscope glass slide. The films were dried quickly by means of a fan, fixed with methanol and stained with a 10% solution of Giemsa (Hopkin and Williams Ltd.) in buffered distilled water of pH 7.2 ( $16.71\text{g Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O} + 2.72\text{g KH}_2\text{PO}_4$  in one litre of distilled water) for 40-50 min in perspex staining plates (Lumsden *et al.*, 1973). The slides were then washed under tap water and allowed to dry.

b. Parasite counting

The stained blood film was examined under the oil immersion (x 100) objective of a Wild M20 microscope. The average number of red blood cells in each microscope field with a uniform covering of cells was an estimated 400. The number of parasitised red cells seen in 20 fields or the number of fields in which 50 infected cells were found, whichever was reached first, was determined and the result was expressed as a percentage of the total number of RBC scanned.

c. Red blood cell counting

The blood to be examined was diluted 1:200 with formol-citrate solution (1% formalin + 3.3 g/l trisodium citrate solution) in a red cell pipette. The diluent was then introduced into a counting chamber with an improved Neubauer ruling. The number of erythrocytes was expressed in millions per ml of blood.

d. Preparation of infective inocula

The contents of one capillary of the required stabilate was mixed with 0.6 ml of chilled solution A and injected intraperitoneally in 0.2 ml aliquots into 3 donor mice. When the parasitaemia reached 10-20%, the mice were bled from the heart into a syringe containing 4 units of heparin for each mouse. The blood was pooled and the parasitaemia and red cell counts were made. The blood was then diluted accordingly with solution A to provide the number of infected cells required for infection (Lumsden et al., 1973).

e. Reticulocyte counting

Thin blood films were prepared on cleaned glass slides coated with 0.3% brilliant cresyl blue in 95% ethyl alcohol. They were air-dried and then incubated in a moist chamber for 5-10 minutes, air-dried again, fixed with methanol and stained in Giemsa solution (Viens et al.,1971). With this method, malaria parasites together with reticulocytes can be seen and counted. Reticulocytes were recorded as a percentage of the total number of red blood cells.

f. Concomitant infections

To lessen the chances of bacterial infections, the CBA/lac mice were given aureomycin (Cyanamid, England) in their drinking water (0.02% solution of powder) until they were weaned. To ensure that the animals were free from Eperythrozoon coccoides or Haemobartonella muris, random samples from new stock mice were splenectomised and their blood films were stained with Giemsa and examined on alternate days for two weeks. Malaria infected control groups of mice were also examined for evidence of undue scattering of their parasitaemias which might reflect a concurrent infection.

No evidence of Eperythrozoon or Haemobartonella infections was ever seen.

g. Detection of subpatent parasitaemias

(1) Splenectomy

The mouse was anaesthetised with ether and the left side costal marginal area was shaved. The mouse was positioned

e. Reticulocyte counting

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No evidence of Eperythrozoon or Haemobartonella infections was ever seen.

g. Detection of subpatent parasitaemias

(1) Splenectomy

The mouse was anaesthetised with ether and the left side costal marginal area was shaved. The mouse was positioned

on a cork board with its left flank uppermost. The front limbs were held together with one paper clamp and hind limbs with another. Both clamps were attached to rubber bands which were secured by pins. After cleaning the shaved area with 70% alcohol an incision of 1 cm in length was made over the spleen, which was visible through the body wall. The spleen was held with blunt forceps and its vessels ligated with cat gut. The spleen was removed after cutting the stalk and the peritoneum and abdominal skin were closed with suture clips.

(ii) Blood and tissue passage

The mouse to be tested for the subpatent parasitaemia was deeply anaesthetized with ether and pinned down on a cork board. Through the anterior approach, the thoracic cavity was cut open and the blood was sucked from the left ventricle into a two ml syringe containing 5 units of heparin in 0.1 ml of PBS. Depending on the size of the mouse 1 ml to 2 ml of blood was obtained in this way and was injected into two young mice.

The spleen, kidneys and liver were also removed. The cells were collected by crushing the individual organ in a fine wire mesh with the help of a small test tube and were flushed through the sieve with chilled TC 199 (10%) solution. The cells collected from each organ were injected into two uninfected young mice as before. Blood films were taken twice a week from the mice for at least 2 weeks and were examined for the presence of malaria infection.

## B. T-cell deprivation of mice

Two methods were used. Dr E. Leuchars, Chester Beatty Research Institute, introduced me to the method of adult thymectomy, irradiation and syngeneic bone marrow reconstitution. This method produces excellent T-cell deprivation. (Davies, 1969; Doenhoff et al., 1970; and Parrott et al., 1970). In later experiments T-cell deprivation was carried out by adult thymectomy and antithymocyte serum (ATS) treatment. Since an irradiation source was not readily available and because this method also provides good T-cell deprivation (Lance 1970; Doenhoff, unpublished data) it was used in most of the experiments.

### 1. Thymectomy

The method of adult (6-8 weeks) thymectomy is used routinely in the Immunology Laboratory of the Edna McConnell Clark Schistosomiasis Research Unit at Winches Farm and is based on that of Kaplan (1950), Miller (1960) and Leuchars (1966).

The mouse was anaesthetised with ether and stretched out ventral side uppermost on a cork board with its limbs attached to paper clamps, connected to rubber bands. The neck was also stretched by a wire loop hooking over the upper incisors and pinned down. A bandage roll of 2 x 4 cm was placed under the shoulders. After swabbing with 70% alcohol a mid-line incision was made in the skin from 1 cm below the chin to approximately the level of the third rib. Using two small blunt forceps, the salivary glands were

separated from the median fissure until the trachea and its fascia was exposed. A wedge shape incision, 3-4 mm long was made on the manubrium sterni with small blunt scissors and was removed carefully. Then the fascia overlying the trachea was pinched and cut out as a rectangular piece about 3 mm wide and extending as far down the thoracic opening as possible.

Slight pressure was applied below the diaphragm, to bring up the thymus and to minimise the pneumothorax. Using a Pasteur pipette with a diameter of 2-3 mm (i.e. slightly smaller than a lobe of the thymus) and connected to a negative pressure vacuum pump with a pressure of 600 mm Hg, the thymic lobes were sucked out one at a time. The front limbs were freed, the roller bandage removed and the incision was closed with No 7 Michel clips by means of an Auto-clip applier (Clay-Adams, Inc. N.Y.).

The mortality rate was less than 5%, deaths being due to over anaesthesia, pneumothorax and haemorrhages.

Sham thymectomy was carried out in a similar way but without removing the thymus.

## 2. Anti-thymocyte serum (ATS): preparation and use

ATS was prepared in albino rabbits by injection of thymocytes from 3-4 week old CBA/lac mice (Levy and Medewar 1966).

Twelve 3-4 week old CBA/lac mice were killed by cervical dislocation. The thoracic cavity was cut open

through the anterior approach. Without rupturing the auricles, lobes of each thymus were dissected out by means of forceps. The thymi were then rinsed and freed from blood and other tissues in a petri-dish containing chilled TC199 (10%) solution.

Thymus cells were pooled by crushing the thymus glands in a fine wire mesh with the help of a small test tube and were flushed through the sieve with chilled TC199 (10%) solution. The cells collected were washed three times in 10 ml of TC199 (150 g x 10 min), then injected i.v. into a rabbit after resuspending in 10 ml of TC199 (10%) solution. The whole process was repeated after two weeks. One week later, the rabbit was killed and bled from the heart. The serum obtained was inactivated at 56°C for 30 min and then stored in a deep freeze at -20°C in 5 ml vials. A single bleeding from a rabbit ensured greater homogeneity in the potency of ATS than multiple bleedings and yielded about 80 ml serum.

The ATS was given subcutaneously in two schedules. In some experiments ATS was given as 0.2 ml doses on three occasions within the 10-12 days after thymectomy (ATS<sub>1</sub> treatment). In other experiments, ATS was given as 0.25 ml doses on 4 occasions within a similar period (ATS<sub>2</sub> treatment).

### 3. Irradiation and bone marrow reconstitution

Irradiation was carried out one week after thymectomy with the use of a Marconi type TM 4574 B irradiation unit (Middlesex Hospital Medical School). The thymectomized and

sham-operated mice were housed in perspex boxes and were exposed to 55 rads/min for a total of 850 rads delivered unfiltered at a distance of 100 cm at 230 kV and 15 mA.

Bone marrow obtained from a syngeneic mouse was used for the reconstitution of irradiated mice. The donor mouse was killed by cervical dislocation and pinned down dorsally. After swabbing with 70% alcohol, an incision was made on both sides of the thigh from the knee to the middle of the lumbar vertebra. The muscle attachments were then freed with curved scissors. The femur was held with Michel clip forceps and the upper extremity of the bone was dissected out; the joint at the lower extremity could then be dislocated easily. The bones were placed in pairs in a petri dish containing a sterile filter paper soaked with TC 199.

A number 25 needle and a two ml. syringe filled with TC 199 was used to flush the marrow down from the cut trochanter end of the femur. The procedure was repeated twice or more until the bone was devoid of marrow. The same 2 mg of TC 199 was used for a pair of bones thus giving  $1.25 \times 10^7$  cells/ml. The bone marrow lumps were broken up by aspiration into a syringe using a number 25G needle. Irradiated mice were injected instantly with 0.4 ml of the cell suspension ( $5 \times 10^6$  cells/mouse). They were all kept on sterile water for 14 days and a standard diet supplemented with sunflower seeds. Mortality was less than 10%. The mice prepared were used 30 days after the irradiation.

#### 4. Tests for the effectiveness of the T-cell deprivation

##### a. Skin grafting

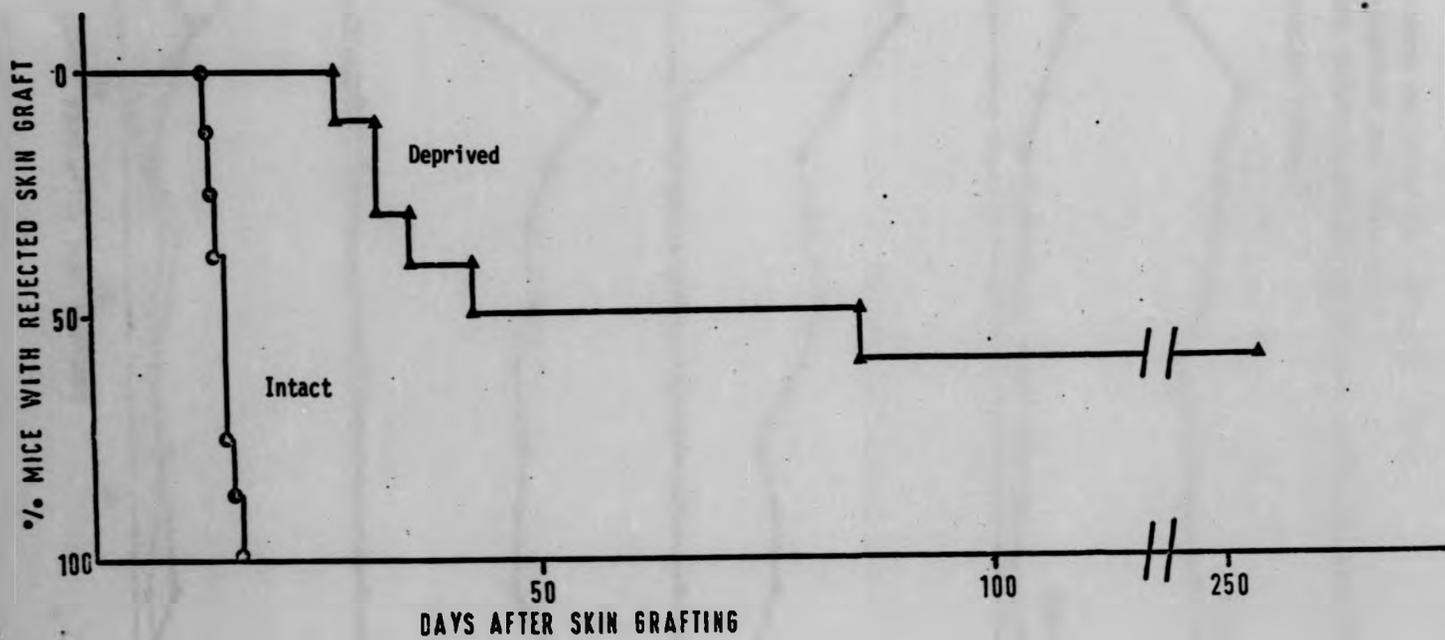
CBA/lac mice were T-cell deprived by thymectomy and  $ATS_2$  treatment. They were then grafted with skin from Parke's mice as described by Billingham and Medawar (1961). In the intact control mice (10), graft rejection began at 15 days and was complete by 19 days : median skin graft survival time was 18 days. In the thymectomy and  $ATS_2$ -treated group the minimum skin graft rejection time was 34 days and the median rejection time was 46 days. Grafts survived in 4 out of 9 mice for up to  $1\frac{1}{2}$  years (see fig 2 p 112 ).

##### b. SRBC antibody response

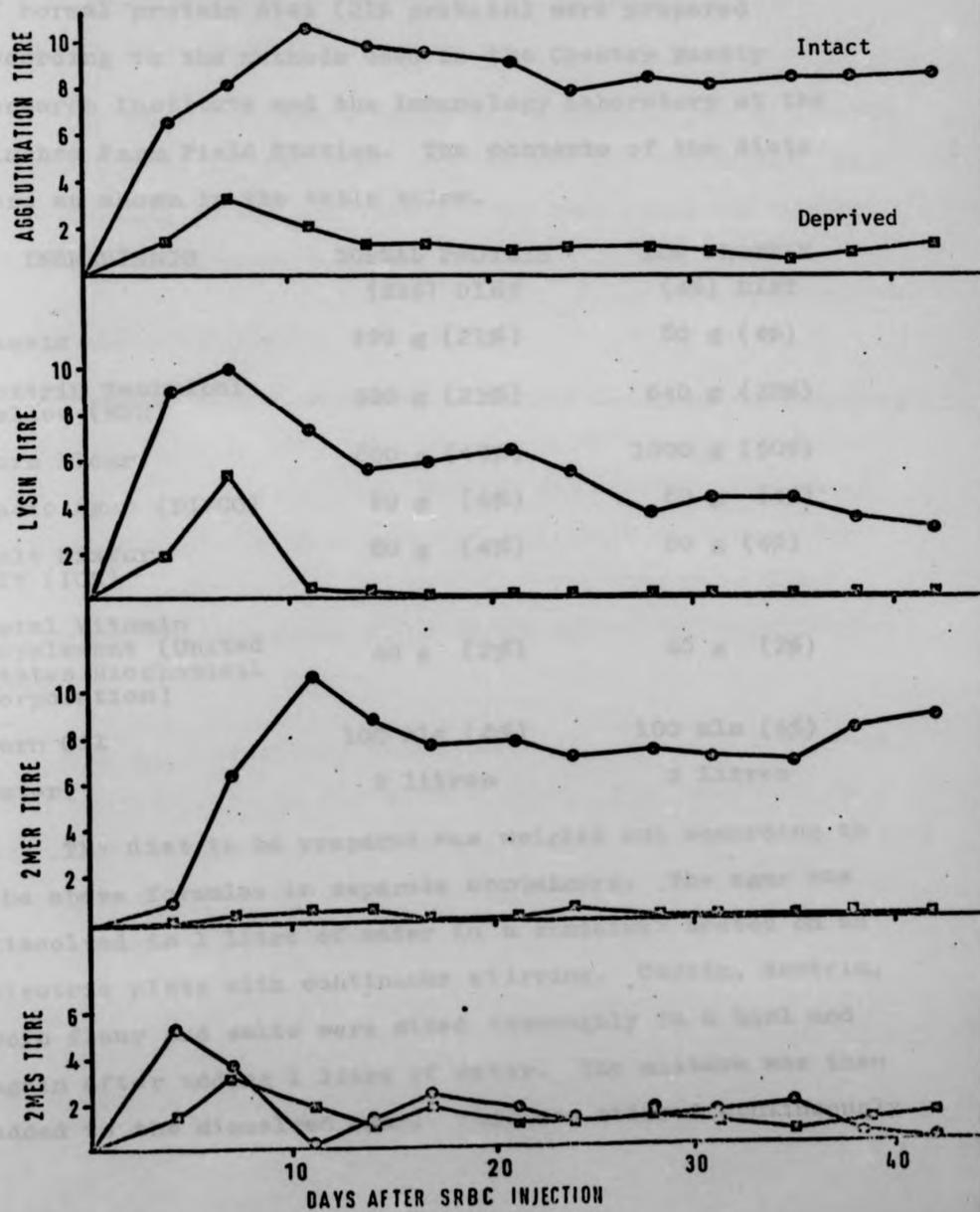
Intravenous injections of 0.2 ml of 2% SRBC were given to the intact control and the T-cell deprived groups. The mice were bled from the retro-orbital plexus bi-weekly and the agglutination titre, haemolysin titre, 2-mercaptoethanol resistant titre (IgG) and 2-mercaptoethanol sensitive titre (IgM) were determined. As shown in Fig 1, the total agglutination titre, haemolysin titre and 2-mercaptoethanol resistant titre (IgG) were very much depressed in the T-cell deprived group of mice compared with those of the intact controls. (see fig 3 p 113 ). (Experiments 4a and b were performed by Mr. R. Musallam).

↓

**FIGURE 2** The percentage of AKR skin-grafts surviving on T-cell-deprived and intact control mice



**FIGURE 3**  $\text{Log}_2$  anti-sheep red blood cell antibody titres in T-cell-deprived and intact mice (2MER/2MES titre=2-mercaptoethanol resistant/sensitive agglutination titres)



## C. Diet preparation and treatment

1. Diet preparation

Low protein calorie diet (4% protein and its control of normal protein diet (21% protein) were prepared according to the methods used in the Chester Beatty Research Institute and the Immunology Laboratory at the Winches Farm Field Station. The contents of the diets were as shown in the table below.

INGREDIENTS	NORMAL PROTEIN (21%) DIET	LOW PROTEIN (4%) DIET
Casein	420 g (21%)	80 g (4%)
Dextrin Technical Yellow (BDH)	500 g (25%)	640 g (32%)
Corn Flour	800 g (40%)	1000 g (50%)
Bacto Agar (DIFCO)	80 g (4%)	80 g (4%)
Salt Mixture XIV (ICN)	80 g (4%)	80 g (4%)
Total Vitamin supplement (United States Biochemical Corporation)	40 g (2%)	40 g (2%)
Corn Oil	100 mls (4%)	100 mls (4%)
Water	2 litres	2 litres

The diet to be prepared was weighed out according to the above formulae in separate containers. The agar was dissolved in 1 litre of water in a container heated on an electric plate with continuous stirring. Casein, dextrin, corn flour and salts were mixed thoroughly in a bowl and again after adding 1 litre of water. The mixture was then added to the dissolved agar. This was stirred continuously

for about 10-15 mins until a paste was formed. The oil was then added and stirred until thoroughly mixed. Stirring was continued while the diet was allowed to cool down. The vitamin supplement was added and mixed at 68°C as some are heat labile.

The diet was spread evenly on a shallow pan to a depth of about 1 to 1½ inches and stored at 4°C overnight. The next morning it was cut into 2 x 2½ inch cakes and stored at -20°C until used.

## 2. Feeding schedules

CBA/lac female mice were randomly assigned to the diet groups as soon as they were weaned at  $21 \pm 3$  days (16-17g) after birth. PABA (Sigma) (0.05%) was added to the water to ensure that the mice were not deficient in PABA. The diets and water were given ad lib. The mice were fed on the diets and the water for 4 weeks before the experiments began.

The mice receiving 4% protein diet consumed less food than the mice on the 21% protein diet. The reduction in the overall calorie intake in addition to protein restriction caused a significant weight loss in the experimental groups (Malave and Layrise, 1976).

### 1. Preparation and use

The three drugs used, chloroquine, quinine and pyrimethamine were all administered intraperitoneally (i.p.) using a 1 ml syringe and 25G needle.

#### a. Chloroquine

Chloroquine was used as chloroquine sulphate injection form, commercially prepared as Nivaquine (May and Baker). One ml contains 40 mg base. This was diluted with normal saline to give the dose required in 0.1 ml/10 g of mouse and was stored at 4°C.

#### b. Quinine

Quinine hydrochloride (BDH) was used. It was not easily soluble in water and so was dissolved in absolute ethyl alcohol (1g base in 0.8 ml). Distilled water was then added to give the required amount of the base of the drug in 0.1 ml/10 g body weight of mouse, and was then put on the magnetic stirrer until fully dissolved. Fresh solution was prepared for every experiment.

#### c. Pyrimethamine

Pyrimethamine BP 120U powder (Wellcome) was used. This failed to form a stable suspension either in normal saline or in water even with the aid of Tween 80. A stock solution of pyrimethamine 25 mg/ml was prepared by homogenising with 0.5% methylcellulose and then was ultrasonicated for about 5 seconds twice and was stored at 4°C (Peters, 1975). The required working dilution

was obtained by diluting the stock solution with methyl cellulose (0.5%) to a dilution of the required dosage in 0.1 ml/10 g of mouse. Since each mouse weighed  $20 \pm 3$  g at the time of treatment it received the drug in  $0.2 \pm 0.03$  ml.

## 2. Drug testing

### a. Therapeutic tests

Therapeutic inoculations were given i.p. as daily doses for 4-14 days. Full details are included as part of the procedure for each experiment.

### b. Suppressive tests

A 4-day suppressive test (Peters 1965a,b) was used to determine suitable dosages during initial experiments and to retest the drug sensitivity of relapse populations isolated after courses of treatment.

Male TO mice were inoculated i.p. with  $1 \times 10^7$  infected erythrocytes of the required parasite and were then grouped randomly into 5 groups of 6 mice each. One group was kept as an untreated control group, the others were treated with different dose levels of a drug daily for 4 days starting 3-5 hrs (day 0) after inoculation of the parasites. The drug dosages used were worked out from the 50% and 90% effective dosages ( $ED_{50}$ ,  $ED_{90}$ ) of previous work done by Peters (1967a, 1975).

The blood films were taken on day 4 and the mean erythrocyte infection rate of each group of mice was measured.

c. Calculations

i. Effective doses

The mean parasitaemia of each treated group was expressed as a percentage of that of the control group. The data were plotted on log-probit paper, the Y axis being the probit of suppression of parasitaemia and the X axis the total log dose of the drug used for 4 days. From that, the drug dosages that reduced the parasitaemia by 50% (ED<sub>50</sub>) and 90% (ED<sub>90</sub>) were worked out and were expressed as a single daily dose in mg/kg.

ii. Index of resistance

When comparisons of drug resistance of parent and relapse strains were made, the 4-day suppressive test was carried out with both strains and an index of resistance (I<sub>50</sub> and I<sub>90</sub>) worked out according to the following formula (Peters, 1975).

$$\text{Index of resistance (I}_{50}\text{)} = \frac{\text{ED}_{50} \text{ of drug against relapse strain}}{\text{ED}_{50} \text{ of drug against parent strain}}$$

iii. Mean survival time

Mean survival time is calculated by dividing the total survival time in days by the number of experimental animals.

$$\text{Mean survival time} = \frac{\text{total survival time in days}}{\text{no. of mice}}$$

However the percentage increased survival time is calculated according to the following formula (Ott, 1969).

$$\% \text{ Increased survival time} = \left( \frac{\text{survival time (days)}}{\text{mean survival time of controls (days)}} - 1 \right) \times 100$$

E. Schistosoma mansoni infections1. Maintenance in the snails

Schistosoma mansoni (Puerto Rican strain) was maintained in Biomphalaria glabrata in the Schistosomiasis unit of Winches Farm Field Station. The infected snails were kept in glass aquaria at 24-26°C and in darkness to prevent shedding of cercariae. When required, the infected snails were stimulated to shed their cercariae by putting them into a beaker at 30°C and under a light source. The cercariae obtained were used within two hours. Single sex cercariae were obtained from snails infected with single miracidia. The sex of the worm was checked previously by infecting some mice and perfusing them.

2. Infection of mice with cercariae

Cercariae were concentrated in a millipore apparatus (XX1004704) using a filter of 8  $\mu$ m pore size. When the cercarial suspension was introduced into the apparatus, the cercariae migrated into the top 1 cm layer of water under the influence of an artificial light and the excess water was removed from below by means of a vacuum pump.

Mice were anaesthetized with 54 mg kg<sup>-1</sup> of veterinary Nembutal (May and Baker 60 mg ml<sup>-1</sup> diluted with normal saline). The abdomens were shaved and the mice placed on their backs between wooden strips, fixed to a base board. Nickel-plated brass rings ( $\frac{1}{2}$  inch diameter by  $\frac{3}{8}$  inch high and 11.5 g in weight) were positioned and secured with 1 inch wide cellotape on the abdomen. The cercarial suspension was stirred vigorously and pipetted into each ring. Twenty

minutes was allowed for the cercariae to penetrate and the unabsorbed water was sucked out, the animals were returned to cages and revived under warm conditions.

### 3. Recovery of adult worms

The adult worms were recovered from the hepatic portal system and the liver by perfusion with heparinised citrate saline (sodium citrate 1.5%, sodium chloride 0.88%, heparin 2 unit/ml) by a modification of the method described by Smithers and Terry 1965 (James and Taylor, 1976).

Each mouse was killed by intraperitoneal injection of about 10 mg of Nembutal (60 mg/ml containing 25 unit/ml of heparin). This caused all the worms to lose hold on the walls of the blood vessels (Smithers and Terry 1965). The mouse was skinned, its abdomen and thoracic cavity were opened and the rib cage was removed.

The mouse was stretched on a vertical sheet of perspex by means of the two spring clips attached onto one hind and one fore limb.

The hepatic portal vein was cut, the perfusion needle (19G) was placed in the left ventricle of the heart and, by means of a 50 ml syringe, 20 ml of the perfusion fluid was injected forcefully and steadily. The perfused blood and fluid was collected in a 25 ml universal tube, allowed to settle for 10 min and the supernatant removed leaving the sediment of about 1 ml. The red cells were lysed with 2-3 drops of saponin (2.5%), the fluid decanted into a Sedgewick-Rafter chamber, and the worms were counted under a dissecting microscope.

## F. Serology

1. Haemagglutination testa. Antigen

Sheep red blood cells (SRBC) were obtained in sterile Alsevers solution in 25 ml bottles (Tissue Culture Services, Slough, Bucks) and were kept at 4°C and used within 14 days. The required amount of cell suspension was washed 3 times with 1 in 10 normal saline (0.9%) by centrifugation (1000g x 10 min).

b. Immunization of mice

The red cells obtained after washing were resuspended in normal saline to give a 20% suspension. Mice given single immunization doses were inoculated i.p. with 0.2 ml of this suspension ( $1 \times 10^9$  SRBC). Hyperimmunized mice were given the same initial inoculum; single doses were given on 6 further occasions at intervals of 2 weeks.

c. Collection of anti-SRBC serum samples from mice1. Bleeding from the retro-orbital plexus

Blood samples were obtained as described by Jayawardena (1975). Each mouse was lightly anaesthetised with ether and its head was firmly fixed between the thumb and the fore-finger of the left hand, stretching back the skin to make the eye-ball protrude. The tip of a pasteur pipette was introduced to 1 or 2 mm depth into the medial canthus of the eye at an angle of 45°. The pipette was rotated slightly to puncture the venous plexus and then withdrawn

slightly to allow blood to flow into it.

The blood samples obtained were transferred to small disposable plastic tubes (3.5 x 0.5 cm). The serum was separated by keeping the blood at 37°C for 30 min and then at 4°C for 5 min. The clots formed were removed and the tubes were centrifuged at 1000g for 10 min.

The serum was sucked into capillary tubes, inactivated at 56°C for 30 min in a water bath and stored at - 20°C in labelled small test tubes until used.

#### ii. Heart puncture

Mice hyperimmunised with SRBC were killed and bled from the heart. Serum was prepared from this and treated as described above.

#### d. Determination of agglutinin titres

The method used is as described by Scott and Gershon (1970). Doubling dilutions of 20  $\mu$ l of each serum were made with normal saline in a haemagglutination plate. The dilution and application of fluids were carried out with the use of a compu-pet 100 machine (Div. Warner-Lambert Co.). 20  $\mu$ l of a 2% suspension of washed SRBC in saline was added to all wells including controls containing normal saline alone. The suspensions were thoroughly mixed on a whirl mixer at moderate speed for 30 sec.

The tray was covered with a transparent plastic cover and was incubated at 37°C for 1 to 2 hrs. The agglutination

titre was read under a dissecting microscope. The end point was taken as the last well where there was visible agglutination. 20  $\mu$ l of freshly prepared 0.82% solution of 2-mercaptoethanol in normal saline was then added to each well and the suspensions mixed thoroughly by placing the tray over the whirl mixer. The tray was left overnight at room-temperature and re-read on the following morning. 2-mercaptoethanol breaks the disulphide bond holding the polymeric units of the IgM together. The titre of 2-mercaptoethanol-resistant antibody is due mainly to agglutination by IgG and the difference between titres obtained before and after treatment with 2-mercaptoethanol is a measure of agglutination due to IgM antibody.

## 2. Haemolysin test

### a. Complement

Fresh complement was obtained by bleeding normal guinea-pigs from the heart without killing them. Two guinea-pigs were kept in stock and were bled alternately. The animal to be bled was anaesthetised with ether just enough to allow it to be handled. A 19G needle fitted to a 10 ml syringe was introduced into the right side of the heart through the right 3rd intercostal space and 10 ml of blood was withdrawn. Serum was prepared by allowing the blood to clot at 37°C. It was stored at -20°C and always used within a week. The serum was used as a 10% solution in normal saline.

b. Determination of haemolysin titres

The serum to be titrated was inactivated as before at  $56^{\circ}\text{C}$  and doubling dilutions were prepared in a haemagglutination tray as described above. 20  $\mu\text{l}$  of the fresh complement (10% guinea-pig serum) was added to all wells including the wells containing saline. 20  $\mu\text{l}$  aliquots of a freshly prepared 2% suspension of SRBC was added to the wells. The tray was covered and was shaken on a whirl mixer. After incubation at  $37^{\circ}\text{C}$  for 1 to 2 hrs, the titres were determined by examining the plate against a white background. The end point was taken when there was 50% lysis of the SRBC.

## IV. EXPERIMENTAL PROCEDURES AND RESULTS

## A. Patterns of infection

1. P.chabaudia. Infections in different strains of mice

CBA/lac and Swiss TO mice were used in the experiments.

i. CBA/lac mice

These have been used extensively throughout this study. A standard inoculum of  $1 \times 10^7$  infected red blood cells in 0.2 ml was given intraperitoneally. The parasitaemia followed a characteristic and reproducible pattern (fig 4a). The infection is very synchronous and the parasites show a preference for the mature red blood cells and single infections of the host cells predominate, except in late infection. Both single and double chromatin dot nuclei are seen. The parasites appeared first in the blood on the first day of the infection. The parasitaemia then increased rapidly until days 5-7 reaching a peak with 30-50% of the red blood cells infected. It fell sharply between days 6-7 and the blood became aparasitaemic by blood film examination between days 17-20. Gametocytes became more frequent after the peak of infection, approximately 10 days after the infection.

A second peak of parasitaemia was seen on days 26-27 which lasted 2-3 days but reached a peak of less than 0.5%. A further recrudescence of parasitaemia occurred between days 56-60 with the highest parasitaemia being 0.05%. Between these peaks, the parasitaemia was not demonstrable by blood film examination,

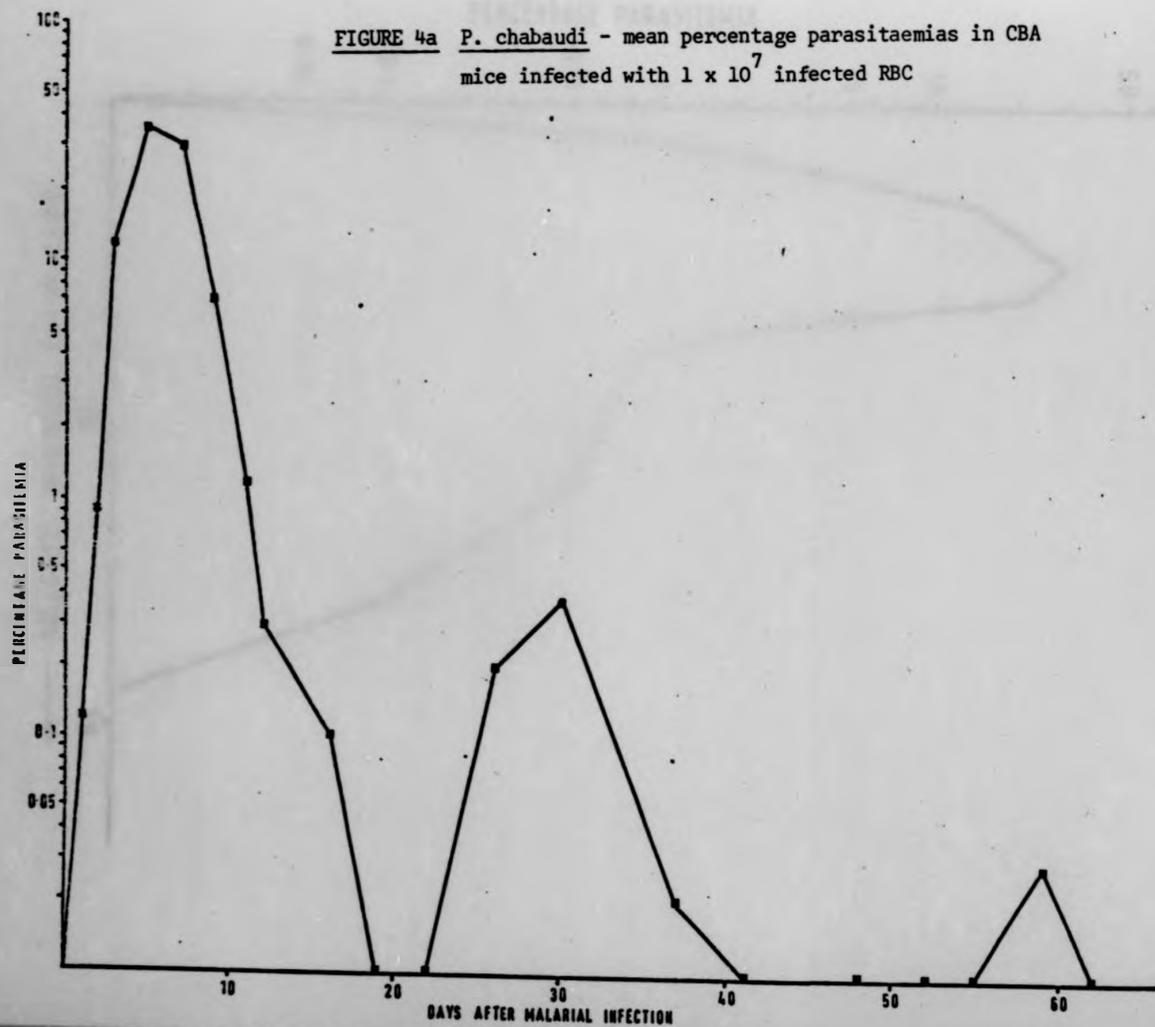


FIGURE 4b Mean percentage parasitaemias in 10 mice after inoculation with  $1 \times 10^7$  *P. chabaudi* infected erythrocytes

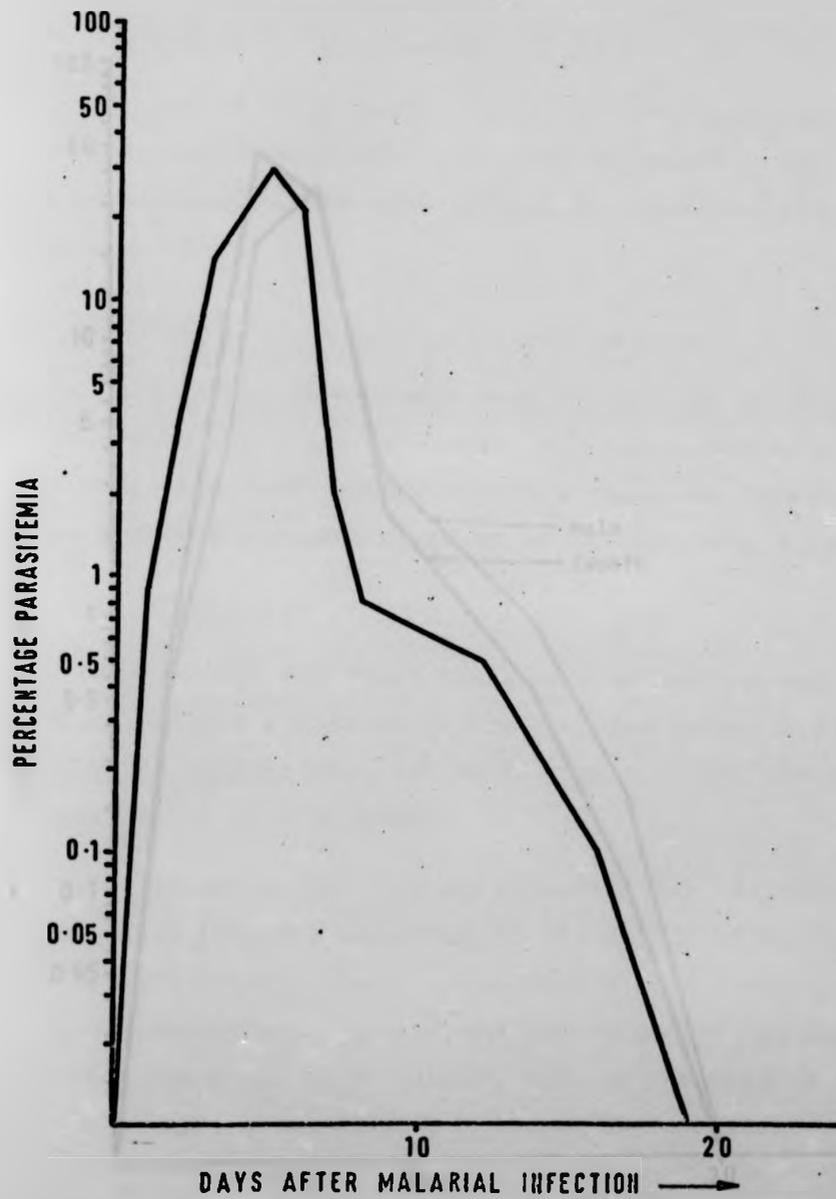
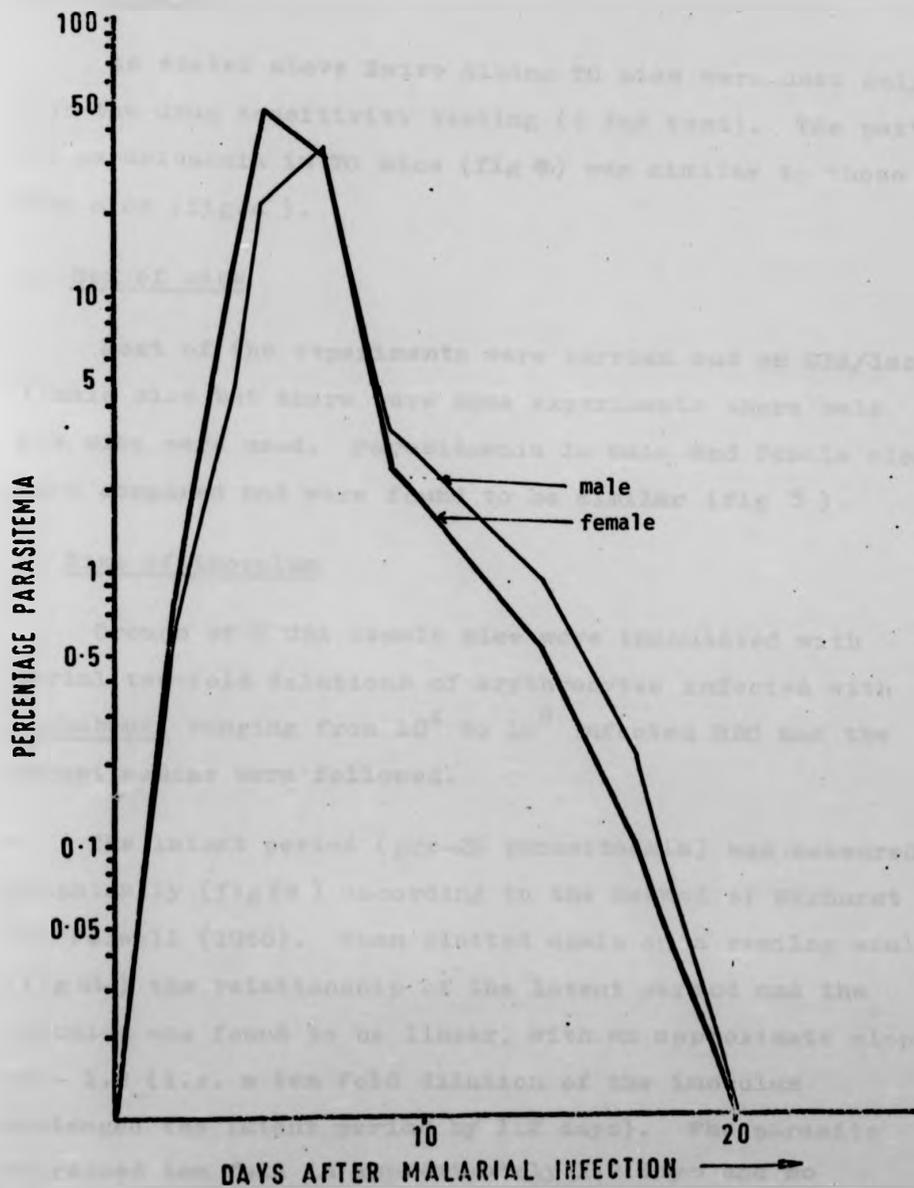


FIGURE 5 Mean percentage parasitaemias of male and female CBA mice after injection of  $1 \times 10^7$  P. chabaudi infected erythrocytes



but infection could be obtained by sub-inoculation of blood to uninfected mice. The parasites could be found by this method up to one week after the last peak of parasitaemia.

ii. TO mice

As stated above Swiss Albino TO mice were used only for the drug sensitivity testing (4 day test). The pattern of parasitaemia in TO mice (fig 4b) was similar to those of CBA mice (fig 4a ).

b. Sex of mice

Most of the experiments were carried out on CBA/lac female mice but there were some experiments where male CBA mice were used. Parasitaemia in male and female mice were compared and were found to be similar (fig 5 ).

c. Size of inoculum

Groups of 5 CBA female mice were inoculated with serial ten-fold dilutions of erythrocytes infected with P.chabaudi ranging from  $10^4$  to  $10^8$  infected RBC and the parasitaemias were followed.

The latent period (pre-2% parasitaemia) was measured graphically (fig 6a) according to the method of Warhurst and Folwell (1968). When plotted again on a semilog scale (fig 6b) the relationship of the latent period and the inoculum was found to be linear, with an approximate slope of - 1.2 (i.e. a ten fold dilution of the inoculum prolonged the latent period by 1.2 days). The parasite increased ten fold in approximately 1.2 days and so

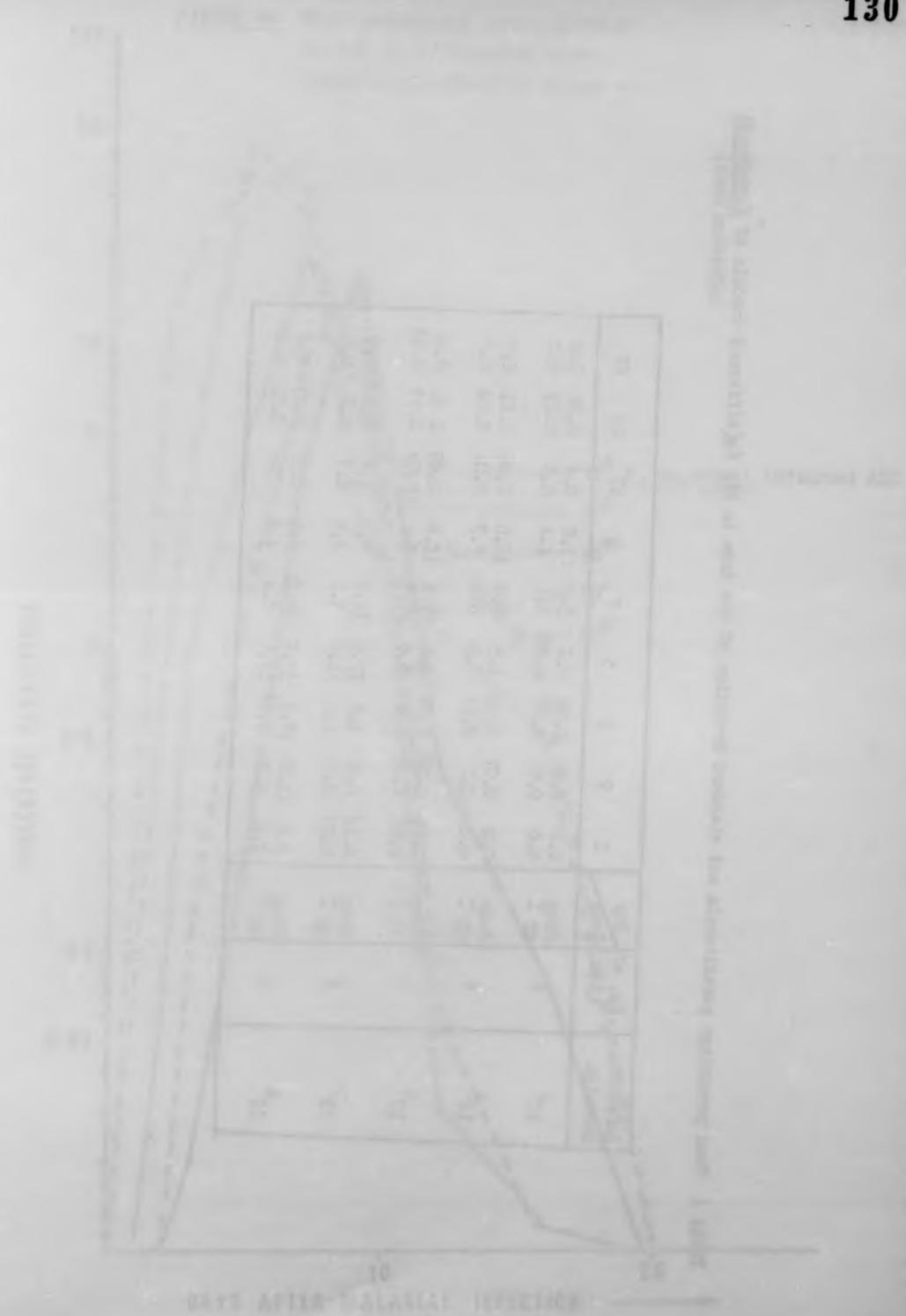


Table I Mean percentage parasitaemia and standard deviation of the data in Fig 6a (different inocula of P.chabaudi infection used)

Infected inoculum	No. of mice	Day Data	1	2	3	5	7	9	12	16	20
			$10^4$	6	Mean SD	0.0 0.0	0.0 0.0	0.02 0.01	0.2 0.08	3.9 3.1	17.0 4.4
$10^5$	6	Mean SD	0.0 0.0	0.01 0.0	0.14 0.11	1.5 2.3	8.8 6.6	16.0 5.7	0.06 0.04	0.01 0.04	0.0 0.0
$10^6$	6	Mean SD	0.01 0.02	0.08 0.07	1.04 1.1	4.6 3.8	19.0 10.2	10.0 3.4	0.03 0.04	0.01 0.02	0.01 0.03
$10^7$	6	Mean SD	0.14 0.07	0.8 0.2	4.8 1.5	47.0 11.0	32.0 5.0	2.3 3.1	1.1 0.7	0.5 0.2	0.0 0.0
$10^8$	6	Mean SD	3.8 3.8	11.8 3.8	19.3 3.6	37.0 19.0	26.0 3.4	5.1 4.0	2.5 0.6	0.07 0.02	0.0 0.0

FIGURE 6a Mean percentage parasitaemias  
in CBA mice injected with  
inocula of different sizes

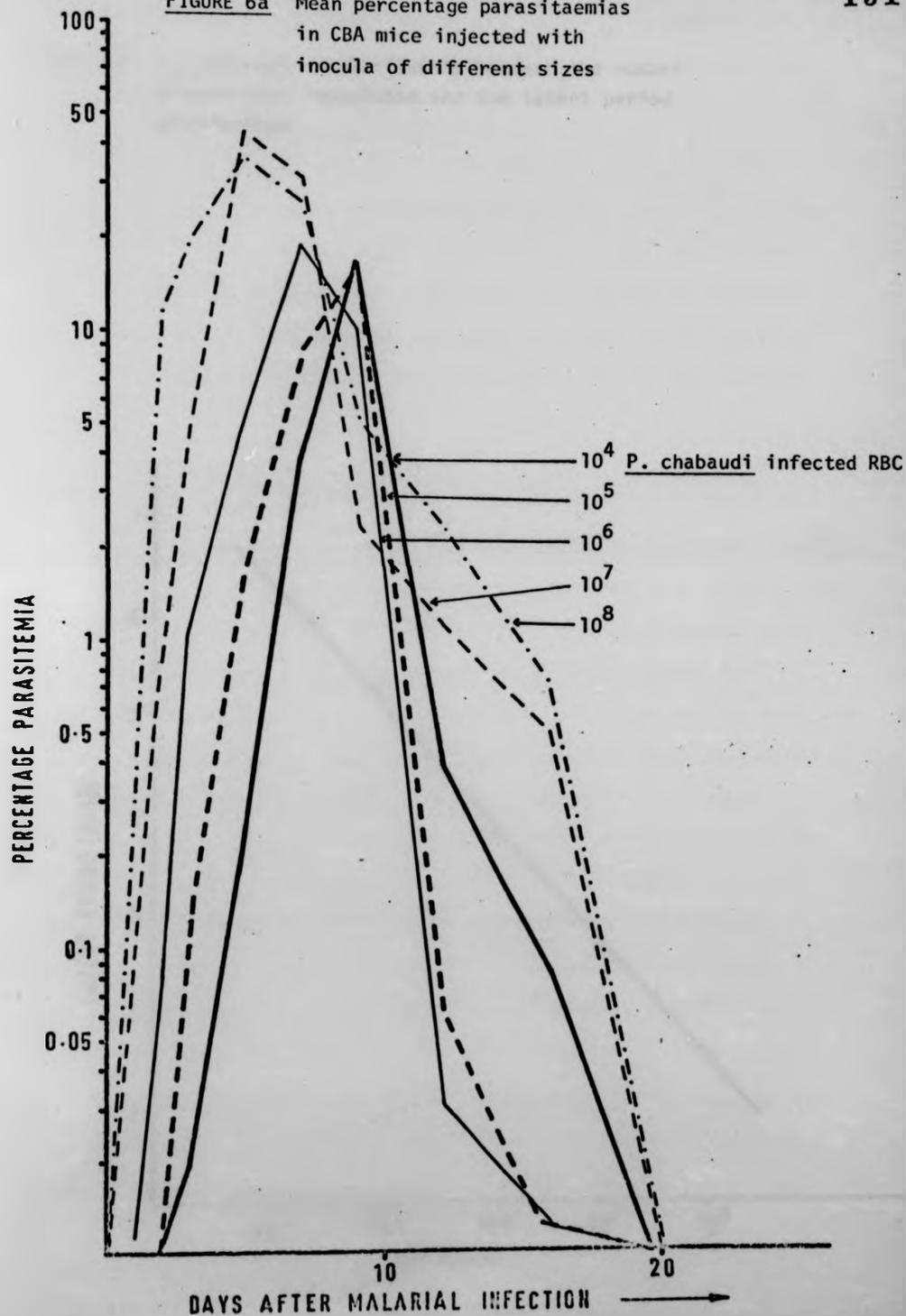
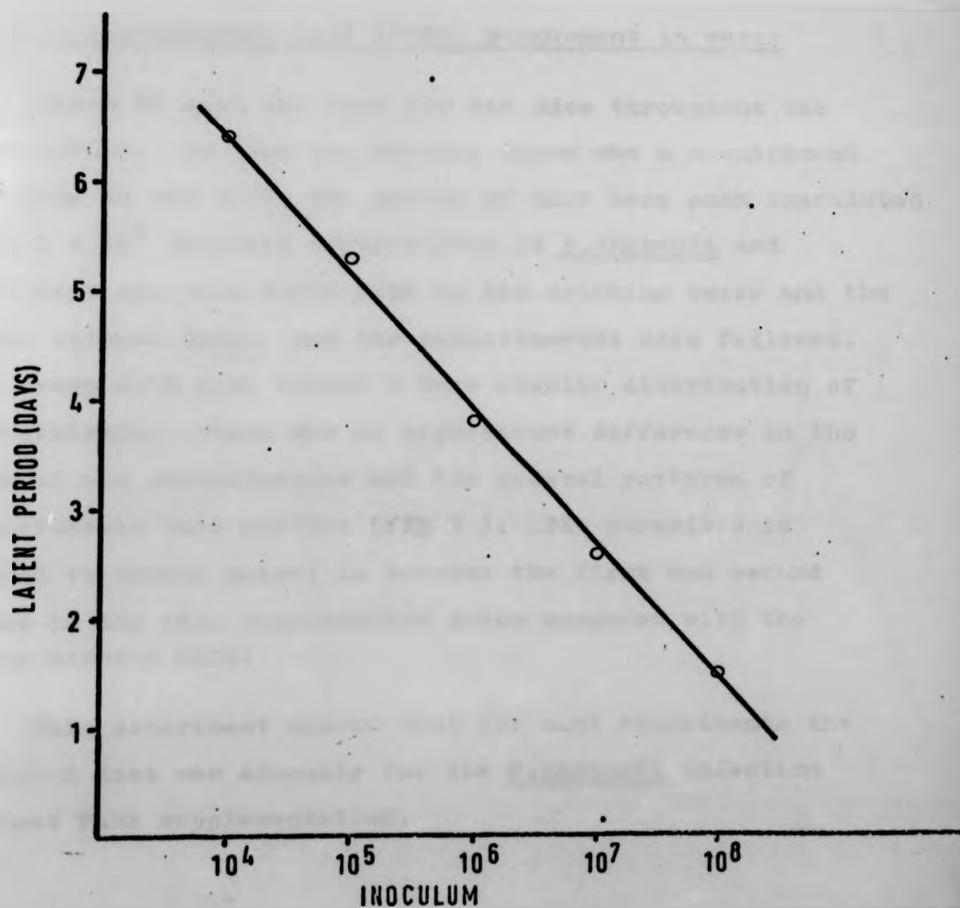


FIGURE 6b *P. chabaudi* - relationship between the number of parasites inoculated and the latent period of infection



the growth rate in hours was  $1/26.8$  hours (Warhurst and Folwell, 1968).

Apart from the delay in the latent period of the parasitaemia, the general patterns of the infection were the same in all the groups. The duration of the first peak of the infection was also similar though with the lower inocula parasitaemias tended to come down earlier to a lower level. There was no mortality in any of the groups.

d. Para-aminobenzoic acid (PABA) supplement in water

Dixon 86 diet was used for the mice throughout the experiments. To find out whether there was a requirement for PABA in the diet, two groups of mice were each inoculated with  $1 \times 10^7$  infected erythrocytes of P.chabaudi and were kept one with 0.05% PABA in the drinking water and the other without PABA, and the parasitaemias were followed. The group with PABA showed a more regular distribution of parasitaemia. There was no significant difference in the peak of the parasitaemias and the general patterns of parasitaemia were similar (fig 7). The parasitaemia tended to remain patent in between the first and second peaks in the PABA supplemented group compared with the group without PABA.

This experiment showed that for most experiments the standard diet was adequate for the P.chabaudi infection without PABA supplementation.

**FIGURE 7** Mean percentage parasitaemias in CBA mice inoculated with  $1 \times 10^7$  infected erythrocytes and given a standard diet with or without a supplement of PABA

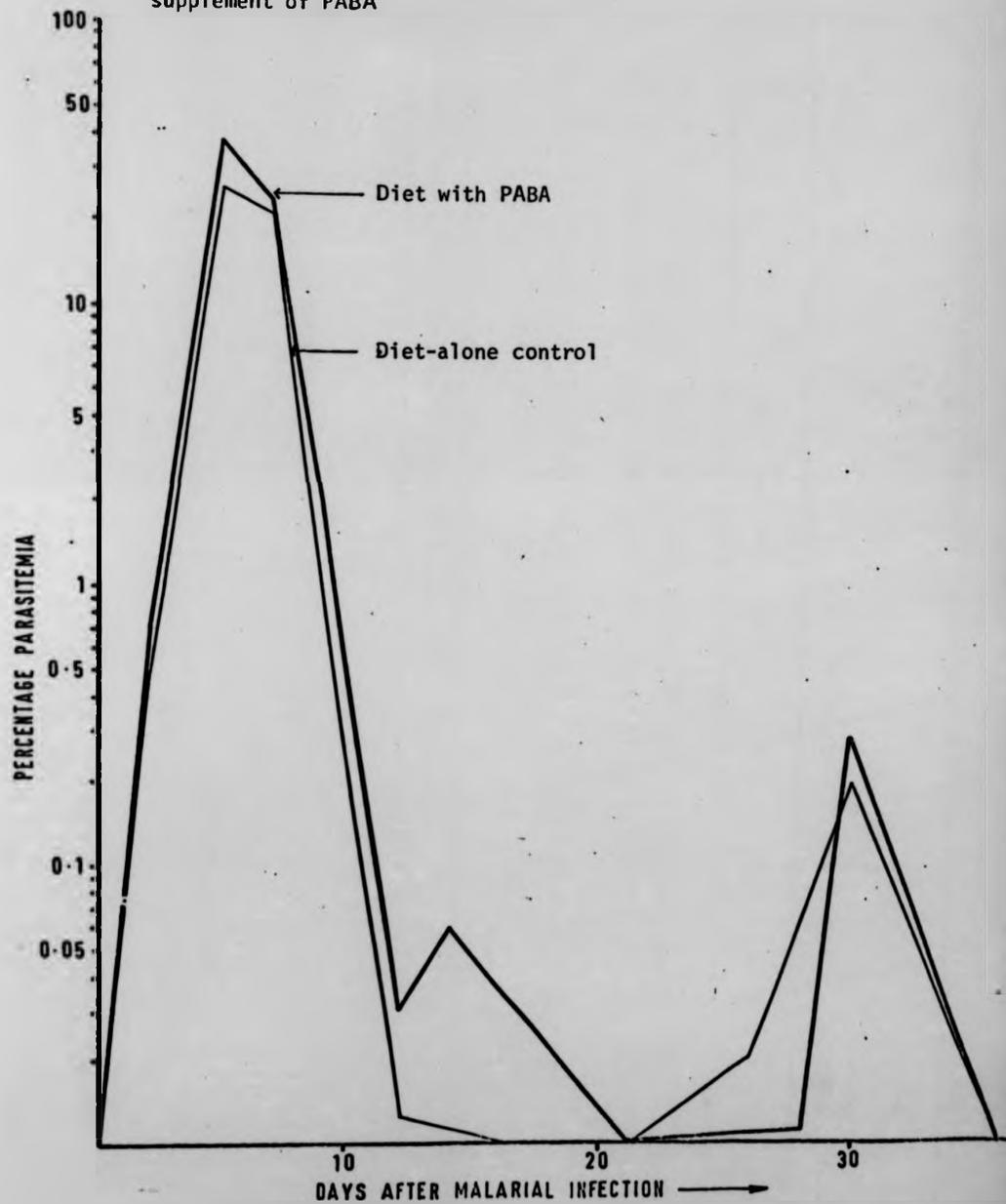


FIGURE 1. A. Daily mean temperature (°C) and precipitation (mm) for the study area. B. Daily mean temperature (°C) and precipitation (mm) for the study area.



Date	Temperature (°C)	Precipitation (mm)
1/1	10	0
1/2	12	0
1/3	15	0
1/4	18	0
1/5	20	0
1/6	22	0
1/7	25	0
1/8	28	0
1/9	30	0
1/10	32	0
1/11	35	0
1/12	38	0
1/13	40	0
1/14	42	0
1/15	45	0
1/16	48	0
1/17	50	0
1/18	52	0
1/19	55	0
1/20	58	0
1/21	60	0
1/22	62	0
1/23	65	0
1/24	68	0
1/25	70	0
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1/27	75	0
1/28	78	0
1/29	80	0
1/30	82	0
1/31	85	0



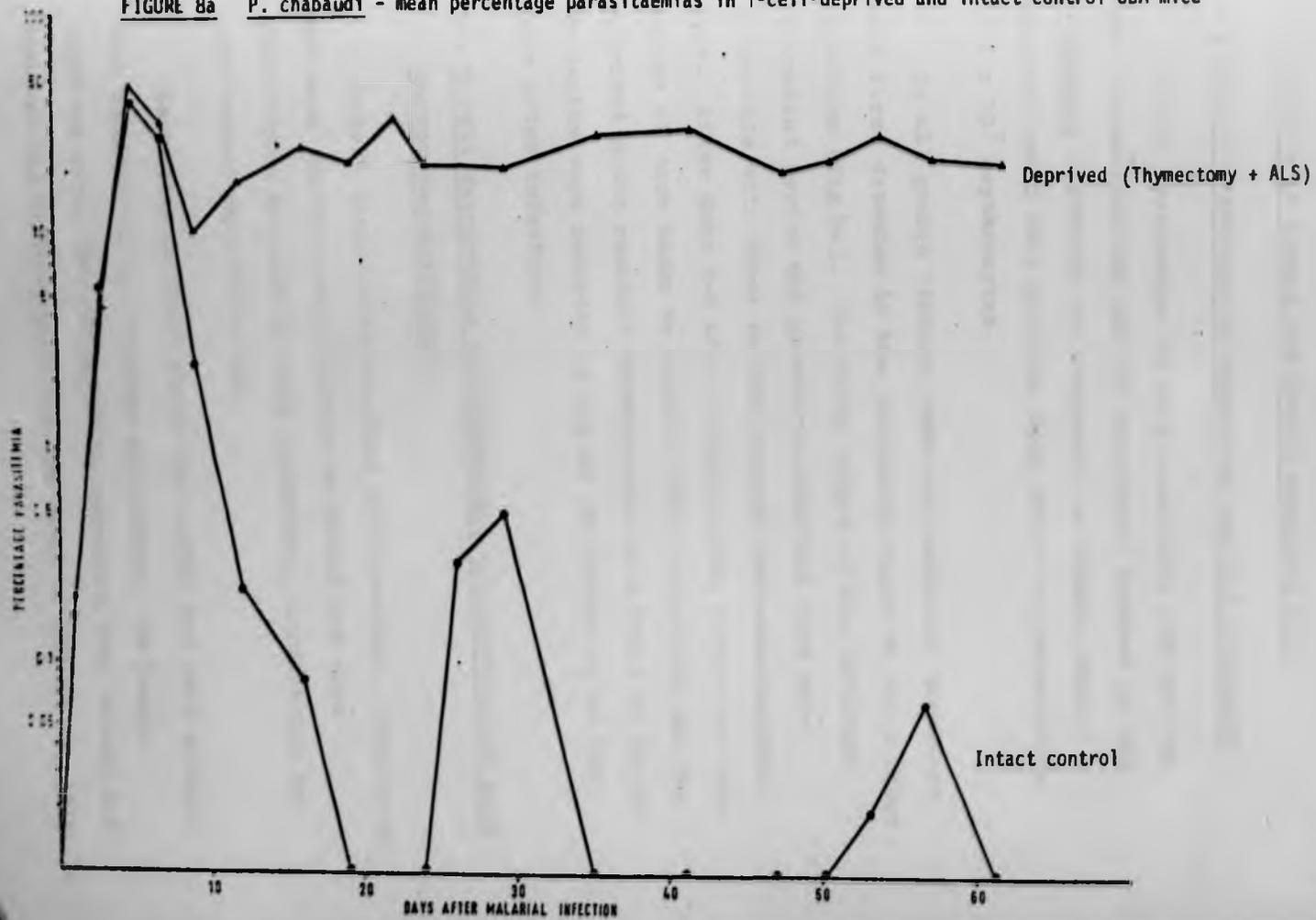
Table II Statistical analysis of the data in Fig 8 a. (n=6)

Group	Day																				
	Data	1	2	3	5	7	9	12	16	19	22	24	26	29	35	41	47	50	53	57	61
Deprived infected	Mean	0.2	0.7	4.4	47.0	33.0	10.0	17.0	27.0	24.0	34.0	22.0	23.0	21.0	30.0	33.0	21.0	23.0	31.0	24.0	23.0
	SD $\pm$	0.1	0.4	1.8	12.0	10.1	2.5	5.1	6.2	3.5	4.6	7.8	5.2	4.3	2.8	3.8	4.1	3.1	5.1	6.2	7.0
Intact infected	Mean	0.16	0.8	5.6	40.0	28.0	2.4	0.22	0.08	0.01	0.0	0.0	0.3	0.5	0.0	0.0	0.0	0.0	0.02	0.07	0.0
	SD $\pm$	0.08	0.4	2.1	9.2	6.4	1.1	0.1	0.02	0.01	-	-	0.1	0.2	-	-	-	-	0.01	0.03	0.0
Significance	P <	NS	NS	NS	NS	NS	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

\*significance between the means of intact and deprived groups  
using student T test.

NS = not significant

FIGURE 8a P. chabaudi - mean percentage parasitaemias in T-cell-deprived and intact control CBA mice



e. Infection in intact and T-cell deprived mice

i. T-cell deprivation by thymectomy and ALS treatment

T-cell deprivation in this experiment was done by adult thymectomy and ALS (2) treatment (stated pp. 108 P.chabaudi infection was compared, in intact, sham-deprived and T-cell deprived mice after the inoculation of  $1 \times 10^7$  erythrocytes.

In all groups (intact, sham and deprived) parasites were first detected in the peripheral blood on day 1 after infection (fig 8a). The early stages of the infection (prepatent period and growth) in deprived mice were comparable with those in both control and sham-deprived mice. After days 5-6 after infection the intact and sham groups of mice began to resolve their infections but the deprived group remained parasitaemic at a level of 20-30%. No deaths were recorded in any of the groups up to 120 days after infection.

ii. T-cell deprivation by thymectomy, irradiation and bone marrow reconstitution

T-cell deprivation was done by thymectomy, irradiation and bone marrow reconstitution as stated and sham deprivation was done by sham thymectomy, irradiation and bone marrow reconstitution.

Parasitaemia patterns in the normal and sham groups were similar to the previous experiment. In T-cell deprived mice, the parasitaemia increased very slowly and reached the maximum parasitaemia level of 52% at day 32 (fig 8b).

TABLE 1. Summary statistics for the 1000 simulated data sets.

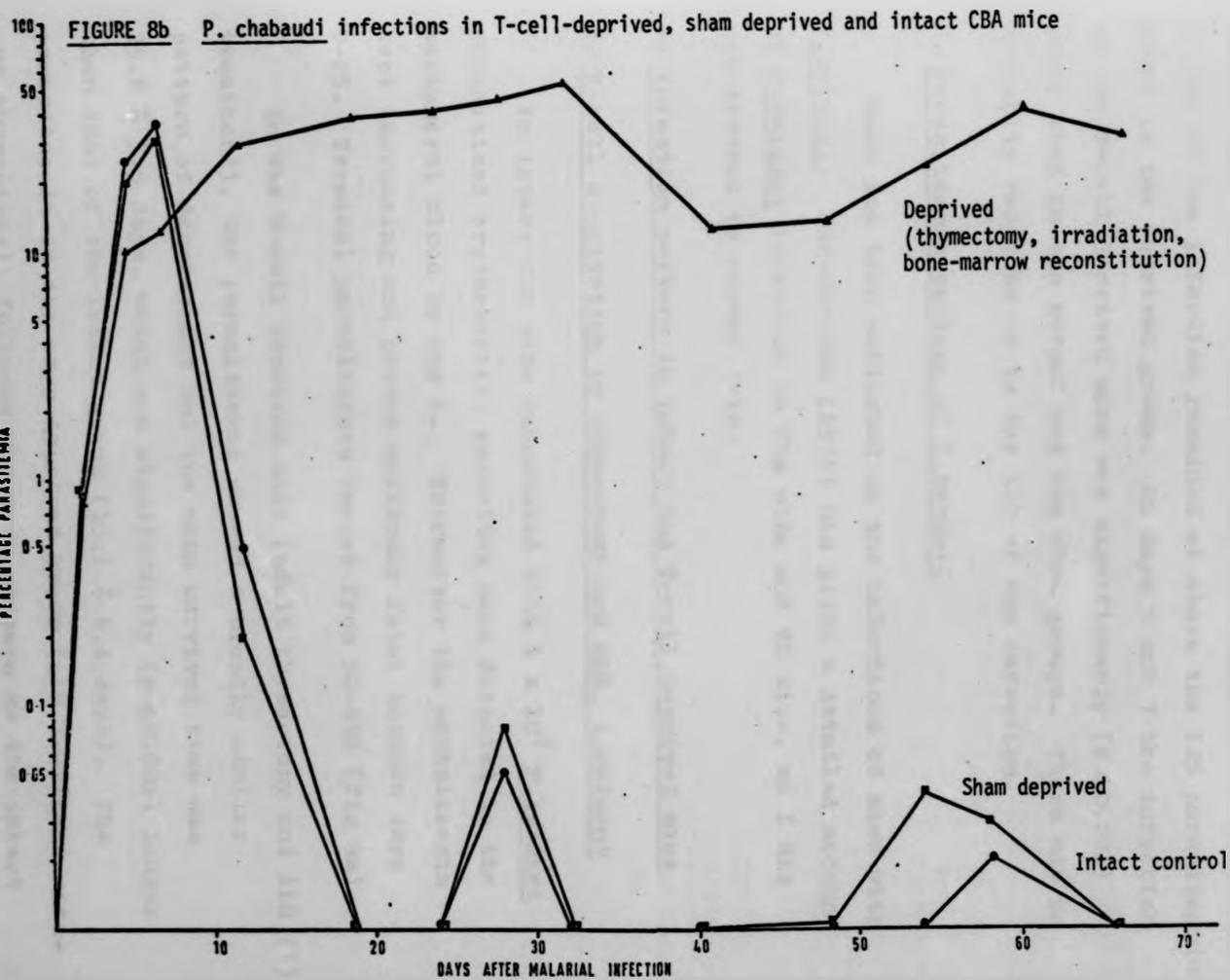
Statistic	1	2	3	4	5	6	7	8	9	10
Mean	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Std. Dev.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Skewness	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kurtosis	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Shapiro-Wilk	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Anderson-Darling	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chi-Square	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kolmogorov-Smirnov	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00



Table III Statistical analysis of the data in Fig 8b. (n=6)

Group	Day Data	2	5	7	12	20	24	28	32	41	48	54	58	66
Deprived infected	Mean	0.7	10.5	12.5	29.0	38.0	40.0	44.0	52.0	12.0	13.2	23.0	38.0	29.0
	SD $\pm$	0.4	4.3	6.7	3.4	9.8	7.1	8.5	17.0	3.2	2.1	5.2	11.0	8.5
Intact infected	Mean	0.8	26.0	38.0	0.5	0.0	0.0	0.05	0.0	0.0	0.0	0.0	0.02	0.0
	SD $\pm$	0.3	3.8	7.2	0.2	0.0	0.0	0.02	0.0	0.0	0.0	0.0	0.01	0.0
Significance	P	NS	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Sham infected	Mean	0.85	20.0	31.0	0.2	0.0	0.0	0.08	0.0	0.0	0.0	0.04	0.03	0.0
	SD $\pm$	0.5	7.2	5.4	0.1	0.0	0.0	0.03	0.0	0.0	0.0	0.02	0.02	0.0
Intact infected	Mean	0.8	26.0	38.0	0.5	0.0	0.0	0.05	0.0	0.0	0.0	0.0	0.02	0.0
	SD $\pm$	0.2	3.2	7.3	0.2	0.0	0.0	0.02	0.0	0.0	0.0	0.0	0.01	0.0
Significance	P <	NS	NS	NS	0.01	NS	NS	NS	NS	NS	NS	0.001	NS	NS

\* see table II



Though the parasitaemia resolved in the intact control group on day 19 the infection remained at above the 12% parasitaemia level in the deprived group. On days 5 and 7 the infection in the T-cell deprived mice was significantly ( $P < 0.001$ ) lower than in the normal and the sham groups. There was no mortality recorded up to day 120 of the infection.

## 2. Parasitaemia pattern of *P.berghei*

Much has been published on the infections of mice with *P.berghei*. Jayawardena (1975) has given a detailed account of *P.berghei* infection in CBA mice and TO mice, so I did not attempt to repeat this.

### a. Infection pattern in intact and T-cell deprived mice

#### i. T-cell deprivation by thymectomy and ALS<sub>2</sub> treatment

In intact CBA mice inoculated with  $1 \times 10^7$  *P.berghei* parasitized erythrocytes, parasites were detected in the peripheral blood by day 1. Thereafter the parasitaemia kept increasing and proved uniformly fatal between days 9-25. Terminal parasitaemia ranged from 50-65% (fig 9a).

In the T-cell deprived mice (adult thymectomy and ALS (1) treatment), the parasitaemia showed a broadly similar pattern of development but the mean survival time was  $25.8 \pm 3.5$  days, which was significantly ( $P < 0.001$ ) longer than that of the intact group ( $18.1 \pm 6.4$  days). The parasitaemia in the sham deprived group (ALS treatment without thymectomy). followed the same pattern as the intact group with a mean survival time of  $20.4 \pm 2.7$  days (fig 9a<sub>2</sub>).



Table IVA Statistical analysis of the data in Fig 5a. (n=8)

Group	Day Data	1	3	7	10	14	17	19	23	27
Deprived infected	Mean	0.6	3.0	17.0	13.0	25.0	29.0	50.0	60.0	52.0
	SD $\pm$	0.2	1.8	3.1	3.7	8.2	6.3	5.7	3.5	1.5
Intact infected	Mean	1.1	3.4	20.0	19.0	38.0	35.0	56.0	57.0	-
	SD $\pm$	0.2	0.8	3.1	4.0	9.0	8.6	4.2	3.5	
Significance	P <	0.001	NS	NS	0.01	0.01	NS	NS	NS	
Sham infected	Mean	0.8	4.5	15.0	17.0	33.0	32.0	59.0	61.0	
	SD $\pm$	0.3	1.1	5.3	3.9	12.8	10.8	19.0	5.2	
Intact infected	Mean	1.1	3.4	20.0	19.0	38.0	35.0	56.0	57.0	
	SD $\pm$	0.2	0.8	3.1	4.0	9.0	8.6	4.2	3.5	
Significance	P <	0.05	0.01	0.05	NS	NS	NS	NS	NS	

\*see table II

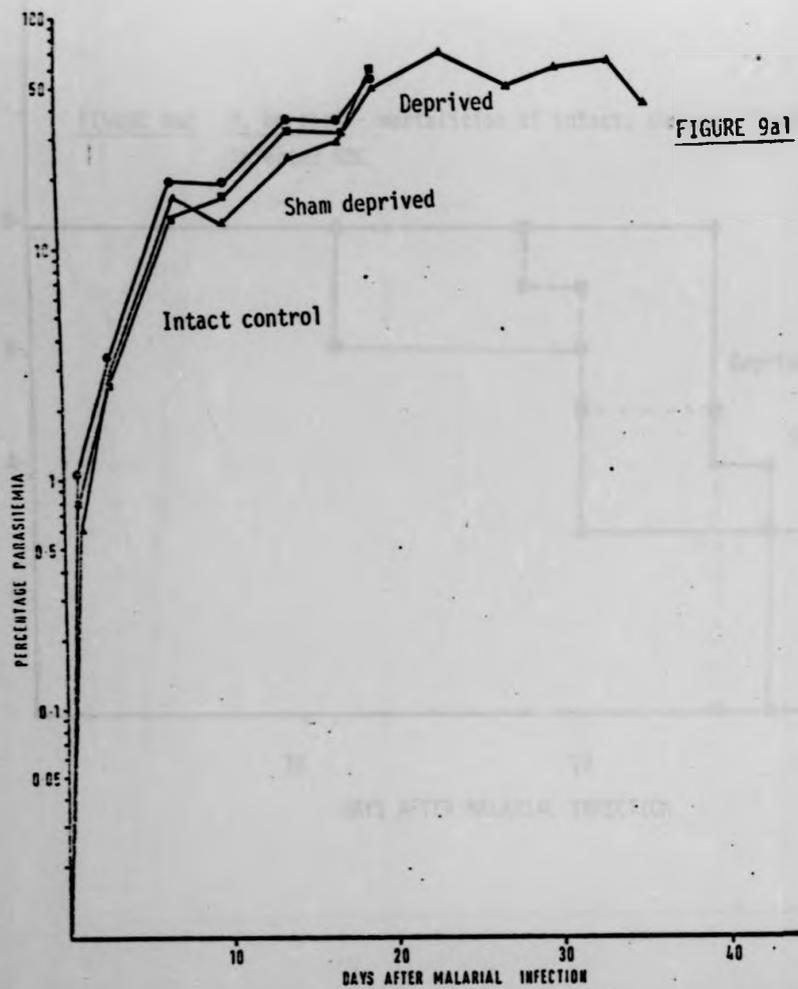
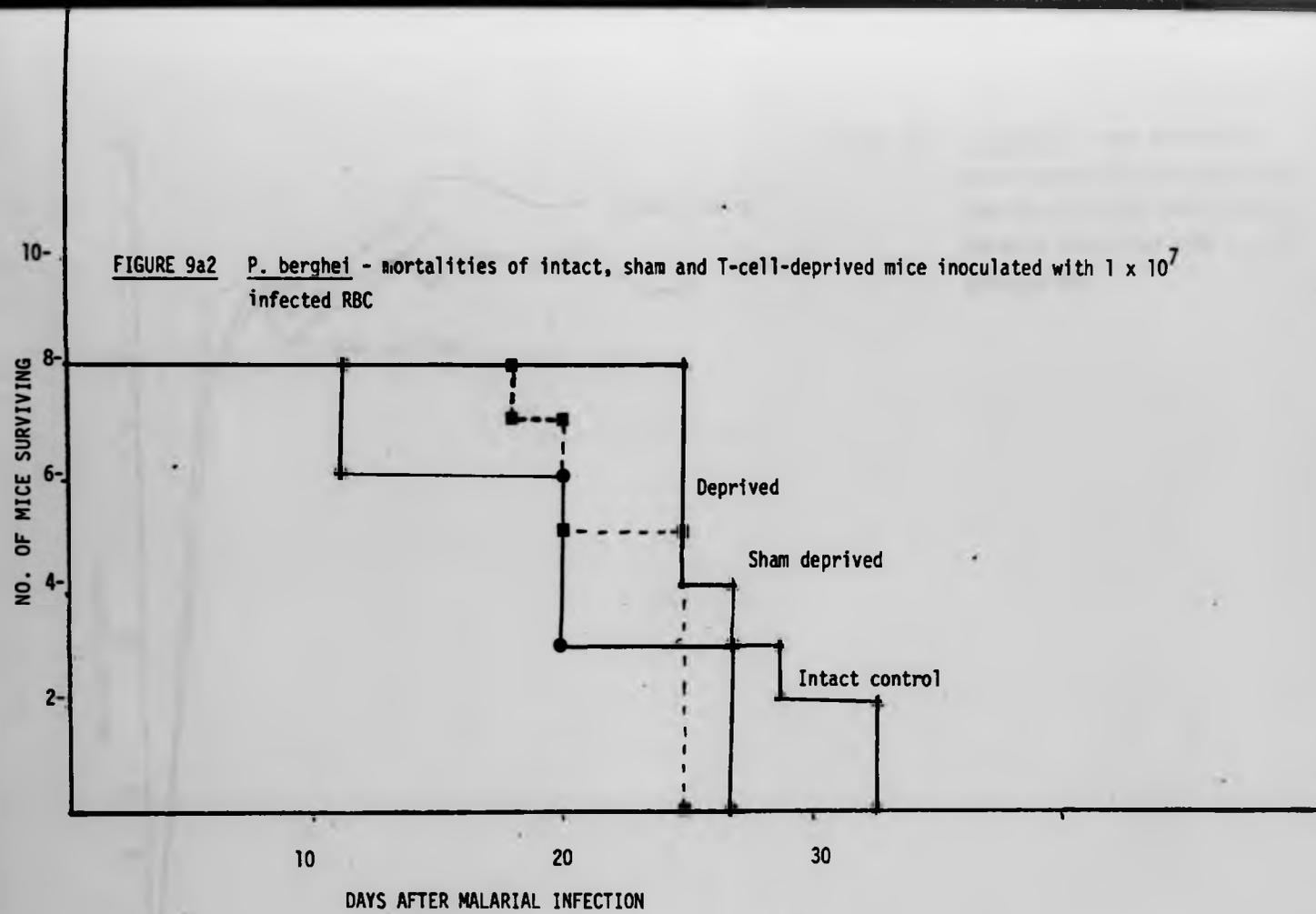


FIGURE 9a1 *P. berghei* - mean percentage parasitaemias in T-cell-deprived, sham deprived and intact control CBA mice inoculated with  $1 \times 10^7$  infected RBC



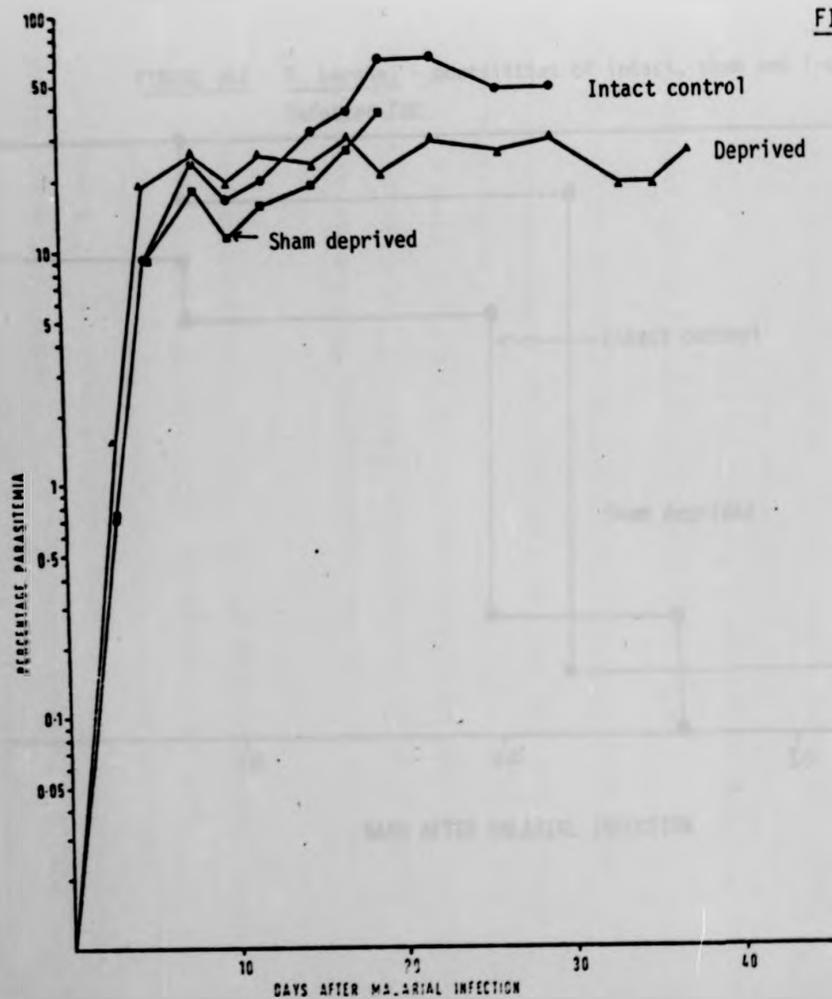
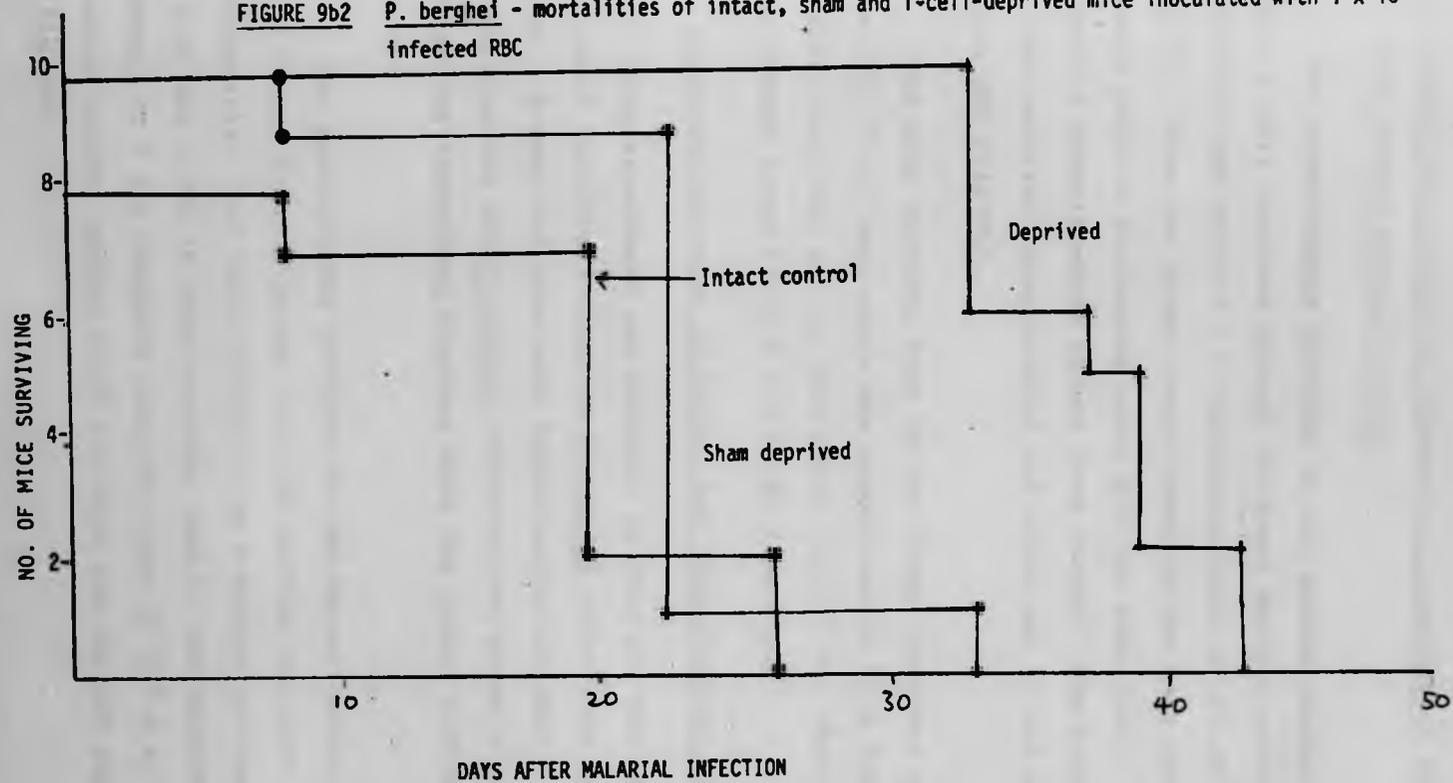


FIGURE 9b1 *P. berghei* - mean percentage parasitaemias in T-cell-deprived, sham deprived and intact control CBA mice inoculated with  $1 \times 10^7$  infected RBC

FIGURE 9b2 P. berghei - mortalities of intact, sham and T-cell-deprived mice inoculated with  $1 \times 10^7$  infected RBC



ii. T-cell deprivation by thymectomy, irradiation and bone marrow reconstitution

The parasitaemia patterns in all groups (normal, sham, T-cell deprived groups) followed similar patterns of infection and reached a parasitaemia level of 18-25% on day 12. Then the parasitaemia level in the sham and intact groups kept on increasing until all the mice died. The terminal parasitaemias ranged from 50-60%. The parasitaemia in the deprived group leveled out after day 12 and remained below 30% (fig 9b<sub>1</sub>).

The mean survival time of the T-cell deprived group was  $37.2 \pm 4.2$  days which was significantly ( $P < 0.001$ ) higher than that of the sham group ( $18.4 \pm 5.6$  days) and the intact group ( $15.2 \pm 8.7$  days) (fig 9b<sub>2</sub>).

b. Infection patterns in intact and thymectomised mice

This experiment was designed to find out the effects of adult thymectomy alone on P.berghei infections in CBA mice. 8 week old mice were thymectomised or sham thymectomised and P.berghei infection was given 4 days after the thymectomy together with the intact control group.

The parasitaemia patterns in the thymectomised, sham and intact control groups were all similar and were comparable. But there seemed to be a slight increase (2.3% and 1.9%) in mean survival time in the thymectomised group,  $21 \pm 3.1$  compared with the sham,  $17.2 \pm 5.4$ , and intact control groups  $17.8 \pm 5.1$  days but was not significant. (fig 10).

Figure 20  
 (1) Temperature vs. Time  
 (2) Heat flux vs. Time  
 (3) Heat transfer coefficient vs. Time



Time (min)	Temperature (°C)	Heat flux (W/m²)	Heat transfer coefficient (W/m²K)
0	20	0	0
1	40	20	2
2	60	40	4
3	70	60	6
4	75	70	7
5	78	75	7.5
6	79	78	7.8
7	80	80	8
8	80	80	8
9	80	80	8
10	80	80	8

Figure 20. (1) Temperature vs. Time, (2) Heat flux vs. Time, (3) Heat transfer coefficient vs. Time.

Heat transfer coefficient vs. Time

Table V Statistical analysis of the data in Fig 10a. (n=12)

Group	Day Data	2	4	6	9	12	16	19
Thymectomised infected	Mean	1.7	11.8	13.2	12.8	30.2	48.4	54.0
	SD $\pm$	0.5	6.6	4.3	2.6	4.6	8.5	8.5
Intact infected	Mean	1.3	12.6	14.8	11.7	32.0	42.3	60.3
	SD $\pm$	0.6	3.9	6.5	0.6	7.0	4.9	0.7
Significance	P <	NS	NS	NS	NS	NS	NS	NS
Sham infected	Mean	1.4	9.8	16.4	15.7	35.3	50.0	56.0
	SD $\pm$	0.5	3.6	6.0	3.1	6.8	3.0	11.3
Intact infected	Mean	1.3	12.6	14.8	11.7	32.0	42.3	60.3
	SD $\pm$	0.6	3.9	6.5	0.6	7.0	4.9	0.7
Significance	P <	NS	NS	NS	NS	NS	NS	NS

\* see table II

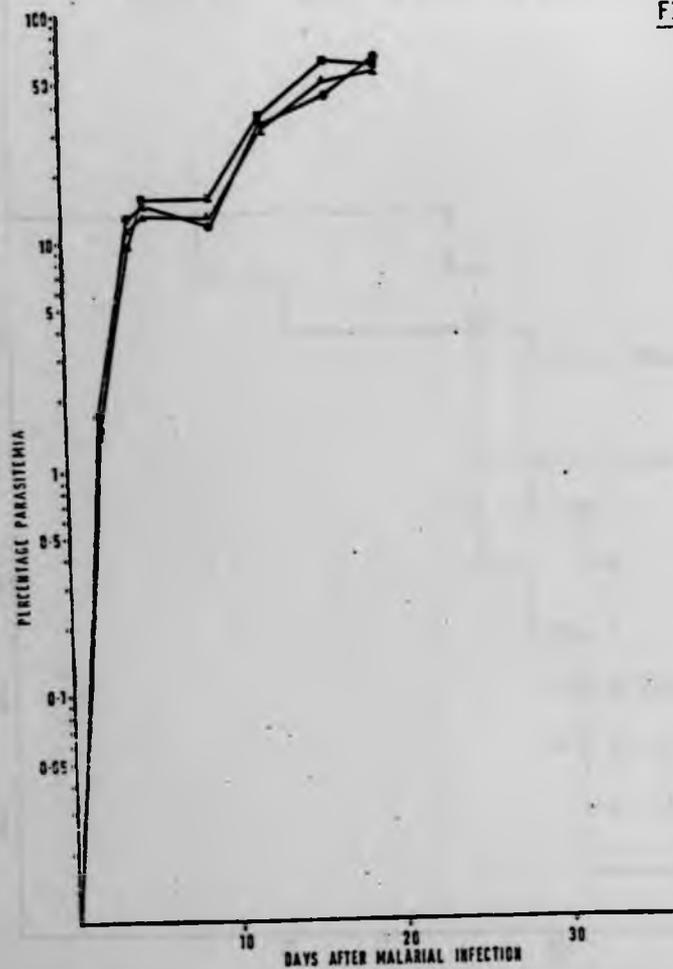
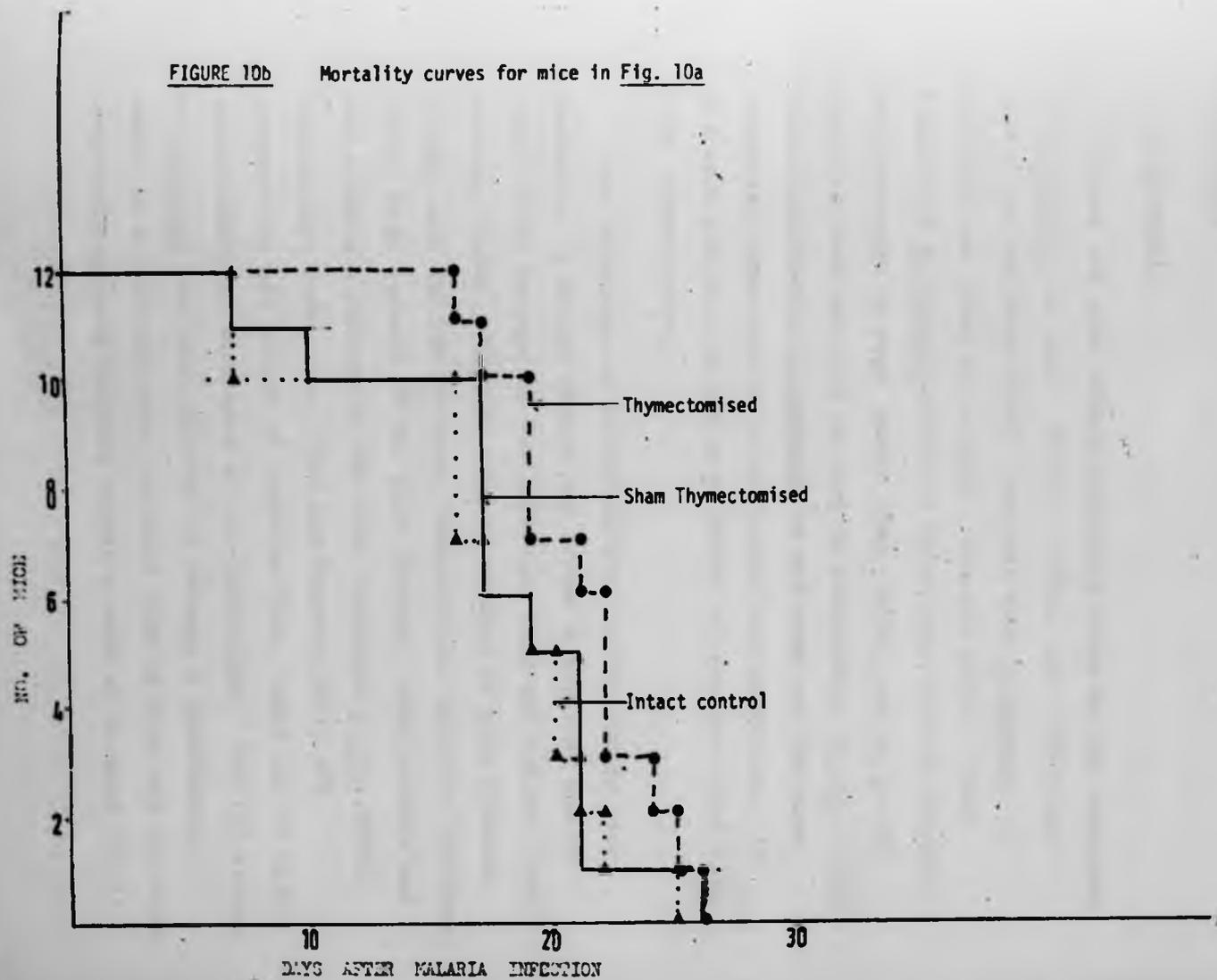


FIGURE 10a *P. berghei* - mean percentage parasitaemias in thymectomised, sham-thymectomised, intact control CBA mice inoculated with  $1 \times 10^7$  infected RBC

- ▲— = thymectomised
- = sham-thymectomised
- = intact control

FIGURE 10b Mortality curves for mice in Fig. 10a



## DISCUSSION

### 1. P.chabaudi

There are some widely differing views on the virulence of P.chabaudi in mice. Peters (1967a), Ott (1969) and Carter and Walliker (1975) reported that P.chabaudi is virulent and often kills mice. Cox and Voller (1966) found that P.chabaudi-infected white mice usually recover, but there may be some deaths (Cox, 1970), and up to 20% mortality was recorded in mice by Nussenzweig *et al.* (1966). In my experiments, P.chabaudi in 6-8 week old CBA mice invariably gave rise to a self-resolving infection. In 3-4 week old weaning CBA mice 70-80% of the mice died with high parasitaemia.

The virulence of rodent malaria infections is very unstable. A benign strain can become a virulent one by rapid blood passage of the infection (Cox and Voller, 1966; Garnham, 1966). Factors such as strains of mice (Peters, 1967a), age and sex of mice, temperature, humidity (Garnham, 1966), PABA content of the diet (Peters, 1970a) protein and and vitamin A content of the diet (Krishnan *et al.*, 1976), concomitant infections, (Ott and Stauber, 1967) and preparation and dosage of inoculum (Ott, 1969) can be major determinants of virulence of the infections. The 17X strain of P.yoelii has been observed to undergo a spontaneous shift to a virulent state in which 100% of mice may die with overwhelming parasitaemias within a week or 10 days (Yoeli

and Hargreaves, 1974).

To standardise the infections as far as possible clones were prepared and stabilised in large numbers. CBA/lac inbred strains of mice were used because they give a reproducible pattern of infection with both P.chabaudi and P.berghei infections and because they have been used extensively by other workers for their immunological studies (Jayawardena et al., 1975, 1977; and Whitmore, 1974)

P.chabaudi in CBA mice gives rise to a characteristic reproducible chronic type of infection which persists for about 60 days. The parasite produces a synchronous type of infection, has no preference for reticulocytes and makes a good experimental model for human malaria caused by P.falciparum (Rosario, 1976). The parasite was not seen by blood examination in the intervals between the peaks of parasitaemia. It could be demonstrated, however, by blood passaging into uninfected recipient mice. The parasite was obtained in this way up to 6 days after the last peak of patency. Injection of kidney, spleen or liver tissue did not improve any positives revealed by blood passage. However, Jayawardena et al. (1977) showed that P.yoelii infections persist in about 60% of mice in their kidneys for about 4 weeks after disappearance of parasitaemia from the blood. Sometimes blood collected between the 2nd and 3rd peaks did not produce infections in recipients. This could mean that the parasitaemia is very low and that parasites occur outside the circulation.

Nussenzweig et al. (1966) showed that 80% P.chabaudi

infected CF1 mice produced a chronic infection with three peaks of parasitaemia. The first peak was between 8 to 12 days with 10-40% parasitaemia. The second peak at the 3rd to 4th week with 10% parasitaemia and the third peak in the 6th week with 30% parasitaemia. According to the authors, the majority of the mice died at the third peak of parasitaemia because of a sudden high parasitaemia.

Use of inocula of different sizes did not affect the outcome of the infection. Even with a higher inoculum ( $10^8$  infected erythrocytes), there was no mortality. The time to the first appearance of the parasite (pre 2% latent period differed but apart from that the general pattern of the infection was similar.

Sengers et al. (1971b) have also shown that there was no correlation between the log inoculum and the number of deaths in P.berghei infections in Swiss mice. Graphically calculated (Warhurst and Folwell, 1968) the pre 2% latent period was found to be 1.2 days (26.8 hrs) (fig 6 a ). Carter and Walliker (1975) gave a latent period of 24 hours in P.berghei infections in mice. The difference in latent period may be due to the different laboratory conditions, strains of mice and lines of parasites or may be because their figure was an approximate one.

It has been shown by several workers that the malaria parasite requires PABA for normal growth (Hawking, 1953; Kretschmar, 1965). Carter (1972) showed that chloroquine resistance in P.voelii is dependent on the concentration of PABA in the diet. Also Kirakosyan (1970) demonstrated that chloroquine was more effective against P.berghei in

mice when PABA was absent from the host.

T-cell deprivation by adult thymectomy, irradiation and syngeneic bone marrow reconstitution, (Miller, Doak and Cross, 1963) has been used for experiments on P.berghei and P.yoelii infections in mice (Jayawardena et al., 1977). Although leaving a residual 10% T-cell population (Doenhoff et al., 1970) this technique has the advantages of ease of performance, reliability in results and avoidance of the major inconvenience of 'wasting' in long term experiments (Davies, 1969). This method was used for some experiments initially but had to be abandoned because of the lack of a convenient irradiation source. T-cell deprivation by adult thymectomy and ALS treatment was used extensively in my experiments. Lance (1970) has presented the evidence to indicate that anti-lymphocyte serum (which is often synonymous with anti-thymocyte serum) acted by selectively depleting the recirculating lymphocyte pool. Doenhoff and Leuchars (1977) showed that the cytotoxic action of ATS is directed mainly against the recirculating thymus derived T-cell since it depletes the blood borne PHA-responsive cell pool. The action of ATS on antigen-reactive thymus-derived cells has been demonstrated directly by the use of chromosomally-marked lymphocyte subpopulations (Leuchars, Wallis and Davies, 1968). Intact mice treated with ATS eventually reconstitute their T-cell pool (PHA responsive cells) after 100 days. But in thymectomised and ATS treated mice recovery of PHA responses is only marginal and takes 400 days (Doenhoff and Leuchars, 1977). The fact that thymectomy followed by ATS treatment resulted in good immunodepression is shown by the experiments

on p 111 . The skin graft rejection was delayed in 4 out of 10 mice for more than  $1\frac{1}{2}$  years and levels of agglutination, haemoglobin and IgG titres were suppressed in the deprived group in comparison with those of the controls.

The immune response of CBA mice to P.chabaudi infection is thymus dependent. P.chabaudi infection in the immunologically intact mouse is a resolving and then chronic type of infection. In mice T-cell deprived by thymectomy and ATS treatment the parasitaemia was the same as that of the normal controls until day 7 of the infection but then instead of resolving it remained relatively constant. Generally the parasitaemia rose to about 50% but then came down and formed a plateau at a level of 12-30%. The mice survived for more than 120 days.

The T lymphocytes appear to perform two major roles in the induction of the immune response to plasmodia. One role is the helper function in initiating the events in the B-cells which lead to antibody secretion, the other role is to secrete lymphokines which bring about activation of the macrophages (Kreier, 1976). Brown (1974) has suggested that T-cells might be important as helpers in establishing variant-transcending immunity. He suggested that during malarial infection, T-cells become primed to a determinant common to all plasmodial variants characteristic of the strain producing the infection.

P.yoelii infection in CBA mice is also T-cell dependent. In normal mice the first wave of parasitaemia resolves in 15-17 days but in T-cell deprived mice the

infection resulted in a progressive parasitaemia and proved fatal in 35-40 days (Jayawardena et al., 1977). A similar slow increase of parasitaemia to that with P.chabaudi was shown in thymectomised, irradiated and bone marrow reconstituted mice infected with P.yoelii (Jayawardena et al., 1977). This might be due to the effect of irradiation in the rapidly dividing cells of the gastro-intestinal tract causing reduced absorption of nutrients like PABA, but this has not been proved.

It has been shown that the haematocrit level correlates very well with the parasitaemia level of P.yoelii infections in mice (Lwin, 1975; Jayawardena et al., 1977); again the reticulocytosis is correlated with the haematocrit value (Jayawardena et al., 1977). The higher the parasitaemia the lower the haematocrit level and as a result the higher the relative reticulocyte count. Since P.yoelii has a great preference for reticulocytes and because of lack of T-cell response, the decrease in haematocrit value and increase of reticulocytes forms a vicious circle until the mouse dies of anaemia. P.chabaudi infection has no predilection for reticulocytes and this might be why a plateau is established and the mice survive.

## 2. P.berghei

P.berghei infections in mice are invariably fatal. (Greenberg et al., 1953). The parasitaemia levels of P.berghei in mice deprived by both methods were similar to that of infected intact controls (figs 9a1 & 9b1). However, the mean survival time for the group T-cell deprived by

thymectomy, irradiation and bone marrow reconstitution was  $36.2 \pm 3$  days which was significantly ( $P < 0.001$ ) longer than that of the normal and sham control mice. The group T-cell deprived by thymectomy and ALS treatment had a mean survival time of  $25.8 \pm 3.5$  days which was again significantly longer ( $P < 0.05$ ) than that of the intact and sham controls. This may be due to the differing effects of ATS and irradiation. ATS selectively depletes the circulating T-cells (Lance, 1970) but irradiation affects the T cells, (PHA responsive cells) and B cells (LPS responsive cells) (Doenhoff and Leuchars, 1977) as well as the rapid dividing cells of the body like gastro-intestinal cells. Experiments were also done at two separate times with different sexes of mice; male mice were used for the thymectomy, irradiation and bone marrow reconstitution experiments and female mice for the other. These factors might play a role in the difference in mean survival times.

Jayawardena et al. (1975) have shown the increased mean survival time in T-cell deprived mice infected with P.berghei. Adult thymectomy alone did not alter the infection nor the survival rate very much. This shows that the thymus has no direct influence through hormones on the infection (Trainin, 1974). Wright (1968) and Wright et al. (1971) have shown that either neonatal thymectomy or ALS treatment in hamsters infected with P.berghei prolonged the survival time. According to them, the deposition of antigen-antibody complexes on the brain vessels in the intact mice shortened the survival time. But the effect of suppressor T-cells on the immune response of the host in the intact animal must also be considered.

Jayawardena and Waksman (1977) demonstrated the presence of T-suppressor cells in a T.brucei infection by showing the suppressive effects of mitogenic responses due to con A and phytohaemagglutinin (PHA), lipopolysaccharide (LPS) and allogenic cells in vitro by the T.brucei infected spleen cells. Since P.berghei produced a fatal infection in mice like T.brucei, T-suppressor cells might play a role.

The two plasmodia, P.chabaudi and P.berghei behave totally differently immunologically. P.chabaudi does not resolve in T-cell deprived mice though it does in the intact mouse. Conversely T-cell deprived mice survived longer than normal mice when infected with P.berghei. The two contrasting parasite-host relationship systems thus formed good models for subsequent studies.

B. Immunodenressive effects of P.chabaudi infection

Since no work has been done on the immunodepressive effects of P.chabaudi, I intended to find out its effects on SRBC. All the experimental mice received an inoculum of  $1 \times 10^7$  P.chabaudi infected erythrocytes on day 0. 0.2 ml of 20% SRBC ( $1 \times 10^9$ ) was given intraperitoneally to both the experimental and the control groups of mice at various intervals during the infections according to the experiment. Sera were obtained from the retro-orbital plexus of mice at various intervals after SRBC injection and the agglutination titre, lysin titre, 2-mercaptoethanol resistant and sensitive titres were determined.

1. The effect of P.chabaudi infection on the primary immune response of mice to SRBC

The experiments were designed to find out the immunodepressive effects of P.chabaudi on the primary immune response to SRBC given at the following levels of parasitaemia.

a. SRBC given at peak parasitaemia

SRBC were given on day 5 (peak) of P.chabaudi infection to both the experimental and the control groups. The results are shown in fig 11 and table VI.

The agglutination titre was between  $\log_2 9.4 (\pm 0.9)$  and  $10.5 (\pm 0.6)$  from days 11-28 and declined to  $\log_2 6 (\pm 0.8)$  on day 63 in the uninfected control group. In the P.chabaudi infected mice the mean agglutination titre was comparable to that of the control group initially on day 8 but from day 11

Year	Month	Day	Time	Place	Remarks
1900	Jan	1	10:00	...	...
1900	Jan	2	10:00	...	...
1900	Jan	3	10:00	...	...
1900	Jan	4	10:00	...	...
1900	Jan	5	10:00	...	...
1900	Jan	6	10:00	...	...
1900	Jan	7	10:00	...	...
1900	Jan	8	10:00	...	...
1900	Jan	9	10:00	...	...
1900	Jan	10	10:00	...	...
1900	Jan	11	10:00	...	...
1900	Jan	12	10:00	...	...
1900	Jan	13	10:00	...	...
1900	Jan	14	10:00	...	...
1900	Jan	15	10:00	...	...
1900	Jan	16	10:00	...	...
1900	Jan	17	10:00	...	...
1900	Jan	18	10:00	...	...
1900	Jan	19	10:00	...	...
1900	Jan	20	10:00	...	...
1900	Jan	21	10:00	...	...
1900	Jan	22	10:00	...	...
1900	Jan	23	10:00	...	...
1900	Jan	24	10:00	...	...
1900	Jan	25	10:00	...	...
1900	Jan	26	10:00	...	...
1900	Jan	27	10:00	...	...
1900	Jan	28	10:00	...	...
1900	Jan	29	10:00	...	...
1900	Jan	30	10:00	...	...
1900	Jan	31	10:00	...	...

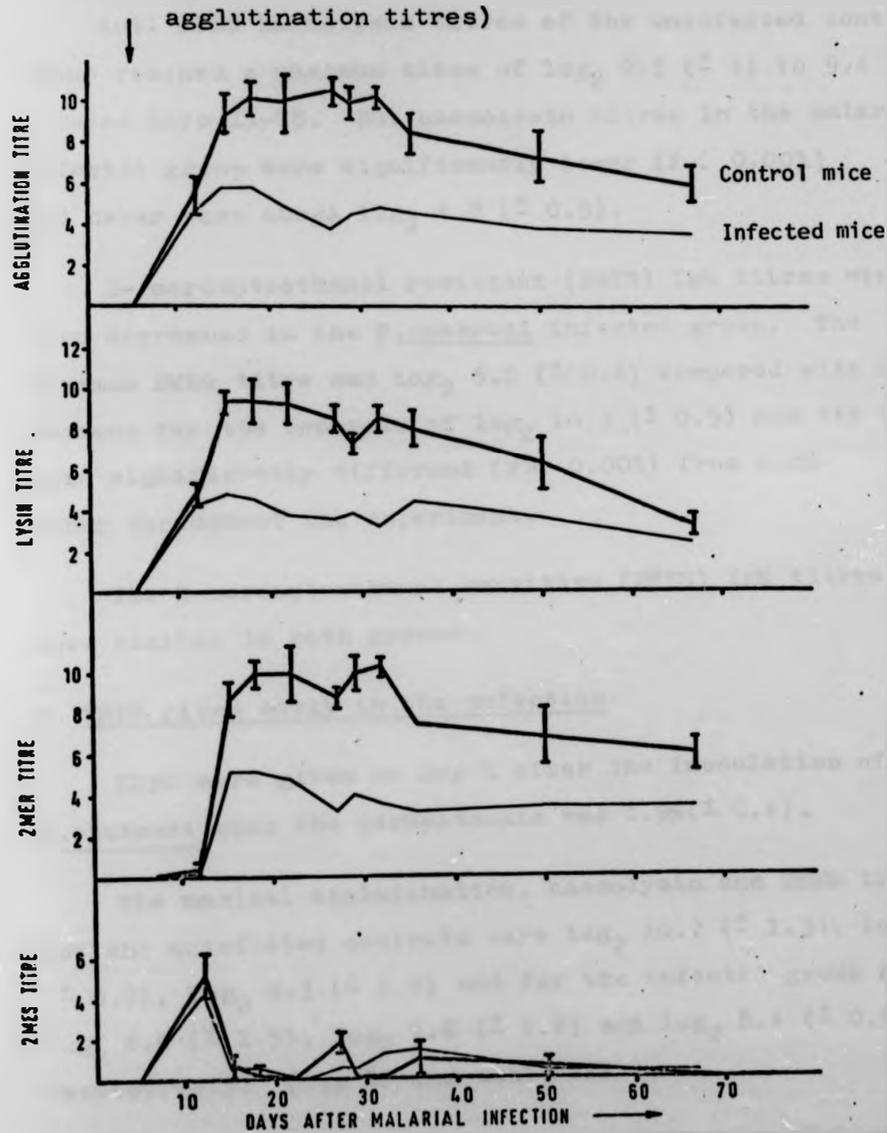
Table VI Statistical analysis of the data in Fig 11 (n=5)

Test	Group	Day Data	8	11	14	18	22	25	28	32	46	63
			Agglutination titre	Control	Mean	5.4	9.4	10.2	10.0	10.5	10.0	10.3
SD +	0.9	0.9			0.8	1.4	0.6	0.8	0.6	1.0	1.3	0.8
Malaria infected	Mean	4.8		5.8	5.8	4.8	3.8	4.6	4.8	4.6	3.8	3.6
	SD +	0.5		0.5	0.5	0.5	0.8	0.6	0.8	0.6	0.5	0.6
Significance	P <	NS	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Lysin titre	Control	Mean	4.8	9.4	9.4	9.3	8.5	7.3	8.5	8.0	6.3	3.3
		SD +	0.5	0.6	1.1	1.0	0.6	0.5	0.6	0.8	1.3	0.5
	Malaria infected	Mean	4.4	4.8	4.6	3.8	3.6	3.8	3.2	4.4	3.6	2.4
		SD +	0.6	0.5	0.6	0.5	0.6	0.5	0.8	0.6	1.1	0.6
Significance	P <	NS	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.05	
ME resistant titre	Control	Mean	0.2	8.8	10.0	10.0	8.8	10.0	10.3	7.5	6.8	6.0
		SD +	0.5	0.8	0.7	1.0	0.5	0.8	0.5	0.6	1.5	0.8
	Malaria infected	Mean	0.2	5.2	5.2	4.8	3.2	4.0	3.6	3.2	3.4	3.4
		SD +	0.5	0.8	0.5	0.5	0.5	0.5	0.9	0.5	0.6	0.6
Significance	P <	NS	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	
ME sensitive titre	Control	Mean	5.2	0.6	0.2	0.0	1.8	0.0	0.0	1.0	0.5	0.0
		SD +	1.1	0.9	0.5	0.0	0.5	0.0	0.0	0.8	0.6	0.0
	Malaria infected	Mean	4.4	0.6	0.6	0.0	0.6	0.6	1.2	1.4	0.4	0.2
		SD +	0.6	0.6	0.6	0.0	0.6	0.6	0.5	0.6	0.6	0.5
Significance	P <	NS	NS	NS	NS	0.01	NS	0.001	NS	NS	NS	

\*Significance between the means of control and experimental group using student T test.

NS - not significant

FIGURE 11 Log<sub>2</sub> antibody titres to sheep red-blood cells given at the peak of *P. chabaudi* infection  
(2MER/2MES titres=2-mercaptoethanol resistant/sensitive agglutination titres)



onwards the agglutination titres were significantly lower ( $P < 0.001$ ) and never exceeded  $\log_2 5.8 (\pm 0.5)$ .

Anti SRBC haemolysin titres of the uninfected control group reached a maximum titre of  $\log_2 9.3 (\pm 1)$  to  $9.4 (\pm 1.1)$  between days 11-18. But haemolysin titres in the malaria infected group were significantly lower ( $P < 0.001$ ) and never rose above  $\log_2 4.8 (\pm 0.5)$ .

2- mercaptoethanol resistant (2MER) IgG titres were also depressed in the P.chabaudi infected group. The maximum 2MER titre was  $\log_2 5.2 (\pm 0.8)$  compared with a maximum for the controls of  $\log_2 10.3 (\pm 0.5)$  and the titres were significantly different ( $P < 0.001$ ) from each other throughout the experiment.

The 2-mercaptoethanol sensitive (2MES) IgM titres were similar in both groups.

b. SRBC given early in the infection

SRBC were given on day 1 after the inoculation of P.chabaudi when the parasitaemia was  $1.9\% (\pm 0.4)$ .

The maximal agglutination, haemolysin and 2MER titres for the uninfected controls were  $\log_2 10.2 (\pm 1.3)$ ,  $\log_2 9.8 (\pm 0.8)$ ,  $\log_2 9.3 (\pm 1.5)$  and for the infected group were  $\log_2 8.6 (\pm 1.5)$ ,  $\log_2 7.8 (\pm 1.2)$  and  $\log_2 8.4 (\pm 0.9)$  respectively. (fig 12 and table VII).

Though there were slight depressions of agglutination, haemolysin and 2MER titres as a result of malaria infection, they were not as significantly different as the previous experiment.

Station	Time	Temp	Wind	Pressure	Humidity	Clouds	Remarks
1	0800	75	10	30.0	70	100	
2	0900	76	12	30.0	72	100	
3	1000	77	15	30.0	75	100	
4	1100	78	18	30.0	78	100	
5	1200	79	20	30.0	80	100	
6	1300	80	22	30.0	82	100	
7	1400	81	25	30.0	85	100	
8	1500	82	28	30.0	88	100	
9	1600	83	30	30.0	90	100	
10	1700	84	32	30.0	92	100	
11	1800	85	35	30.0	95	100	
12	1900	86	38	30.0	98	100	
13	2000	87	40	30.0	100	100	
14	2100	88	42	30.0	100	100	
15	2200	89	45	30.0	100	100	
16	2300	90	48	30.0	100	100	
17	0000	91	50	30.0	100	100	
18	0100	92	52	30.0	100	100	
19	0200	93	55	30.0	100	100	
20	0300	94	58	30.0	100	100	
21	0400	95	60	30.0	100	100	
22	0500	96	62	30.0	100	100	
23	0600	97	65	30.0	100	100	
24	0700	98	68	30.0	100	100	
25	0800	99	70	30.0	100	100	
26	0900	100	72	30.0	100	100	
27	1000	101	75	30.0	100	100	
28	1100	102	78	30.0	100	100	
29	1200	103	80	30.0	100	100	
30	1300	104	82	30.0	100	100	
31	1400	105	85	30.0	100	100	
32	1500	106	88	30.0	100	100	
33	1600	107	90	30.0	100	100	
34	1700	108	92	30.0	100	100	
35	1800	109	95	30.0	100	100	
36	1900	110	98	30.0	100	100	
37	2000	111	100	30.0	100	100	
38	2100	112	102	30.0	100	100	
39	2200	113	105	30.0	100	100	
40	2300	114	108	30.0	100	100	
41	0000	115	110	30.0	100	100	
42	0100	116	112	30.0	100	100	
43	0200	117	115	30.0	100	100	
44	0300	118	118	30.0	100	100	
45	0400	119	120	30.0	100	100	
46	0500	120	122	30.0	100	100	
47	0600	121	125	30.0	100	100	
48	0700	122	128	30.0	100	100	
49	0800	123	130	30.0	100	100	
50	0900	124	132	30.0	100	100	

Date of observation: 10/10/1910

Table VII Statistical analysis of the data in Fig 12 (n=5)

Test	Group	Day Data	4	8	11	14	18	22	25	28	32	46	63
Agglutination titre	Control	Mean SD <sup>+</sup>	5.2 0.5	9.3 0.7	8.8 0.5	10.2 1.3	9.8 1.1	8.8 1.1	8.6 1.1	9.3 1.5	6.7 1.3	6.3 0.6	4.8 0.8
	Malaria infected	Mean SD <sup>-</sup>	3.6 0.6	8.6 0.9	8.6 1.5	8.0 1.0	7.6 1.1	7.2 1.3	7.0 1.0	7.0 0.7	6.4 1.1	5.6 1.9	3.8 0.6
	Significance	P <	0.02	NS	NS	0.02	0.02	NS	0.05	0.02	NS	NS	NS
Lysin titre	Control	Mean SD <sup>+</sup>	7.2 0.8	9.8 0.8	7.2 0.5	7.0 2.0	8.0 1.0	7.8 1.3	8.0 1.2	7.0 1.4	6.5 1.3	5.6 0.6	3.2 0.5
	Malaria infected	Mean SD <sup>-</sup>	5.0 1.0	7.8 1.9	8.0 1.2	5.8 0.8	6.4 1.1	5.8 0.8	5.4 0.9	5.6 0.9	5.4 1.1	5.0 1.2	2.8 0.6
	Significance	P <	0.01	NS	NS	NS	0.05	0.02	0.01	NS	NS	NS	NS
ME resistant titre	Control	Mean SD <sup>+</sup>	0.0 0.0	8.8 0.8	7.8 1.3	9.0 1.9	8.0 1.0	8.0 0.7	8.6 1.1	9.3 1.5	5.5 0.6	6.0 0.0	4.6 0.9
	Malaria infected	Mean SD <sup>-</sup>	0.0 0.0	8.4 0.9	7.2 1.3	8.2 3.1	7.6 1.1	6.6 1.1	7.0 1.0	6.6 1.1	4.8 1.5	5.4 1.1	3.8 0.6
	Significance	P <	0.0	NS	NS	NS	NS	0.05	0.05	0.02	NS	NS	NS
ME sensitive titre	Control	Mean SD <sup>+</sup>	5.2 0.5	0.6 0.8	1.0 1.0	1.2 1.1	0.0 0.0	0.8 0.5	0.0 0.0	0.0 0.0	1.3 1.0	0.3 0.6	0.2 0.5
	Malaria infected	Mean SD <sup>-</sup>	3.6 0.6	0.2 0.5	1.4 0.6	0.8 1.3	0.0 0.0	0.6 0.6	0.0 0.0	0.4 0.6	1.6 0.6	0.2 .5	0.0 0.0
	Significance	P <	0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*Significance between the means of control and experimental group using student T test.

NS = not significant



FIGURE 12

FIGURE 12  $\text{Log}_2$  antibody titres to sheep red-blood cells given at day 1 of *P. chabaudi* infection

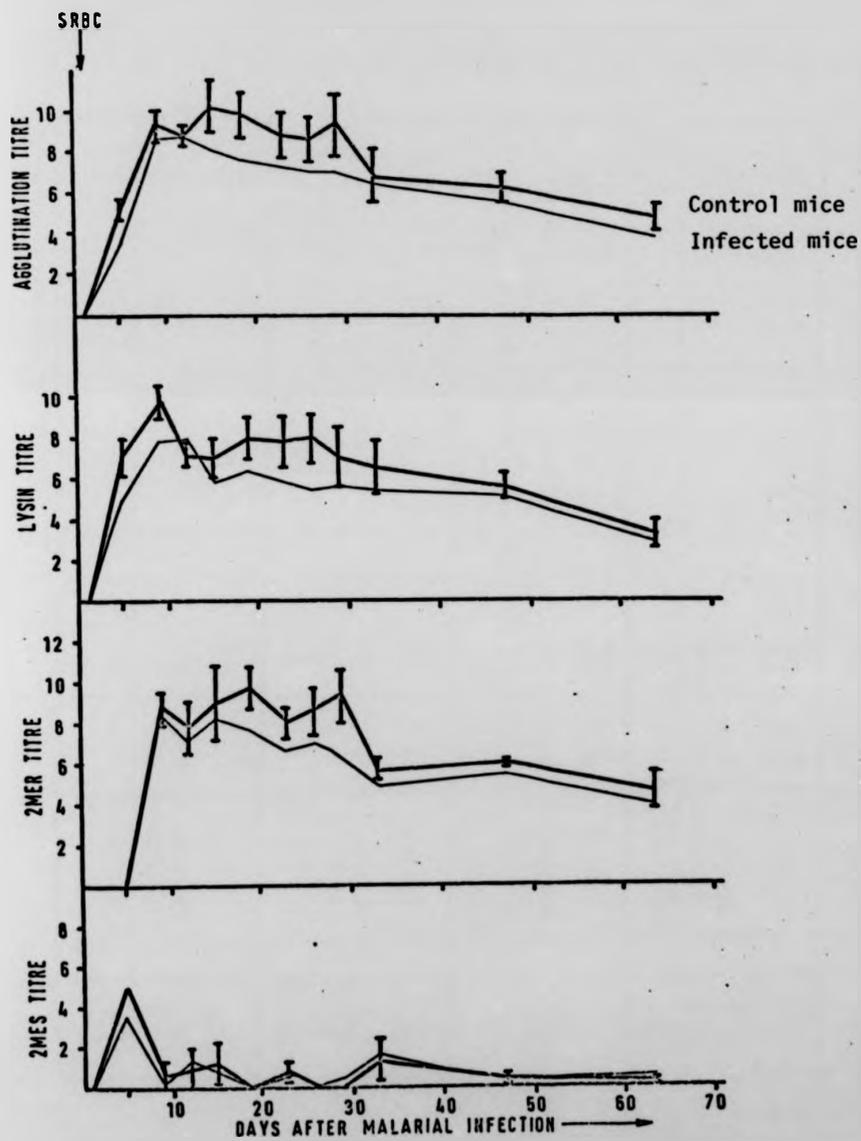


Fig. 10. The amount of water vapor in the air (in grams per cubic meter) during the day on the 10th of June 1922.

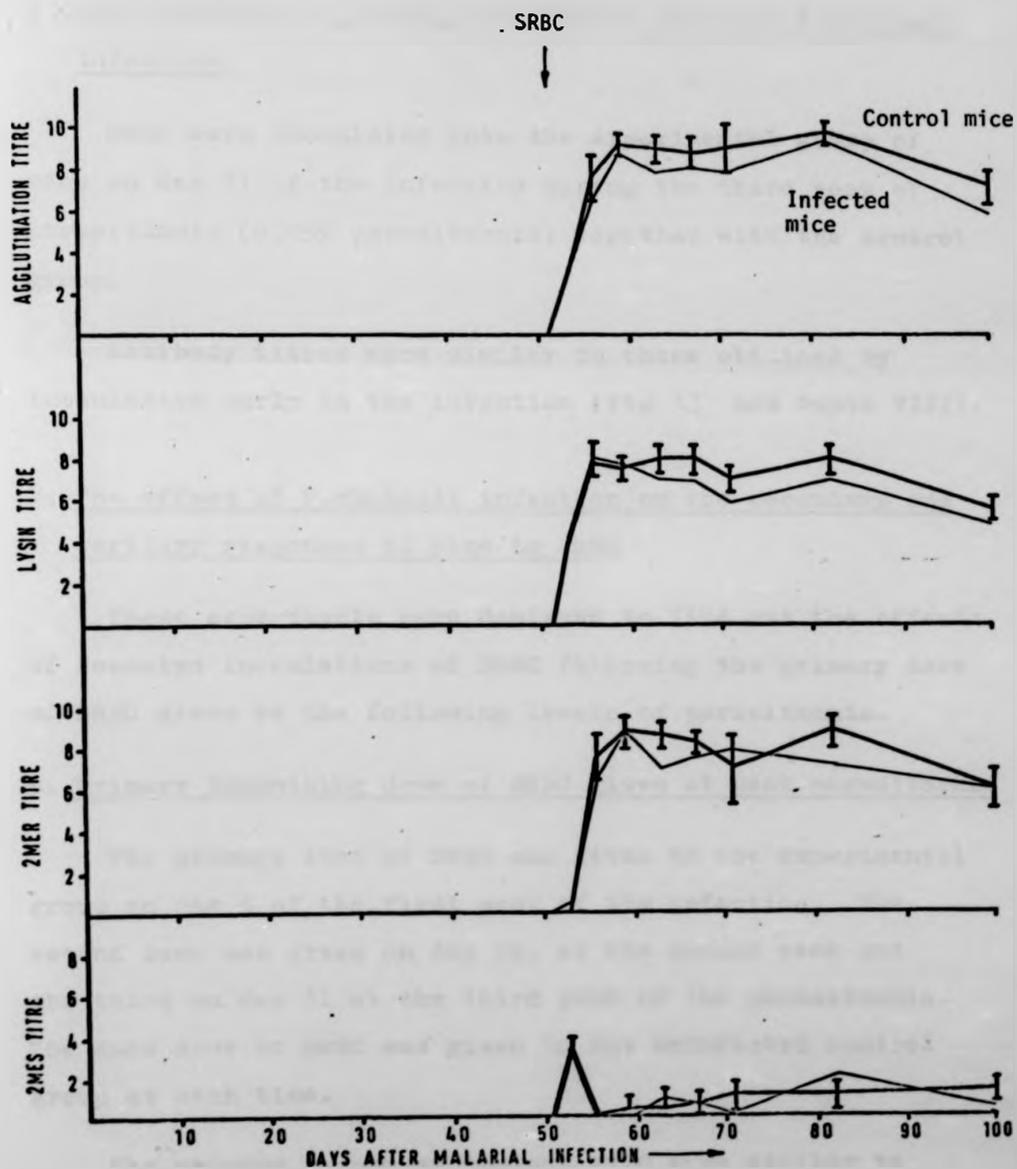


Table VIII Statistical analysis of the data in Fig 13 (n=5)

Test	Group	Day Data	53	56	59	63	67	71	82	101
			Agglutination titre	Control	Mean SD -	3.2 0.5	7.6 1.1	9.2 0.5	9.0 0.7	8.8 0.8
Malaria infected	Mean SD -	3.2 0.5		6.6 1.5	8.8 0.5	7.8 0.5	8.0 0.8	7.8 0.5	9.3 1.0	5.8 1.0
Significance	P <	NS		NS	NS	0.02	NS	NS	NS	0.05
Lysin titre	Control	Mean SD -	4.0 0.0	7.8 0.8	7.6 0.6	8.0 0.7	8.0 0.7	8.4 0.6	8.0 0.7	5.6 0.6
	Malaria infected	Mean SD -	4.0 0.0	8.0 1.2	7.8 1.0	7.0 0.0	7.0 0.8	5.8 3.3	7.0 0.8	4.8 1.0
	Significance	P <	NS	NS	0.02	NS	NS	NS	NS	NS
ME resistant titre	Control	Mean SD -	0.0 0.0	7.6 1.1	8.8 0.8	8.6 0.6	8.2 0.5	7.0 2.7	8.8 0.8	6.0 1.0
	Malaria infected	Mean SD -	0.4 0.9	6.6 1.5	8.8 0.5	7.0 1.4	7.5 1.0	7.8 0.5	7.0 1.4	5.8 1.0
	Significance	P <	NS	NS	NS	NS	NS	NS	0.05	NS
ME sensitive titre	Control	Mean SD -	3.2 0.5	0.0 0.0	0.4 0.6	0.4 0.9	0.6 0.6	1.0 0.7	1.0 0.7	1.2 0.5
	Malaria infection	Mean SD -	3.2 0.5	0.0 0.0	0.0 0.0	0.8 1.0	0.5 0.6	0.0 0.0	2.0 0.5	0.3 0.5
	Significance	P <	NS	NS	NS	NS	NS	0.01	NS	0.05

\*see table VI

FIGURE 13  $\text{Log}_2$  antibody titres to sheep red-blood cells given during the chronic phase of *P. chabaudi* infection



The 2MES titre was too low in both the groups to point out any significant difference between the groups.

c. SRBC inoculation during the chronic phase of P.chabaudi infection

SRBC were inoculated into the experimental group of mice on day 51 of the infection during the third peak of parasitaemia (0.05% parasitaemia) together with the control group.

Antibody titres were similar to those obtained by inoculation early in the infection (fig 13 and table VIII).

2. The effect of P.chabaudi infection on the secondary and tertiary responses of mice to SRBC

These experiments were designed to find out the effects of repeated inoculations of SRBC following the primary dose of SRBC given at the following levels of parasitaemia.

a. Primary immunizing dose of SRBC given at peak parasitaemia

The primary dose of SRBC was given to the experimental group on day 5 of the first peak of the infection. The second dose was given on day 29, at the second peak and the third on day 51 at the third peak of the parasitaemia. The same dose of SRBC was given to the uninfected control group at each time.

The primary responses against SRBC were similar to those of the previous experiment (Exp B. 1a) i.e. there

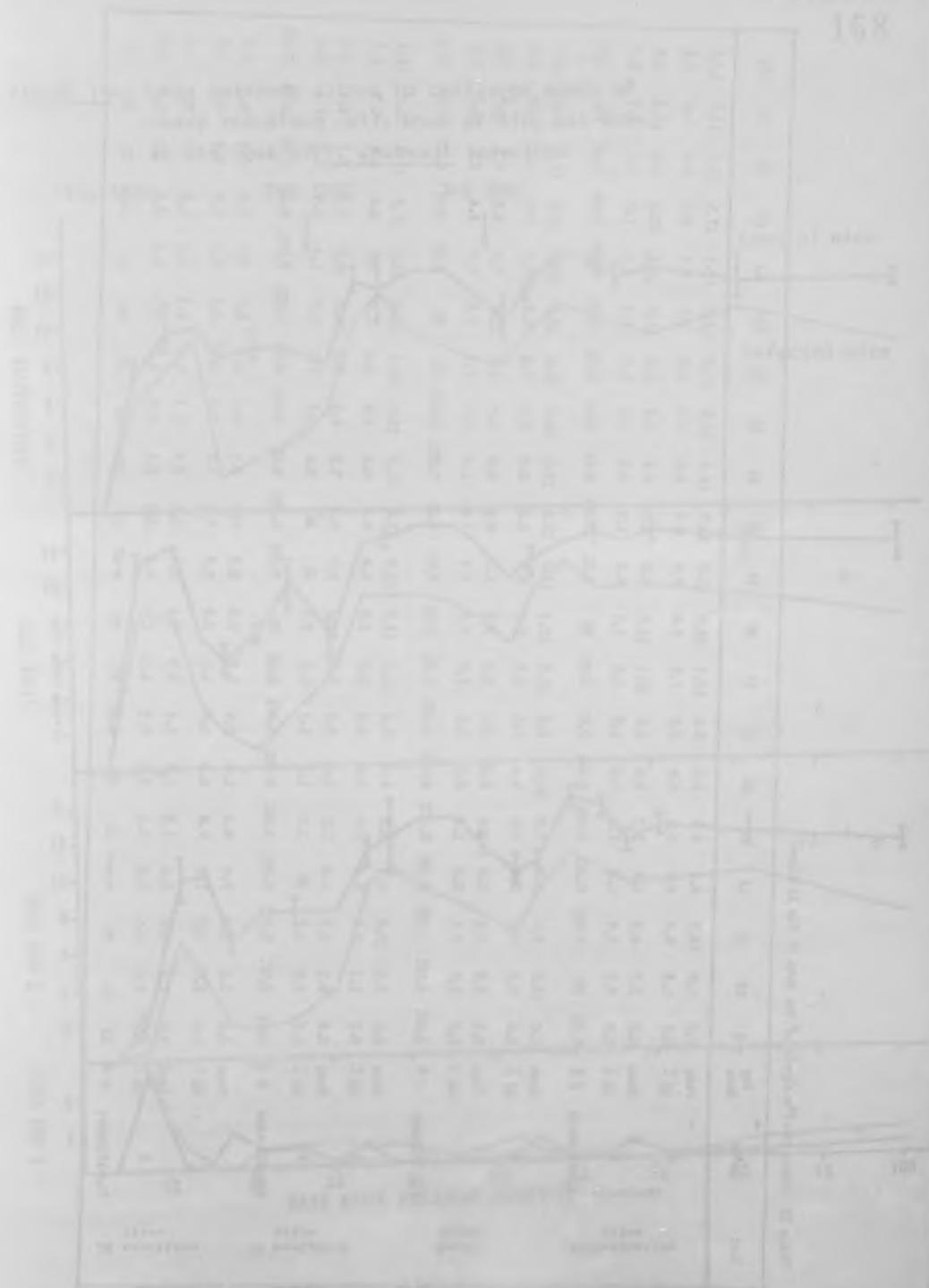
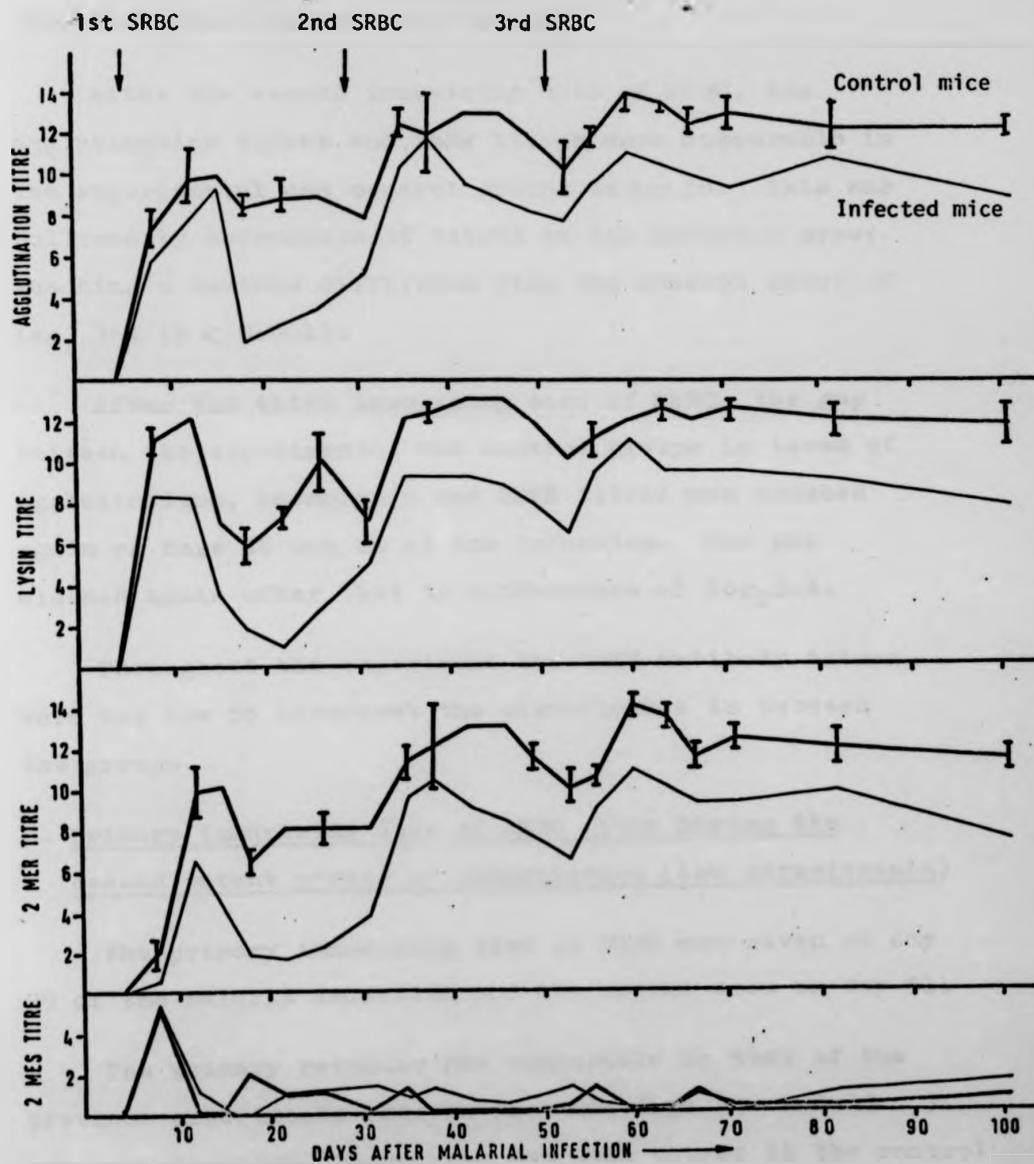


Table IX Statistical analysis of the data in Fig 14 a(n=6)

Test	Group	Day Data	8	12	15	18	22	26	31	35	38	42	46	49	53	56	60	64	67	71	82	102	
Agglutination titre	A	Mean	7.2	9.8	10.0	8.4	8.8	9.0	8.0	12.5	12.0	13.0	13.0	12.0	10.3	11.8	14.0	13.5	12.5	13.0	12.3	12.3	
		SD ±	0.8	1.3	0.0	0.6	0.8	0.0	0.0	0.6	2.0	0.0	0.0	0.0	1.3	0.5	1.0	0.6	0.6	0.8	1.0	0.5	
	B	Mean	5.8	7.7	4.3	1.8	2.7	3.4	5.2	10.4	11.8	9.6	8.8	8.3	7.8	9.5	11.0	10.5	10.0	9.3	10.8	9.8	
		SD ±	0.8	2.3	1.0	1.0	1.4	1.5	1.8	1.8	1.6	2.2	1.7	2.2	1.7	1.7	1.4	1.3	1.2	2.5	1.7	2.0	
	Significance	P <	0.02	NS	0.001	0.001	0.001	0.001	0.01	0.05	NS	0.01	0.001	0.01	0.02	0.01	0.01	0.001	0.02	0.01	NS	0.01	
	Lysin titre	A	Mean	11.0	12.0	7.0	5.8	7.2	10.0	9.0	12.0	12.3	13.0	13.0	12.0	10.0	11.0	12.0	12.8	12.0	12.5	12.0	11.0
SD ±			0.6	0.0	0.0	0.8	0.5	1.4	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.8	0.0	0.5	0.0	0.5	0.8	1.0	
B		Mean	8.3	8.2	3.5	1.8	1.0	2.8	5.0	8.2	9.2	8.2	8.8	8.0	6.5	9.5	11.0	9.5	9.5	9.5	9.0	7.8	
		SD ±	0.8	1.2	0.6	0.8	1.6	0.5	1.2	2.2	2.3	2.3	2.5	1.6	1.7	1.7	1.6	1.7	2.4	2.4	2.7	1.5	
Significance		P <	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.01	0.01	0.01	0.01	0.001	0.001	NS	NS	0.01	0.05	0.02	0.05	0.001
HE resistant titre		A	Mean	2.0	9.8	10.0	6.4	8.0	8.0	8.0	11.2	12.0	13.0	13.0	11.5	10.0	10.5	14.0	13.5	11.5	12.5	12.0	11.5
	SD ±		0.7	1.3	0.0	0.6	0.0	0.8	0.0	0.8	2.0	0.0	0.0	0.6	0.8	0.6	1.0	0.6	0.6	0.6	0.8	0.6	
	B	Mean	0.6	6.5	4.2	1.8	1.7	2.2	3.8	9.6	10.4	9.0	8.3	7.5	6.5	9.0	10.8	10.0	9.3	9.3	9.8	7.3	
		SD ±	0.6	0.8	1.2	1.0	1.2	1.1	1.9	1.5	2.1	2.1	2.1	2.4	1.3	0.8	1.0	1.8	1.5	2.5	2.2	1.5	
	Significance	P <	0.01	0.001	0.001	0.001	0.001	0.001	0.001	0.05	NS	0.001	0.001	0.01	0.001	0.01	0.001	0.001	0.02	0.02	0.05	0.001	
	HE sensitive titre	A	Mean	5.2	0.0	0.0	2.0	0.8	1.0	0.0	1.2	0.0	0.0	0.0	0.5	0.3	1.3	0.0	0.0	1.0	0.3	0.3	0.8
SD ±			0.5	0.0	0.0	1.0	0.8	0.8	0.0	1.1	0.0	0.0	0.0	0.6	0.5	0.5	0.0	0.0	1.2	0.5	0.8	0.5	
B		Mean	5.3	1.2	0.2	0.0	1.0	1.2	1.4	0.8	1.4	0.6	0.5	0.8	1.3	0.5	0.2	0.3	1.0	0.0	1.0	1.5	
		SD ±	0.8	2.0	0.5	0.0	0.6	0.8	0.9	1.3	1.5	0.6	0.6	0.5	1.0	1.0	0.5	0.5	0.8	0.0	1.4	1.3	
Significance		P <	NS	NS	NS	0.001	NS	NS	0.01	NS	NS	0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

\*see table VI

FIGURE 14a  $\log_2$  antibody titres to challenge doses of sheep red-blood cells when priming was done at the peak of *P. chabaudi* infection



was significant depression of agglutination titres, haemolysin titres and 2MER titres in the infected group ( $P < 0.001$ ) especially between days 15-26 after malaria infection (fig 14a and table IX ).

After the second immunizing dose of SRBC, the agglutination titres and 2MER titres were comparable in the experimental and control groups on day 38. This was followed by depression of titres in the infected group reaching a maximum difference from the control group of  $\log_2 3-4$  ( $P < 0.001$ ).

After the third immunizing dose of SRBC, the gap between the experimental and control groups in terms of agglutination, haemolysin and 2MER titres was reduced again on days 56 and 60 of the infection. The gap widened again after that to differences of  $\log_2 3-4$ .

Throughout the experiment the 2MES antibody titres were too low to interpret the significance in between the groups.

b. Primary immunizing dose of SRBC given during the second patent period of parasitaemia (low parasitaemia)

The primary immunizing dose of SRBC was given on day 29 of the malaria infection and the second dose on day 51.

The primary response was comparable to that of the previous experiments (see B. 1b,c and figs 12 and 13 ). The agglutination, haemolysin and 2MER titres in the control and the experimental groups were not different on days 31 and 35 (fig 14b and table X ). Later these titres

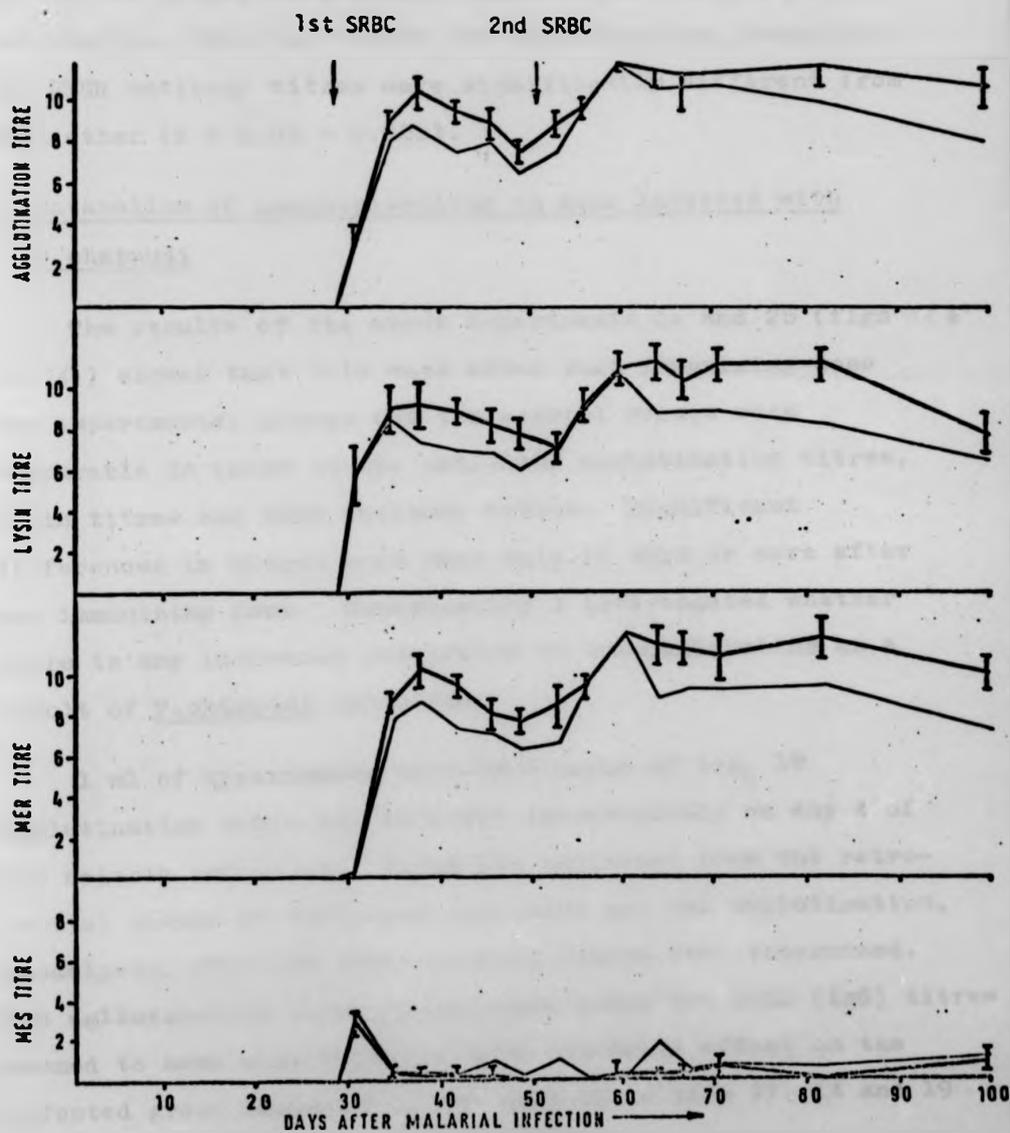
Date	Description	Debit	Credit	Balance
1880				
Jan 1	Balance			
Jan 15	...			
Jan 30	...			
Feb 15	...			
Feb 28	...			
Mar 15	...			
Mar 31	...			
Apr 15	...			
Apr 30	...			
May 15	...			
May 31	...			
Jun 15	...			
Jun 30	...			
Jul 15	...			
Jul 31	...			
Aug 15	...			
Aug 31	...			
Sep 15	...			
Sep 30	...			
Oct 15	...			
Oct 31	...			
Nov 15	...			
Nov 30	...			
Dec 15	...			
Dec 31	...			

Table X Statistical analysis of the data in Fig 14b (n=6)

Test	Group	Day														
		Data	31	35	38	42	46	49	53	56	60	64	67	71	82	101
Agglutination titre	Control	Mean	2.8	9.0	10.4	9.6	9.0	7.6	8.5	9.8	12.0	11.6	11.4	11.4	11.8	10.8
		SD	0.5	1.0	0.9	0.6	0.7	0.6	1.0	0.8	0.5	1.1	1.1	1.3	1.0	1.0
	Malaria infected	Mean	3.5	8.0	8.8	7.5	8.0	6.5	7.6	9.7	12.0	10.7	9.8	9.8	10.0	8.2
		SD	1.2	1.3	1.3	0.8	0.9	0.6	0.6	0.5	0.0	0.8	1.2	1.2	0.7	0.8
	Significance	P <	NS	NS	0.05	0.001	NS	0.02	NS	NS	NS	NS	0.05	NS	0.01	0.001
Lysin titre	Control	Mean	5.8	9.0	9.2	8.8	8.2	7.8	7.0	9.2	11.0	11.4	10.4	11.2	11.2	7.8
		SD	0.5	0.0	0.8	0.8	0.8	0.5	1.4	0.5	0.8	0.9	0.9	0.8	0.8	1.0
	Malaria infected	Mean	5.5	8.5	7.4	7.0	6.8	6.0	7.2	9.0	11.0	10.7	8.8	8.8	8.2	6.4
		SD	1.4	1.1	1.1	0.6	0.8	0.6	0.8	0.6	0.8	1.4	1.2	0.8	1.3	0.9
	Significance	P <	NS	NS	0.02	0.01	0.02	0.001	NS	NS	NS	NS	0.05	0.001	0.001	0.05
ME resistant titre	Control	Mean	0.0	8.6	10.7	9.4	8.8	7.6	8.5	9.4	12.0	11.4	11.2	10.8	11.8	10.0
		SD	0.0	0.6	0.8	0.6	0.8	0.6	1.0	0.6	0.0	0.9	0.8	1.1	1.0	0.8
	Malaria infected	Mean	0.2	7.8	8.6	7.3	7.0	6.3	6.6	9.3	12.0	8.8	9.2	9.2	9.4	7.2
		SD	0.4	1.2	1.5	1.0	0.6	0.8	0.6	0.8	0.0	0.8	1.5	1.0	1.3	0.8
	Significance	P <	NS	NS	0.02	0.01	0.01	0.02	0.01	NS	NS	0.001	0.02	0.05	0.01	0.001
ME sensitive titre	Control	Mean	2.8	0.4	0.2	0.2	0.2	0.0	0.0	0.4	0.0	0.2	0.2	0.6	0.0	0.8
		SD	0.5	0.6	0.5	0.6	0.5	0.0	0.0	0.6	0.0	0.5	0.5	0.6	0.0	0.5
	Malaria infected	Mean	3.3	0.2	0.2	0.2	1.0	0.2	1.0	0.2	0.0	1.8	0.5	0.7	0.6	1.0
		SD	0.8	0.6	0.6	0.6	0.6	0.4	0.0	0.4	0.0	1.3	0.6	0.5	0.9	0.7
	Significance	P <	NS	NS	NS	NS	NS	NS	0.001	NS	NS	0.02	NS	NS	NS	NS

\* see table VI

**FIGURE 14b**  $\text{Log}_2$  antibody titres to a challenge dose of sheep red-blood cells when priming was done at low *P. chabaudi* parasitaemias



reduced more in the infected group and became significantly different from the control group on day 49 ( $P < 0.02 - 0.001$ ).

After the second immunizing dose the antibody titres in the two groups were almost identical on days 56 and 60 but starting from day 71-101 the agglutination, haemolysin and 2MER antibody titres were significantly different from each other ( $P < 0.05 - 0.001$ ).

### 3. Catabolism of immunoglobulins in mice infected with P.chabaudi

The results of the above experiments 2a and 2b (figs 14a and 14b) showed that 7-10 days after each immunizing dose the experimental groups and the control groups were comparable in terms of the anti-SRBC agglutination titres, lysin titres and 2MER antibody titres. Significant differences in titres were seen only 10 days or more after the immunizing dose. Consequently I investigated whether there is any increased catabolism of immunoglobulins as a result of P.chabaudi infection.

1 ml of hyperimmune anti-SRBC serum of  $\log_2 18$  agglutination titre was injected intravenously on day 4 of the malaria infection. Serum was collected from the retro-orbital plexus at different intervals and the agglutination, haemolysin, 2MER and 2MES antibody titres were determined. The agglutination titre, haemolysin titre and 2MER (IgG) titres seemed to have significantly more catabolic effect on the infected group compared to the control on days 11, 14 and 19 ( $P < 0.05-0.001$ ) (fig 15 and table XI).

FIGURE 2. The effect of ...  
 ...  
 ...



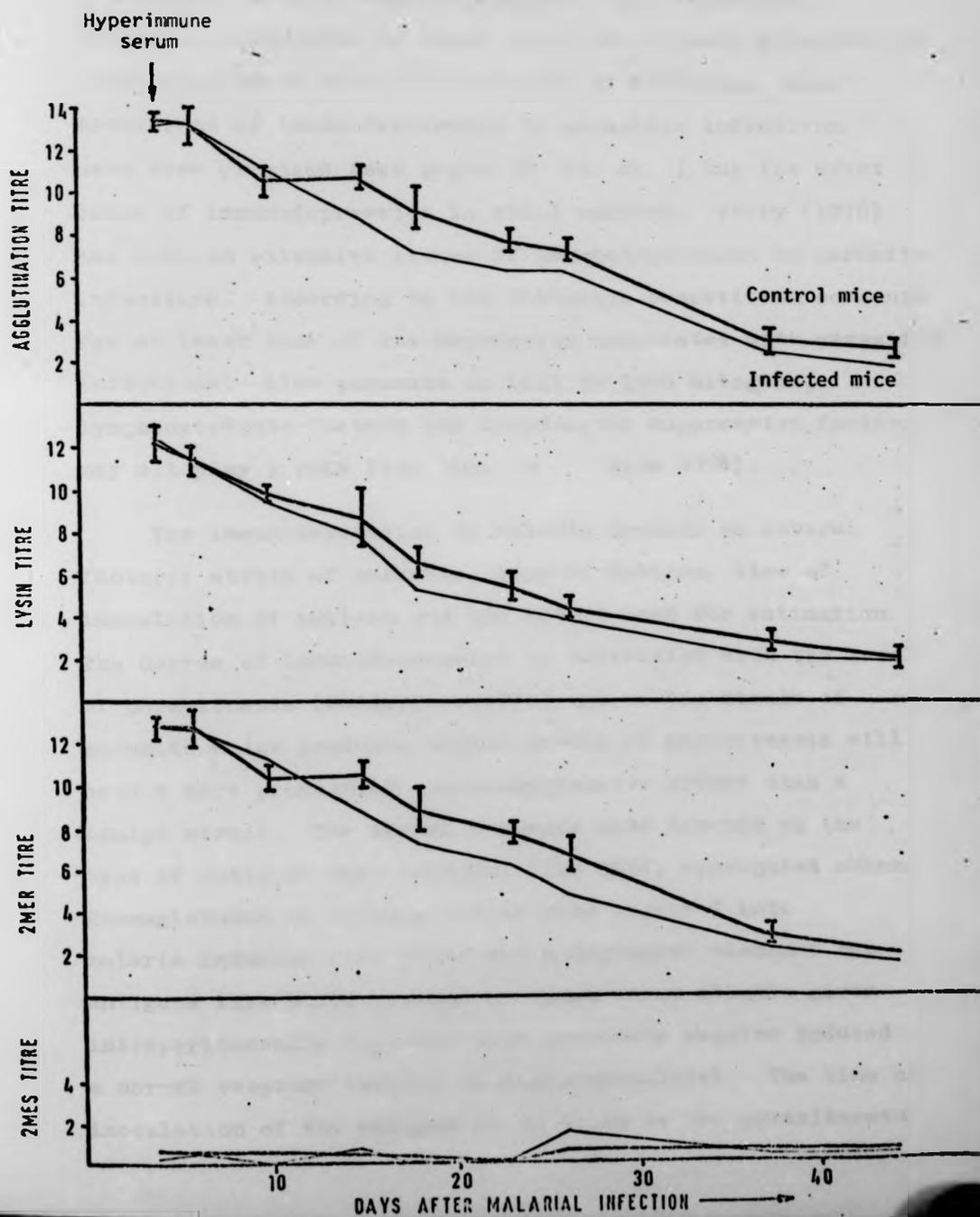
FIGURE 2. The effect of ...  
 ...  
 ...

Table XI Statistical analysis of the data in Fig 15 (n=6)

Test	Group	Day	0	2	6	11	14	19	22	33	40
		Data									
Agglutination titre	Control	Mean	13.3	13.2	10.5	10.7	9.2	7.7	7.3	3.0	2.7
		SD	0.5	1.0	0.8	0.5	1.0	0.5	0.5	0.6	0.5
	Malaria infected	Mean	13.0	13.2	11.0	8.8	7.2	6.3	6.3	2.5	1.8
		SD	0.7	0.4	0.6	0.4	1.0	0.5	0.5	0.5	0.2
Significance	P <	NS	NS	NS	0.001	0.01	0.01	0.01	NS	0.01	
Lysin titre	Control	Mean	12.2	11.4	9.8	8.7	6.5	5.4	4.3	2.8	2.0
		SD	0.8	0.6	0.4	0.6	0.6	0.6	0.5	0.5	0.0
	Malaria infected	Mean	12.4	11.4	9.4	7.8	5.3	4.3	3.8	2.0	1.8
		SD	0.9	0.6	0.6	0.4	0.5	0.5	0.2	0.6	0.8
Significance	P <	NS	NS	NS	0.05	0.01	0.02	NS	NS	NS	
ME resistant titre	Control	Mean	12.8	12.8	10.3	10.5	8.8	7.7	6.7	2.8	2.0
		SD	0.4	0.8	0.5	0.6	1.0	0.5	0.8	0.5	0.0
	Malaria infected	Mean	12.8	12.7	11.3	8.8	7.2	6.2	4.8	2.2	1.5
		SD	0.5	0.5	0.8	0.4	1.0	0.4	0.8	0.5	0.6
Significance	P <	NS	NS	NS	0.05	0.05	0.05	0.01	NS	NS	
ME sensitive titre	Control	Mean	0.5	0.3	0.5	0.2	0.3	0.0	0.7	0.3	0.5
		SD	0.6	0.5	0.6	0.4	0.8	0.0	0.6	0.5	0.6
	Malaria infected	Mean	0.2	0.5	0.0	0.7	0.0	0.2	1.5	0.3	0.3
		SD	0.5	0.6	0.0	0.5	0.0	0.4	0.6	0.5	0.5
Significance	P <	NS	NS	NS	NS	NS	NS	0.05	NS	NS	

\* see table VI

FIGURE 15 The effect of *P. chabaudi* infection on the catabolism of passively transferred antibody to SRBC injected on day 4 of *P. chabaudi* infection (titres expressed as  $\log_2$ )

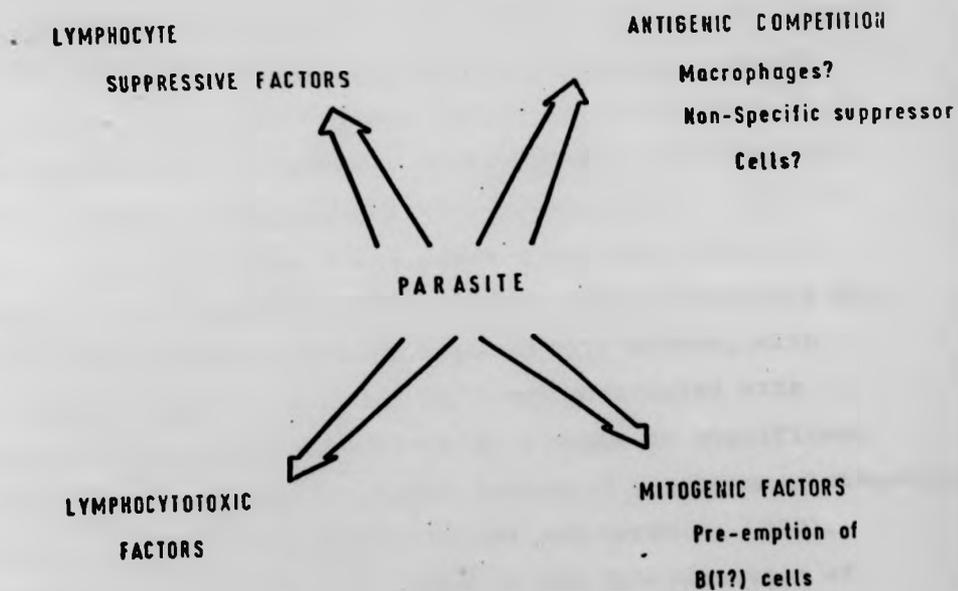


DISCUSSION

Antibody production is the result of the co-operation of T-cells, B-cells and macrophages. Any depletion, damage or inhibition of these cells or of their co-operation will bring about reduced production of antibody. Many mechanisms of immunodepression in parasitic infections have been proposed (see pages 38 to 42 ) but the exact cause of immunodepression is still unknown. Terry (1978) has made an extensive review of immunodepression in parasitic infections. According to him antigenic competition accounts for at least some of the depression associated with parasitic infections. Also parasite or host derived mitogens, lymphocytotoxic factors and lymphocytic suppressive factors may all play a role (see fig. 16 page 178).

The immunodepression in malaria depends on several factors; strain of parasite, type of antigen, time of inoculation of antigen and the method used for estimation. The degree of immunodepression is correlated with the level of parasitaemia (Wedderburn, 1974) and so the strain of parasite which produces higher levels of parasitaemia will have a more pronounced immunosuppressive effect than a benign strain. The immune response also depends on the type of antigen; when antigens like SRBC, aggregated human gammaglobulin or tetanus toxoid were injected into malaria infected mice there was a depressed response but antigens like bacteriophage or human serum albumin given intraperitoneally together with pertussis vaccine induced a normal response (review by Wedderburn, 1974). The time of inoculation of the antigen in relation to the parasitaemia

**FIGURE 16** Possible mechanisms of parasite-associated immunodepression (Terry, 1978)



level of the malaria infection played a major role in depression of the level of the immune response (Whitmore, 1974). SRBC given in the early part of the malaria infection enhanced the PFC response rather than depressing it and then the switch from enhancement to depression occurred rapidly (Poels and Van Niekerk, 1977). The spleen plaque assay method was used by the majority of workers (Salaman et al., 1969; Wedderburn, 1974; and Whitmore, 1974). Weidanz and Rank (1975) demonstrated a severely depressed splenic antibody response to SRBC and that this had little effect on antibody formation in the lymph nodes during P.yoelii infection in mice. The spleen plaque assay thus does not seem to reflect the degree of immunity elsewhere in the body. In my experiments, the method of estimation of the immunodepressive effects of P.chabaudi infection was by measurement of agglutination and haemolysin titres to SRBC. 2-mercaptoethanol was added after recording the endpoints of the agglutination titre, and resuspending the agglutinated cells. A comparison of this method, with existing procedures in which sera are pretreated with mercaptoethanol, has been shown to produce no significant differences in the MR-resistant titres of the haemagglutination response to several antigens (Scott and Gershon, 1970). The same method has been applied in the determination of the response to SRBC in P.yoelii infected mice by Greenwood et al. (1971a).

The immunodepressive effects of P.chabaudi infection on the primary immune response to SRBC gave the expected results. The degree of suppression depended on the time when antigen was injected relative to infection, the time

at which the response was measured and the class of antibody estimated as discussed earlier. When antigen (SRBC) was given at the height of the parasitaemia (expt. B la p 158), there was a significant depression of agglutination, haemolysin and 2 ME-resistant (IgG) titres, and this depression was still present 70 days after infection. The titres of malaria infected and control groups were very significantly different 15-35 days after infection, but less so between days 35 and 70 (fig 11 ).

When SRBC were given at periods of low parasitaemia either early or during the chronic phase of the infection, the agglutination, haemolysin and 2-ME-resistant titres were generally lower in the infected group than in the controls (figs 12 and 13 ), though they were not reduced to the same extent as when SRBC were given during periods of high parasitaemia (fig 11 p 160). I found no enhancement of IgM (ME sensitive) titres in the early stages of malaria infection like Poels and Niekirk (1977) who demonstrated increased splenic plaques at this stage. Also my result differed from that of Hudson *et al.* (1976) who found increased IgM splenic PFCs in early T. brucei infections in mice. The method I used may not have been sufficiently sensitive to detect such differences. The background agglutination and haemolysin titres of unchallenged and uninfected as well as malaria infected groups were very low ( $\log_2 0 - \log_2 1$ ) throughout the experiment and so were taken as 0.

The effect of P. chabaudi infection on the secondary and tertiary responses of mice to SRBC depended on the

timing of the initial priming. When the priming with SRBC was done at the peak of parasitaemia (5 days after infection), the primary response was markedly suppressed (fig 14 a p 168), but when another challenge of SRBC (secondary) was given, the initial antibody response was comparable with that of the controls for 7-10 days. Immunodepression became pronounced only after 10 days (fig 14 a p 168). Tertiary responses to a further challenge were similar. When priming was done at a low level of parasitaemia and was followed by a secondary challenge during the chronic stage of malaria, the secondary response in the infected group again matched that of the normal group for 7-10 days. A minor degree of immunodepression was then seen (fig 14 b p 171).

Salaman et al. (1969) have shown that mice in which the primary response was much depressed, by giving SRBC at the peak of P.yoelii infection, responded vigorously to a second dose of SRBC (estimated by the 4 day spleen plaque assay). This result agreed with my result for the period between 7-10 days after the second or third challenge. Subsequent immunodepression following 10 days after the secondary response was comparable with that of Whitmore (1974) who showed that the suppression of IgG and IgM splenic PFCs of the primary response to SRBC by mice previously infected with P.berghei resulted in a diminished response to subsequent challenge with this antigen. Greenwood et al. (1971a) also primed mice infected with P.yoelii infection at the peak of parasitaemia and followed with a secondary dose of SRBC one month later and found a depressed primary type of response with high IgM (ME sensitive

titres). They interpreted the results by measuring the SRBC antibody titres only at one point, 7 days after the SRBC injection. According to their results the total agglutination titres differed by  $\log_2 5.2$  in the primary response and by only  $\log_2 2$  in the secondary responses between the malaria infected groups and the uninfected controls. These results agree with my data (fig 14 a p 168). This shows that memory to the primary infection was not impaired.

Initially comparable levels of antibody occurred against SRBC after the primary or secondary challenges in both infected and control mice, but subsequent lower levels in the infected group made me suspect that there was increased catabolism of SRBC antibodies associated with malaria infection (fig 14 a p 168). But this was not clearly shown in a preliminary passive transfer of hyperimmune serum against SRBC from uninfected donor mice to recipient mice 5 days after infection with malaria. However this should be clarified by further passive transfer experiments transferring the hyperimmune serum to such mice at various times after malaria infection. In addition hyperimmune serum against SRBC raised in malaria infected and uninfected mice could be passively transferred to uninfected recipients and the relative rates of catabolism assessed as shown in expt B 3 p 180 , in order to assess avidity.



Table XIIA Statistical analysis of the data in Fig. 17 (n=5)

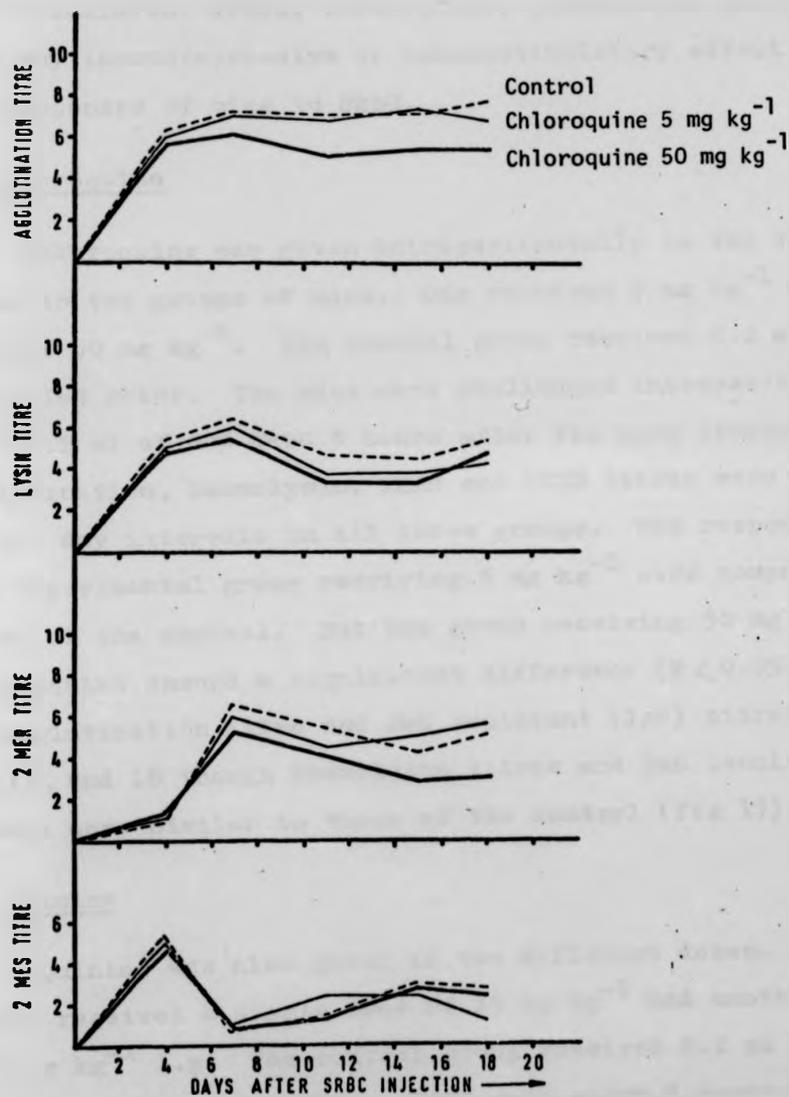
Test	Group	Day					
		Data	4	7	11	15	18
Agglutination titre	Control	Mean SD -	6.3 0.5	7.2 0.9	7.0 0.7	7.2 1.3	7.8 1.3
	Chloroquine 5 mg/kg Significance	Mean SD - P <	6.0 0.7 NS	7.0 0.7 NS	6.7 0.8 NS	7.4 0.6 NS	6.8 1.1 NS
Lysin titre	Control	Mean SD -	5.4 0.6	6.4 0.6	4.6 0.6	4.4 0.9	5.4 0.6
	Chloroquine 5 mg/kg Significance	Mean SD - P <	5.0 0 NS	6.0 0 NS	3.7 0.6 NS	3.8 0.5 NS	4.2 1.1 NS
ME resistant titre	Control	Mean SD -	0.9 0.2	7.2 0.9	5.4 0.6	4.2 0.5	5.0 0.7
	Chloroquine 5 mg/kg Significance	Mean SD - P <	1.0 0.3 NS	7.0 0.7 NS	4.4 0.6 NS	5.0 0.7 NS	5.6 0.6 NS
ME sensitive titre	Control	Mean SD -	5.4 0.5	0.0 0.0	1.6 1.1	3.0 1.0	2.8 1.1
	Chloroquine 5 mg/kg Significance	Mean SD - P <	5.0 0.7 NS	0.0 0.0 NS	2.4 0.5 NS	2.4 0.6 NS	1.2 1.3 NS

Table XIIB Statistical analysis of the data in Fig. 17 (n=5)

Test	Group	Day					
		Data	4	7	11	15	18
Agglutination titre	Control	Mean SD -	6.3 0.5	7.2 0.9	7.0 0.7	7.2 1.3	7.8 1.3
	Chloroquine 50 mg/kg Significance	Mean SD - P <	5.9 0.6 NS	6.2 0.8 NS	5.2 1.6 0.05	5.4 1.1 0.05	5.4 1.4 0.05
Lysin titre	Control	Mean SD -	5.4 0.6	6.4 0.6	4.6 0.6	4.4 0.9	5.4 0.6
	Chloroquine 50 mg/kg Significance	Mean SD - P <	4.8 0.8 NS	5.4 0.6 0.05	3.3 1.7 NS	3.2 1.3 NS	4.6 0.9 NS
ME resistant titre	Control	Mean SD -	0.9 0.2	7.2 0.9	5.4 0.6	4.2 0.5	5.0 0.7
	Chloroquine 50 mg/kg Significance	Mean SD - P <	1.0 0.1 NS	6.2 0.8 NS	4.0 1.0 0.05	2.8 0.5 0.01	3.0 1.2 0.02
ME sensitive titre	Control	Mean SD -	5.4 0.5	0.0 0.0	1.6 1.1	3.0 1.0	2.8 1.1
	Chloroquine 50 mg/kg Significance	Mean SD - P <	4.9 0.6 NS	0.0 0.0 NS	1.2 0.8 NS	2.6 0.9 NS	2.4 1.5 NS

\* See table VI

**FIGURE 17** The effect of chloroquine on the antibody response to SRBC (titres expressed as  $\log_2$ )



C. The effects of antimalarial drugs on the immune response of mice to SRBC

These experiments were designed to find out whether the antimalarial drugs, chloroquine, quinine and pyrimethamine, have any immunodepressive or immunostimulatory effect on the responses of mice to SRBC.

1. Chloroquine

Chloroquine was given intraperitoneally in two different doses to two groups of mice. One received  $5 \text{ mg kg}^{-1}$  and another  $50 \text{ mg kg}^{-1}$ . The control group received 0.2 ml of distilled water. The mice were challenged intraperitoneally with 0.2 ml of 20% SRBC 5 hours after the drug treatment. The agglutination, haemolysin, 2MER and 2MES titres were determined at 3-4 day intervals in all three groups. The responses in the experimental group receiving  $5 \text{ mg kg}^{-1}$  were comparable with those of the control. But the group receiving  $50 \text{ mg kg}^{-1}$  of chloroquine showed a significant difference ( $P < 0.05$  to  $0.01$ ) in agglutination titre and 2ME resistant (IgG) titres on days 11, 15, and 18 though haemolysin titres and 2ME sensitive titres were similar to those of the control (fig 17).

2. Quinine

Quinine was also given in two different doses. One group received a single dose of  $15 \text{ mg kg}^{-1}$  and another  $150 \text{ mg kg}^{-1}$  i.p. The control group received 0.2 ml of distilled water as before. SRBC were given 5 hours after the drug treatments. Results differed little between groups (fig 18).



FIGURE 1



FIGURE 2

Table XIIIa Statistical analysis of the data in Fig 18 (n=5)

Test	Group	Day					
		Data	4	7	11	14	18
Agglutination titre	Control	Mean	4.5	7.8	9.2	8.6	9.6
		SD $\pm$	0.6	0.8	0.8	0.9	0.9
	Quinine $150 \text{ mg kg}^{-1}$	Mean	5.4	7.8	11.0	8.8	9.8
		SD $\pm$	0.9	0.5	1.4	2.2	1.5
Significance	P <	NS	NS	0.05	NS	NS	
Lysin titre	Control	Mean	5.0	7.6	9.4	8.0	7.8
		SD $\pm$	0.7	0.9	1.7	0.0	1.9
	Quinine $150 \text{ mg kg}^{-1}$	Mean	5.8	7.6	10.6	9.0	9.8
		SD $\pm$	0.8	0.9	1.5	0.8	1.5
Significance	P <	NS	NS	NS	0.05	NS	
ME resistant titre	Control	Mean	0.6	7.4	8.4	8.2	9.0
		SD $\pm$	0.9	0.9	1.1	0.8	1.2
	Quinine $150 \text{ mg kg}^{-1}$	Mean	0.2	7.2	11.0	8.8	10.3
		SD $\pm$	0.5	0.8	1.4	2.2	1.3
Significance	P <	NS	NS	0.02	NS	NS	
ME sensitive titre	Control	Mean	4.0	0.4	0.8	0.8	0.6
		SD $\pm$	0.7	0.6	0.8	0.8	0.6
	Quinine $150 \text{ mg kg}^{-1}$	Mean	5.2	0.6	0.0	0.0	0.0
		SD $\pm$	1.1	0.6	0.0	0.0	0.0
Significance	P <	NS	NS	NS	NS	NS	

\* see

Table XIII B Statistical analysis of the data in Fig 18 (n=5)

Test	Group	Day	4	7	11	14	18
		Date					
Agglutination titre	Control	Mean	4.5	7.8	9.2	8.6	9.6
		SD -	0.6	0.8	0.8	0.9	0.9
	Quinine <sub>1</sub> 15 mg kg <sup>-1</sup>	Mean	4.6	7.4	9.4	8.2	9.4
		SD -	0.9	0.9	1.1	1.1	1.1
	Significance	P <	NS	NS	NS	NS	NS
	Lysin titre	Control	Mean	5.0	7.6	9.4	8.0
SD -			0.7	0.9	1.7	0.0	1.9
Quinine <sub>1</sub> 15 mg kg <sup>-1</sup>		Mean	4.8	8.0	8.0	7.6	7.8
		SD -	1.6	0.7	1.0	0.6	0.5
Significance		P <	NS	NS	NS	NS	NS
ME resistant titre		Control	Mean	0.6	7.4	8.4	8.2
	SD -		0.9	0.9	1.1	0.8	1.2
	Quinine <sub>1</sub> 15 mg kg <sup>-1</sup>	Mean	0.4	7.2	8.6	8.0	8.8
		SD -	0.9	0.8	1.1	1.0	0.8
	Significance	P <	NS	NS	NS	NS	NS
	ME sensitive titre	Control	Mean	4.0	0.4	0.8	0.8
SD -			0.7	0.6	0.8	0.8	0.6
Quinine <sub>1</sub> 15 mg kg <sup>-1</sup>		Mean	4.2	0.2	0.8	0.2	0.6
		SD -	1.5	0.4	0.4	0.5	0.6
Significance		P <	NS	NS	NS	NS	NS

table VI

FIGURE 18 The effect of quinine on the antibody response to SRBC (titres expressed as  $\log_2$ )

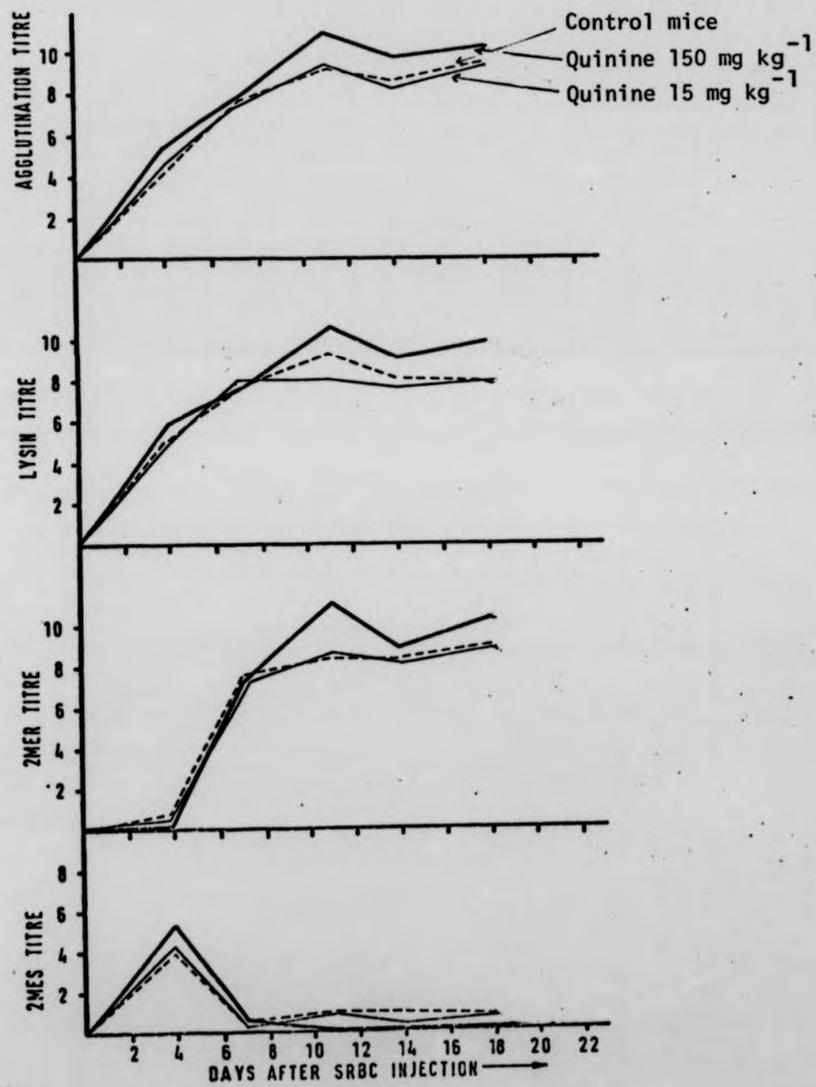




Table XI7A Statistical analysis of the data in Fig 19(n=5)

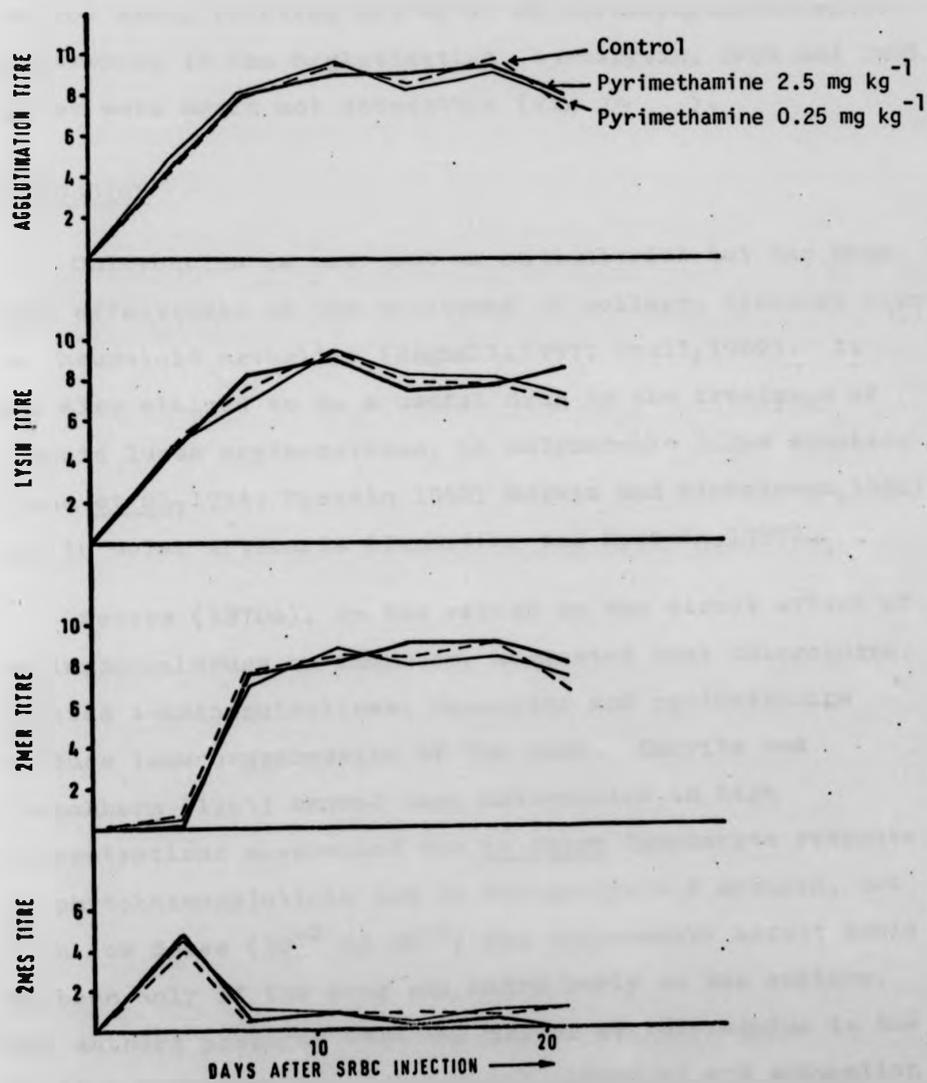
Test	Group	Day	4	7	11	14	18
		Data					
Agglutination titre	Control	Mean	4.5	7.8	9.2	8.6	9.6
		SD	0.6	0.8	0.8	0.9	0.9
	Pyrimethamine 2.5 mg kg <sup>-1</sup>	Mean	4.8	8.0	9.0	9.0	9.4
		SD	1.1	0.7	0.7	0.7	0.7
	Significance	P <	NS	NS	NS	NS	NS
Lysin titre	Control	Mean	5.0	7.6	9.4	8.0	7.8
		SD	0.7	0.9	1.7	0.0	1.9
	Pyrimethamine 2.5 mg kg <sup>-1</sup>	Mean	4.8	8.2	9.2	7.4	7.8
		SD	0.5	0.5	0.8	0.6	0.5
	Significance	P <	NS	NS	NS	NS	NS
ME resistant titre	Control	Mean	0.6	7.4	8.4	8.2	9.0
		SD	0.9	0.9	1.1	0.8	1.2
	Pyrimethamine 2.5 mg kg <sup>-1</sup>	Mean	0.0	7.6	8.2	9.0	9.0
		SD	0.0	0.6	0.8	0.7	0.7
	Significance	P <	NS	NS	NS	NS	NS
ME sensitive titre	Control	Mean	4.0	0.4	0.8	0.8	0.6
		SD	0.7	0.6	0.8	0.8	0.6
	Pyrimethamine 2.5 mg kg <sup>-1</sup>	Mean	4.8	0.4	0.8	0.0	0.4
		SD	1.1	0.6	0.5	0.0	0.6
	Significance	P <	NS	NS	NS	NS	NS

Table IVB Statistical analysis of the data in Fig 19 (n=5)

Test	Group	Day	4	7	11	14	18
		Data					
Agglutination titre	Control	Mean +	4.5	7.8	9.2	8.6	9.6
		SD -	0.6	0.8	0.9	0.9	0.9
	Pyrimethamine 0.25 mg kg <sup>-1</sup>	Mean +	4.8	8.0	9.6	8.2	9.2
		SD -	2.6	1.0	1.1	0.8	1.3
Significance	P <	NS	NS	NS	NS	NS	
Lysin titre	Control	Mean +	5.0	7.6	9.4	8.0	7.8
		SD -	0.7	0.9	1.7	0.0	1.9
	Pyrimethamine 0.25 mg kg <sup>-1</sup>	Mean +	4.8	6.8	9.4	8.2	8.0
		SD -	0.5	0.8	1.8	0.5	1.2
Significance	P <	NS	NS	NS	NS	NS	
ME resistant titre	Control	Mean +	0.6	7.4	8.4	8.2	9.0
		SD -	0.9	0.9	1.1	0.8	1.2
	Pyrimethamine 0.25 mg kg <sup>-1</sup>	Mean +	0.3	7.0	8.8	8.0	8.4
		SD -	0.5	1.0	0.8	1.2	0.9
Significance	P <	NS	NS	NS	NS	NS	
ME sensitive titre	Control	Mean +	4.0	0.4	0.8	0.8	0.6
		SD -	0.7	0.6	0.8	0.8	0.6
	Pyrimethamine 0.25 mg kg <sup>-1</sup>	Mean +	4.5	1.0	0.8	0.2	0.8
		SD -	2.5	0.7	0.8	0.5	0.8
Significance	P <	NS	NS	NS	NS	NS	

see table VI

FIGURE 19 The effect of pyrimethamine in the antibody response to SRBC (titres expressed at  $\log_2$ )



### 3. Pyrimethamine

Pyrimethamine was given intraperitoneally as a single dose at two dose levels to two groups of mice. One group received  $0.25 \text{ mg kg}^{-1}$  and another  $2.5 \text{ mg kg}^{-1}$ . The normal control group received 0.2 ml of 2% carboxymethylcellulose. Differences in the agglutination, haemolysin, 2MER and 2MES titres were again not detectable (fig 19 ).

### DISCUSSION

Chloroquine is not just an antimalarial but has been used effectively in the treatment of collagen diseases such as rheumatoid arthritis (Bagnall, 1957; Scull, 1962). It was also claimed to be a useful drug in the treatment of discoid lupus erythematosus, in polymorphic light eruption (Cahn et al, 1954; Epstein 1960; Merwin and Winkelmann, 1962) and in solar urticaria (Brunsting and Epstein, 1957).

Peters (1970a), in his review on the direct effect of antimalarial drugs on immunity, suggested that chloroquine, related 4-aminoquinolines, mepacrine and pyrimethamine produce immunosuppression of the host. Hurvitz and Hirschhorn (1965) showed that chloroquine in high concentrations suppressed the in vitro lymphocyte response to phytohaemagglutinin and to streptolysin  $\theta$  antigen, but with low doses ( $10^{-2} \text{ mg ml}^{-1}$ ) the suppressive effect could be seen only if the drug was added early to the culture. The authors proposed that the action of chloroquine is due to the stabilization of lysosomal membranes and prevention of release of enzymes required during the initiation of depression of lymphocytes.

According to Peters (1970a), the erythrocytic parasitaemia with chloroquine resistant strains of P. berghei reached higher levels in mice treated with high doses of chloroquine or quinine than in animals that had received lower doses of drugs. This effect, according to him, was due to the immunosuppressive effect of the drugs, which could not be elicited with a drug sensitive strain of plasmodium infection.

In the experiment IV C.1, chloroquine did not show any sign of immunodepression of SRBC antibody titre at a dose level of  $5 \text{ mg kg}^{-1}$ , but with the high dosage of  $50 \text{ mg kg}^{-1}$  there was significant depression of agglutination titres on days 11, 15, 18 and also in the ME resistant titre (IgG) on days 15 and 18.  $50 \text{ mg kg}^{-1}$  dose of chloroquine is much higher than that of the human oral dosage of 1500-1800 mg given over 4 days and equivalent to  $20\text{-}26 \text{ mg kg}^{-1}$  (Ross Institute, 1975). One cannot be sure whether such a dose given over 4 days would produce pronounced immunosuppression or not.

Whitehouse and Bostrom (1965) referring to other workers stated that chloroquine has no effect on the immune response of the rabbit and guinea pig. Thompson and Bartholomew (1964) (reported by Sams, 1967) tested the ability of rabbits to produce circulating antibody to typhoid O and H antigens and to bovine serum albumin. It was found that chloroquine had absolutely no effect on this ability. Kalmanson and Guze (1965) demonstrated that hydroxychloroquine had no effect on either the primary or the secondary antibody response to diphtheria toxoid or on the delayed tuberculin

hypersensitivity reaction in guinea pigs.

Pyrimethamine is an antimetabolite and its effect on plasmodium is by inhibiting some metabolic process like dihydrofolate reductase function for synthesis of tetrahydrofolate co-factors (see page 58 ). Other anti-folic acid substances like methotrexate have marked immunosuppressive properties (Aisenberg, 1970) which might therefore be a feature of chemotherapy with drugs like pyrimethamine.

Experiments using either the high or the low dosages of quinine and pyrimethamine had no significant effect on the antibody titres to SRBC in mice. Chloroquine at  $50 \text{ mg kg}^{-1}$  and quinine at  $150 \text{ mg kg}^{-1}$  i.p. are the highest dose levels that can be given to CBA mice without killing them. Also pyrimethamine at  $2.5 \text{ mg kg}^{-1}$  is much higher than the human therapeutic dosage of  $50 \text{ mg}$  i.e.  $0.08 \text{ mg kg}^{-1}$  (Ross Institute, 1975).

Immunosuppression depends to a considerable extent on the appropriate timing of drug and antigen. Aisenberg (1967) investigated the suppression in mice of the response to a nonreplicating antigen (SRBC) with the alkylating agent cyclophosphamide. According to him cyclophosphamide must be given simultaneously with the antigen or within the preceding 24 hours to achieve complete suppression at a low drug dosage ( $165 \text{ mg kg}^{-1}$ ), while at higher drug levels ( $330 \text{ mg kg}^{-1}$ ) the agent can be given up to 48 hours before or after the antigen. With a replicating antigen, the immunosuppressant must be delayed to allow the antigen to build up to the necessary level (Aisenberg, 1970). In my

experiments, the drugs were given 5 hours before the non replicating antigen (SRBC) and were of both high and low dosages. So if the drugs have an immunosuppressive effect it should be detected because the dose of the drugs and the timing met the criteria put up by Aisenberg (1970).

Chloroquine at a dose of  $50 \text{ mg kg}^{-1}$  produces some immunodepression in mice but  $5 \text{ mg kg}^{-1}$  does not. It is doubtful whether normal therapeutic doses of chloroquine in man are immunosuppressive, but it is wise to look for it especially when this is given in high doses against the chloroquine resistant strains of P.falciparum. Otherwise chloroquine might produce an increased drug resistant strain. It has been shown that reduction of the immune response of the host facilitates the emergence of drug resistant strains of parasites (Peters 1970a) and also the occurrence of a selection of resistant mutants under drug pressure (Peters 1970b). So chloroquine might act both ways by suppressing the immune response of the host and by a constant drug pressure.

D. Resistance to reinfection after drug  
treatment of P.chabaudi infections

These experiments were designed to find out whether suppressive or curative treatment that would still allow the development of a strong immunity was possible.

1. Resistance to reinfection

The experimental group of 12 mice was infected by inoculation of  $1 \times 10^7$  P.chabaudi from two donor mice. The control group was injected with 0.2 ml of PBS per mouse. The parasitaemia is shown in fig 20 . Challenge infections were made on day 66 of the infection on the assumption that there were no more parasites in mice of the test group. (see experiment IVAla p 125 ). The challenge inoculum was  $1 \times 10^7$  infected erythrocytes obtained from two donors infected with the original stabilate (LUMP 1156).

The experimental group showed a solid immunity to reinfection while the uninfected challenge control group of mice gave a normal pattern of parasitaemia with a peak parasitaemia of 32%. (fig 20).

2. Suppressive chemotherapy and immunity to reinfection

Preliminary experiments established that i.p. injections of chloroquine of  $10 \text{ mg kg}^{-1}$  or more for 7 days cured P.chabaudi infections in CBA mice and the maximal dose of a single i.p. injection which would not kill the mouse was  $50 \text{ mg kg}^{-1}$ .

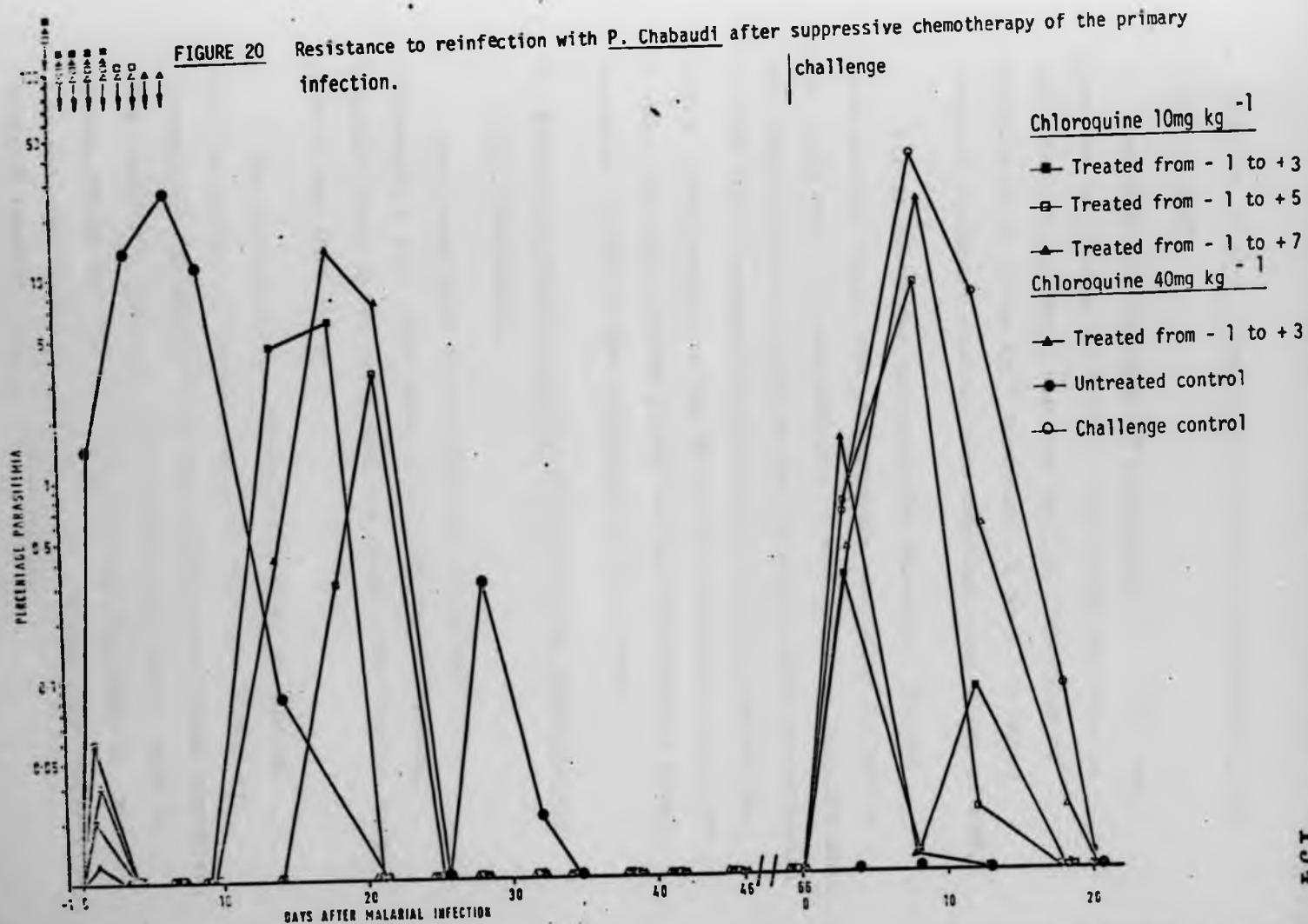


Table XVA Statistical analysis of the data in Fig 20.

Group	No. of mice	Day Data	1	4	7	9	15	18	21	25	28	32	35	66	0	3	8	12	18	20	% reduction parasitaemia
Chloroquine 40mg kg <sup>-1</sup> day <sup>-1</sup> from -1 to +3	8	Mean SD	0.01 0.01	0.0	0.0	0.0	0.4 0.1	13.0 2.8	7.5 3.5	0.0	0.0	0.0	0.0	0.0	0.0	1.5 0.9	0.01 0.01	0.0	0.0	0.0	95%
Chloroquine 10mg kg <sup>-1</sup> day <sup>-1</sup> from -1 to +3	8	Mean SD	0.02 0.02	0.0	0.0	0.0	4.5 2.2	5.9 4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3 0.4	0.01 0.01	0.08 0.2	0.0	0.0	99%
Chloroquine 10 mg kg <sup>-1</sup> day <sup>-1</sup> from -1 to +5	8	Mean SD	0.05 0.8	0.0	0.0	0.0	0.0 0.6	0.3 7.2	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.7 0.3	8.0 7.2	0.2 0.02	0.0	0.0	75%
Chloroquine 10mg kg <sup>-1</sup> day <sup>-1</sup> from -1 to +7	8	Mean SD	0.03 0.04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3 0.2	23.0 12.0	0.4 0.3	0.02 0.04	0.0	28%
Infected control	12	Mean SD	1.3 0.8	13.0 7.0	27.0 12.0	11.0 5.1	0.07 0.03	-	0.0	0.0	0.3 0.2	0.02 0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100%
Challenge control	8	Mean SD	-	-	-	-	-	-	-	-	-	-	-	-	0.0	0.5 0.3	32.0 18.0	7.0 3.2	0.08 0.03	0.01 0.01	-

- the percentage reduction (r) of parasitaemia (maximal) in the experimental groups relative to the mean percentage parasitaemia (maximal) of the control group was calculated by  $r = 100 - \left( \frac{\text{experimental group}}{\text{challenge control group}} \times 100 \right)$

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a. Effects of high doses of suppressive chemotherapy with chloroquine

The standard inoculum of P.chabaudi infection was given to two groups of 8 mice. One group was kept as controls and the other received an i.p. injection of chloroquine at  $40 \text{ mg kg}^{-1}$  from days -1 to 3. A third group of challenge control mice received only 0.2ml of PBS.

Fig 20 shows the parasitaemia patterns. In the experimental group, the parasitaemia was below 0.015% on day 1 and was then undetectable in blood films. Parasitaemia was then detectable again on day 15 with a mean parasitaemia of 0.4% and a maximum of 13% on day 18 of the infection. After rechallenging on day 66 of the infection, there was a very low parasitaemia (1.5%) in the experimental group compared to 32% in the challenge control group.

b. Effects of moderate doses of suppressive chemotherapy with chloroquine

One group (A) of 8 mice received  $10 \text{ mg kg}^{-1}$  of chloroquine i.p. from days -1 to 3 of the infection, another group (B) the same dose per mouse from days -1 to 5, and C from days -1 to 7.

The parasitaemias obtained are shown in fig 20 . All the groups of treated mice had very low levels of parasitaemia (0.02-0.05%) on day 1 which then became negative. The infection remained negative for more than 5 days in group A mice and for more than 10 days in group B. The maximal mean parasitaemias were of 5.9% and 3.2% respectively. Group C remained negative throughout.

1. The first part of the experiment was to determine the effect of temperature on the rate of reaction. The reaction was carried out at three different temperatures: 20°C, 30°C, and 40°C. The rate of reaction was measured by the time taken for a certain amount of product to be formed. The results are shown in the table below.

Temperature (°C)	Time taken for product formation (s)
20	120
30	60
40	30



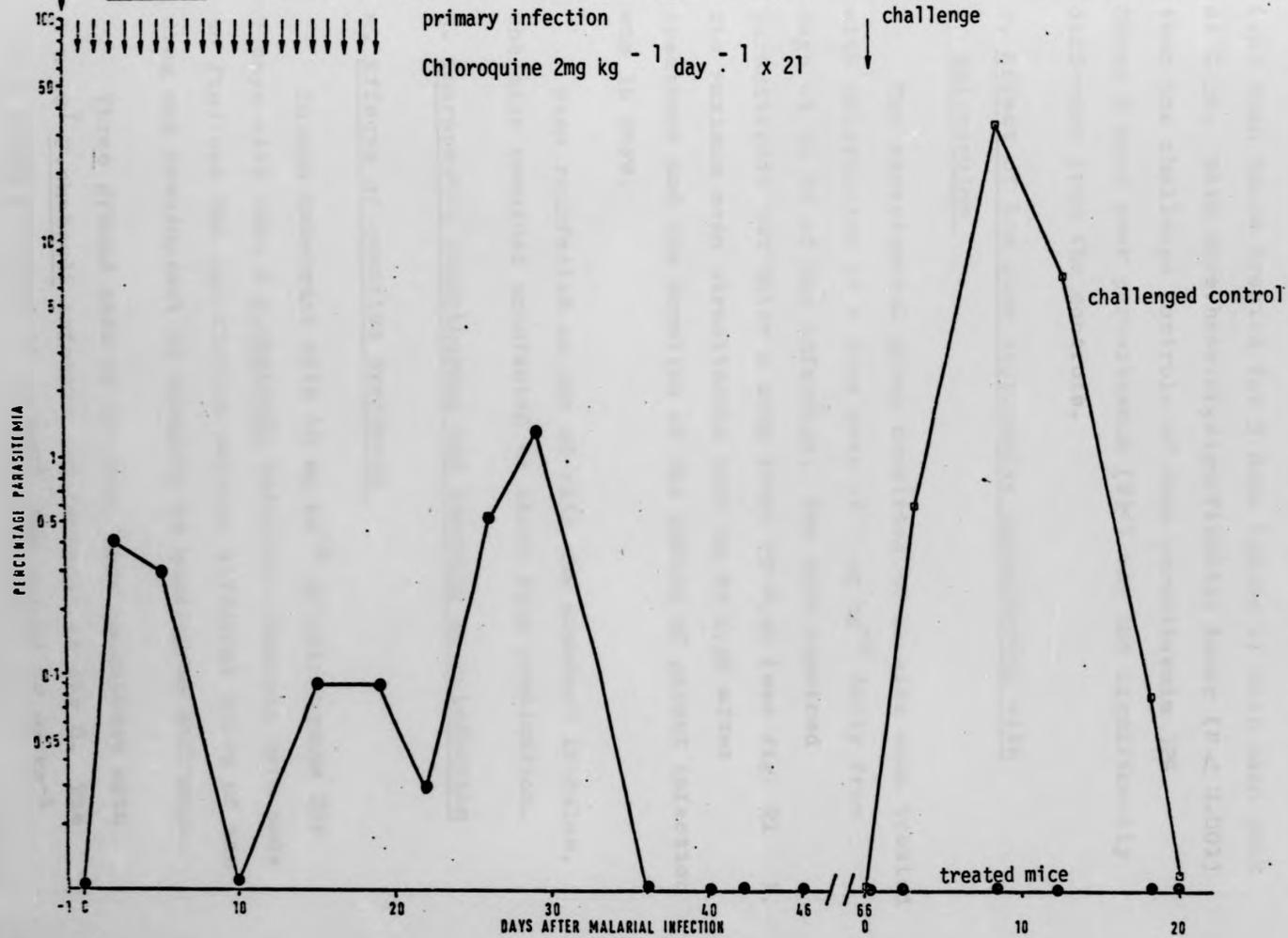
Table XVb Statistical analysis of the data in Fig 21.

Group	No. of mice	Day Date	2	5	10	15	19	22	26	29	33	36	66	0	3	8	11	17	20	% reduction of parasitaemia	
			Rechallenge																		
Chloroquine 2 mg kg <sup>-1</sup> day <sup>-1</sup> from -1 to +19 days	12	Mean	0.4	0.3	0.01	0.09	0.09	0.03	0.5	1.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100%
		SD	0.3	0.01	0.01	0.2	0.02	0.6	2.2	0.4	0.3	-	-	-	-	-	-	-	-	-	
Challenge control	8	Mean	-	-	-	-	-	-	-	-	-	-	-	0.0	0.5	32.0	7.0	0.08	0.01		
		SD	-	-	-	-	-	-	-	-	-	-	-	-	0.3	12.0	3.2	0.03	0.01		

- see table XVA for the calculation of % reduction of parasitaemia.

FIGURE 21 Resistance to reinfection with P. Chabaudi after prolonged suppressive chemotherapy of the primary infection challenge

FIGURE 21 Resistance to reinfection with *P. Chabaudi* after prolonged suppressive chemotherapy of the



On reinfection, the group treated for 7 days (group B) had significantly higher mean ( $P < 0.001$ ) peak parasitaemias (7%) than those treated for 5 days (group A) with mean peak at 0.3%. Both were, however, significantly lower ( $P < 0.001$ ) than the challenge controls of mean parasitaemia 32%. Group C mean peak parasitaemia (23%) was not significantly different from the controls.

c. Effect of low dose suppressive chemotherapy with chloroquine

The experimental group consisted of 12 mice each treated with chloroquine at a dose rate of  $2 \text{ mg kg}^{-1}$  daily from days -1 to 20 of the infection. The mice remained parasitaemic but below a mean level of 0.4% (see fig 21 ). The maximum mean parasitaemia went up to 1.3% after treatment and the duration of the period of patent infection was 36 days.

When reinfected on day 66 with the standard inoculum, the mice remained uninfected by blood film examination.

3. Therapeutic chemotherapy and immunity to reinfection

a. Effects of curative treatment

Though treatment with  $10 \text{ mg kg}^{-1}$  of chloroquine for 7 days will cure a P.chabaudi infection attempts were made to find out the correlation between different doses of the drug and development of immunity to homologous challenge.

Three groups each of 10 mice, were inoculated with  $1 \times 10^7$  P.chabaudi infected erythrocytes on day 0. The first group received  $10 \text{ mg kg}^{-1}$ , the second  $20 \text{ mg kg}^{-1}$

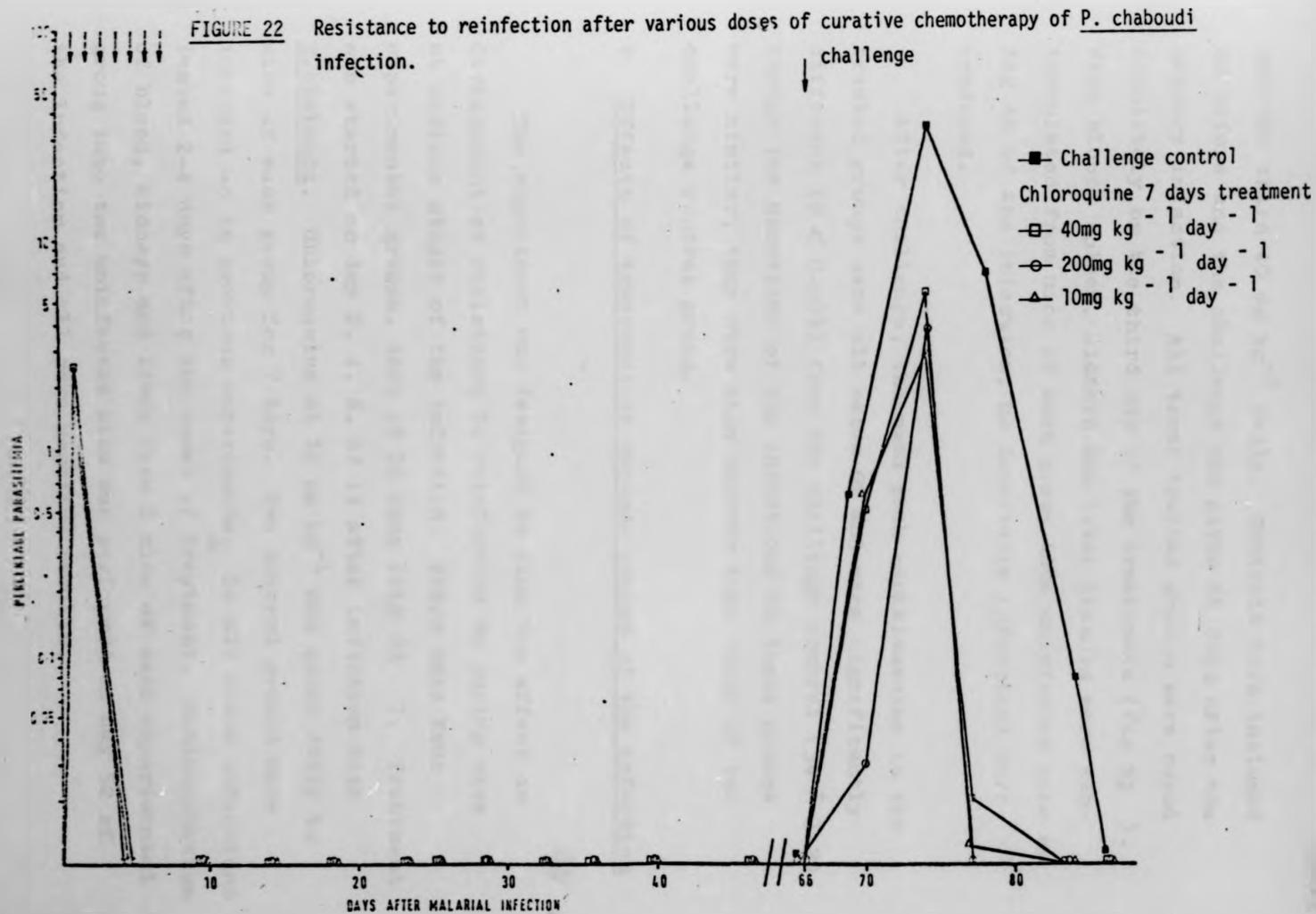


Table XVI Statistical analysis of the data in Fig 22. (n=8)

Group	Day Data	1	5	9	14	18	23	25	28	66	0	3	8	11	17	20	% reduction of parasitaemia
		Chloroquine 40 mg kg <sup>-1</sup>	Mean SD <sup>+</sup> SD <sup>-</sup>	2.5 0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5 0.07	4.5 0.7	0.01 0.01	
Chloroquine 20 mg kg <sup>-1</sup>	Mean SD <sup>+</sup> SD <sup>-</sup>	1.4 0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.03 0.33	3.8 1.0	0.0	0.0	0.0	89%
Chloroquine 10 mg kg <sup>-1</sup>	Mean SD <sup>+</sup> SD <sup>-</sup>	1.5 0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6 0.4	2.9 2.1	0.02 0.01	0.0	0.0	91%
Challenge control	Mean SD <sup>+</sup> SD <sup>-</sup>	-	-	-	-	-	-	-	-	0.0	0.0	0.6 0.2	34.0 3.2	7.0 0.5	0.08 0.03	0.01 0.01	-

- see table XVA for the calculation of % reduction of parasitaemia

FIGURE 22 Resistance to reinfection after various doses of curative chemotherapy of P. chaboudi infection. challenge



and the third 40 mg kg<sup>-1</sup> daily. Controls were included as before and the challenge was given 68 days after the primary infection. All three treated groups were cured completely by the third day of the treatments (fig 22 ). When blood, spleen, kidneys and liver tissues were sub-inoculated from mice of each group into uninfected mice on day 46 of the infection, no detectable infections were produced.

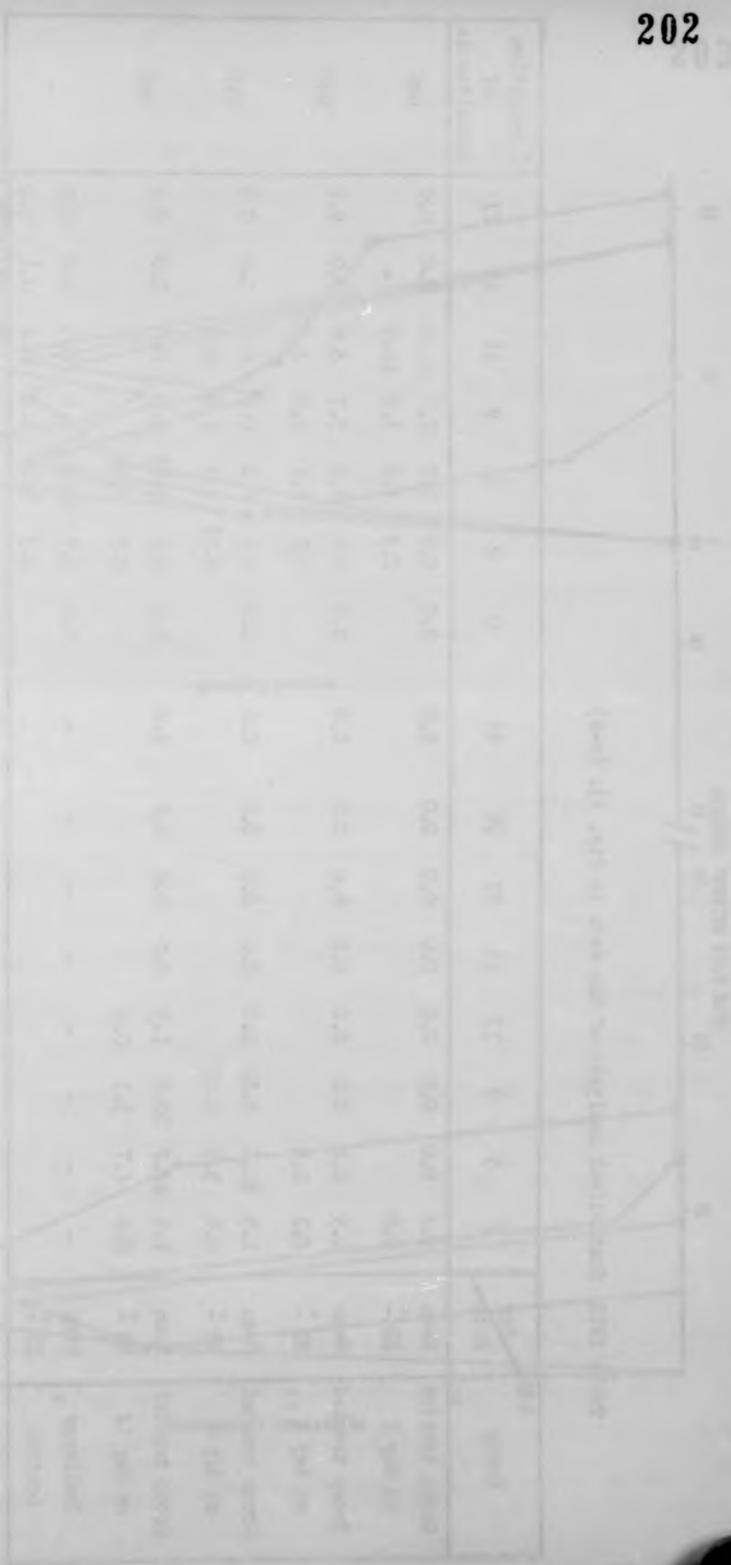
After challenge, the mean peak parasitaemias in the treated groups were all below 6% and were significantly different ( $P < 0.001$ ) from the challenge control ( $34 \pm 3.2$ ). Though the durations of the infections in these groups were similar, they were also shorter than those of the challenge control group.

b. Effects of treatment at various stages of the infection

The experiment was designed to find the effect on development of resistance to reinfection by curing mice at various stages of the infection. There were four experimental groups, each of 10 mice (fig 23 ). Treatment was started on day 2, 4, 6, or 14 after infection with P.chabaudi. Chloroquine at 10 mg kg<sup>-1</sup> was given daily to mice of each group for 7 days. Two control groups were included as in previous experiments. In all cases infections lasted 2-4 days after the onset of treatment. Subinoculation of blood, kidneys and liver from 2 mice of each experimental group into two uninfected mice was performed on day 52 of the infection and all gave negative results.

Figure 1. The effect of vertical distance on pressure (mm Hg) on 1 x 1 ft surface area of 25 different materials in an office environment. (continued)

- 1 - Carpet
- 2 - Linoleum
- 3 - Hardwood
- 4 - Parquet
- 5 - Concrete
- 6 - Glass
- 7 - Metal
- 8 - Plastic
- 9 - Rubber
- 10 - Fabric
- 11 - Paper
- 12 - Wood
- 13 - Brick
- 14 - Stone
- 15 - Cement
- 16 - Asphalt
- 17 - Concrete
- 18 - Glass
- 19 - Metal
- 20 - Plastic
- 21 - Rubber
- 22 - Fabric
- 23 - Paper
- 24 - Wood
- 25 - Brick



Vertical distance (ft)

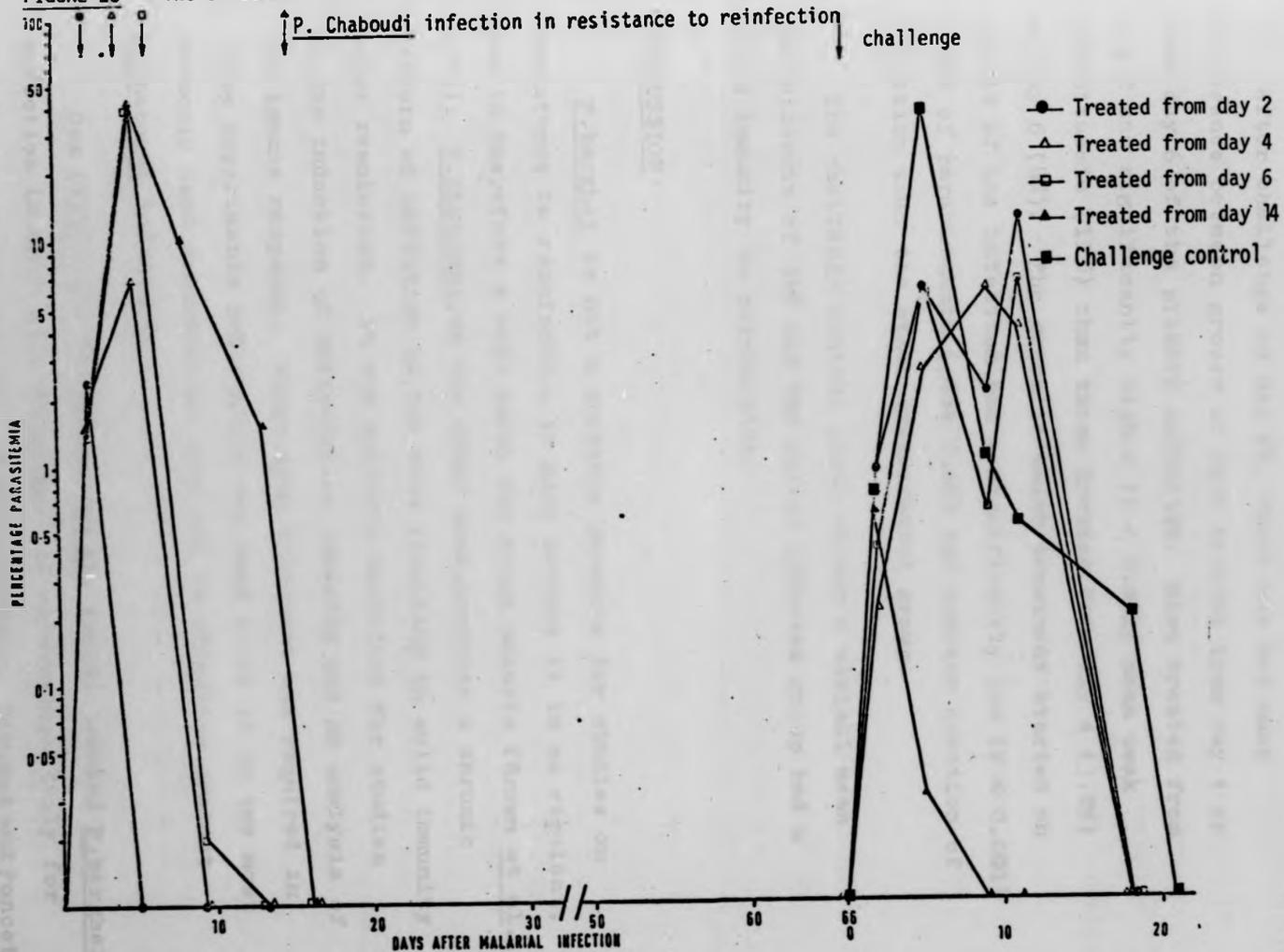
Pressure (mm Hg)

Table XVII Statistical analysis of the data in Fig. 23. (n=8)

Group	Day Data	2	5	9	13	15	22	26	66	0	2	5	9	11	18	21	% reduction of parasitaemia
Group treated on day 2	Mean	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	5.9	2.1	11.5	0.0	0.0	84%
	SD $\pm$	0.8									0.3	3.5	3.2	10.9	-		
Group treated on day 4	Mean	1.9	6.8	0.0	0.0	0.0	0.0	0.0	0.0	Rechallenge	0.0	0.2	2.5	5.7	3.8	0.0	84%
	SD $\pm$	0.2	1.6								0.2	2.4	5.8	4.0			
Group treated on day 6	Mean	1.5	40.0	0.02	0.0	0.0	0.0	0.0	0.0	Rechallenge	0.0	0.4	6.0	0.6	6.5	0.0	81%
	SD $\pm$	0.6	5.7	0.02							0.13	4.7	1.0	8.5			
Group treated on day 14	Mean	1.5	42.2	10.0	1.5	0.0	0.0	0.0	0.0	Rechallenge	0.0	0.6	0.03	0.0	0.0	0.0	98%
	SD $\pm$	0.6	4.7	3.3	0.9						0.5	0.02					
Challenge control	Mean	-	-	-	-	-	-	-	-	Rechallenge	0.0	0.7	36.0	1.0	0.5	0.2	-
	SD $\pm$										0.3	5.1	0.4	0.4	0.1	0.0	

see table XVI for the calculation of % reduction of parasitaemia

FIGURE 23 The effect of curative chloroquine treatment ( $10\text{mg kg}^{-1}\text{ day}^{-1} \times 7$ ) of various stages of



After challenge on day 66, there was not much difference between groups of mice treated from day 4 or from day 6 of the primary infection. Mice treated from day 2 had significantly higher ( $P < 0.001$ ) mean peak parasitaemia (11%) than those treated from day 4 (3.8%) on day 6 (6%). The group in which treatment started on day 14 of the infection had a significantly low ( $P < 0.001$ ) level of parasitaemia (peak 0.6%) and shorter duration of infection than the other experimental group.

The challenge control group showed a maximal mean parasitaemia of 36% and the normal infected group had a solid immunity to reinfection.

#### DISCUSSION

P.berghei is not a suitable parasite for studies on resistance to reinfection in mice because it is so virulent, and is therefore a poor model for human malaria (Brown et al., 1976). P.chabaudi, on the other hand, produces a chronic pattern of infection in CBA mice resulting in solid immunity after resolution. It was suitable therefore for studies on the induction of antimalarial immunity and an analysis of the immune response. Where drug treatment was required in these experiments chloroquine was used since it is the most commonly used antimalarial drug and is effective against P.chabaudi infection.

Cox (1958) and Gilbertson et al. (1970) treated P.berghei infection in mice with  $20 \text{ mg kg}^{-1}$  of chloroquine daily for 5 days but there was still 35-40% mortality. Sargent and Poncet (1956) treated the first attack of P.berghei in mice with

25 mg kg<sup>-1</sup> of chloroquina, but the infection recrudesced and the mice died. A single dose of drug given after malaria infection (the "Rane test") has been used by some workers for screening purposes. Peters (1970a) and Pinder (1973) in their reviews on the "Rane test", concluded that chloroquine is active against P.berghei but is not curative. In my experiments I managed to cure up to 50-75% of the mice with chloroquine (10 mg kg<sup>-1</sup>) given for 14 days, and 13-38% with 7 day treatment.

A solid immunity was demonstrated by challenge 66 days after infection (exp IV D<sup>1</sup> p192). The immunity obtained on that day was likely to be sterile i.e. maintained in the absence of parasites, since blood, kidneys, spleen or liver tissue injected from the infected mice into recipient mice did not produce any infection. Cox (1970) has shown that 80% of the mice infected with P.chabaudi recovered and produced a solid acquired immunity. He also claimed that the immunity was of sterile type after 8 weeks of infection and lasted for over 5 months. The occurrence of a sterile immunity following malaria infections in mice has been reported for P.berghei (Weiss and De Giusti, 1966), for P.vinckei (Cox, *et al.* 1966.) and in P.yoelii (Barker, 1971). Corradetti (1963) also reported a sterile immunity of P.berghei in rats. Jayawardena (1975) has demonstrated the persistence of P.yoelii infection in the kidneys of CBA mice 4 weeks after the regression of the parasitaemia.

So, though a sterile immunity is likely, the possibility of the parasite surviving somewhere in the body must be considered. On the other hand, drug-cured malaria infections

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are a more sure way of showing the existence of a sterile immunity (Cox, 1966a). Chemotherapy from days -1 to 7 of infection suppressed the first wave of parasitaemia. There was no recrudescence of the parasitaemia and no immunity to challenge. There was resistance to reinfection when the suppressive chemotherapy was followed by a recrudescence. Mice given suppressive chloroquine treatment ( $10 \text{ mg kg}^{-1}$ ) from days -1 to 3 recrudesced 5 days earlier and with higher parasitaemia than the group treated from days -1 to 5. On rechallenging, the first group had significantly lower parasitaemia than the second.

The results of the experiment D 3b (p201) supported the previous experiment in some ways by showing that immunity in malaria infection can be achieved by curative treatment early in the infection. The immunity obtained by treating the infected mice on days 1, 2, 4, or 6 was not significantly different (figs 22 and 23). The strongest immunity to challenge was obtained by treating the malaria infection on day 14 (fig 23).

Nussenzweig *et al.* (1966) have shown that, though cross-resistance to P.vinckei infection could be shown 24 hours after a P.chabaudi infection, the maximal protective effect was seen only during the second week of the P.chabaudi infection. Eling and Jerusalem (1977) have also shown that P.berghei infection in Swiss or C3H/ST2 mice produced maximal immunity when treatment began 14 days after the start of the infection. They used sulfathiosole in drinking water at a concentration of  $200 \text{ mg l}^{-1}$  which took 3-5 days to clear the

parasites from the blood. They did get some immunity when treatment started on day 0 but, though their results could not be compared directly with mine, best protection was obtained by treatment at a comparable stage in the infection. When chloroquine was given at a dose rate of  $40 \text{ mg kg}^{-1}$  from days -1 to 3, recrudescence infections were not significantly different after treatment with  $10 \text{ mg kg}^{-1}$  for the same period of infection. On rechallenging also, the parasitaemia levels were not significantly different from each other.

Afridi and Rahim (1959, 1962) have shown less effective protection against human malaria using a single high suppressive dose of pyrimethamine (50 mg) than a lower dose (25 mg). According to Eling and Jerusalem (1977), the degree of immunity is related to the number of surviving parasites in the host which is inversely proportional to the amount of drug administered. So effective drug treatment diminishes the number of parasites and the development of immunity. This general assumption seems to apply in my experiments where complete suppression of parasitaemia with chloroquine from -1 to 7 days did not produce any immunity at all.

When the malaria infection was maintained at low levels for a long period of time by subcurative doses (chloroquine  $2 \text{ mg kg}^{-1}$  daily), the mouse acquired a solid immunity and was aparasitaemic after challenge. Carrescia and Arcoleo (1957) tried to prolong the parasitaemia of P.berghei infection in mice by repeatedly treating them with  $5 \text{ mg kg}^{-1}$  of chloroquine during the primary attack and during each relapse. They managed to demonstrate some immunity by this method.

Gilbertson et al. (1970) suppressed P.berghei infection in mice for 90 days with a PABA-deficient milk diet and were able to demonstrate immunity. According to them, a short high parasitaemia was not enough to promote measurable resistance and they concluded that the duration of a first infection was more important. However, this method of suppression by chemotherapy in the human situation might provoke the development of drug resistant strains if prolonged (Peters, 1970a).

Some resistance to reinfection was obtained by curing the infection from day 1. Upon rechallenge on day 68, the peak parasitaemia was below 6% compared with 33% in the challenge controls. This resistance to reinfection does not seem to depend on the dosage of curative drug used, 10 mg kg<sup>-1</sup> daily for 7 days being as effective as 40 mg kg<sup>-1</sup>. That immunity develops within this short period of infection (24 hours) has been shown by Nussenzweig et al. (1966). According to them, 24 hours after P.chabaudi infection, mice were resistant to rechallenging with a virulent strain of P.vinckei. Though the authors could not prove that this was an immunological response they did show that resistance was not due to PABA deficiency.

In my experiments the inoculum size was very high ( $1 \times 10^7$ ) and if these parasites died a great deal of antigen would be released to stimulate the immune response. Immunity to malaria infection has been induced by using parasitic fractions as immunogens (Zuckerman et al., 1967; Jerusalem and Eling, 1969; D'Antonio et al., 1970; and Hamburger and Zuckerman, 1976). Though chloroquine given at a dose of

10 mg kg<sup>-1</sup> is effective enough to bring down the parasitaemia, 40 mg kg<sup>-1</sup> for 4 days has also been shown to allow a relapse. This means that though the parasite count in the peripheral blood became negative some parasites were still surviving to provoke the immunity.

On the other hand, the soluble antigens may be produced by the phagocytic cells after taking up the parasites (Seitz, 1976). Soluble malarial antigens have been shown with different species of plasmodium infections (p.37). Seitz (1976) showed that antibodies directed against the soluble antigens are more reliable indicators of the immune status of the animals than the fluorescent antibody. He also demonstrated that plasmodial extracts prepared by breaking up parasite material by physical means contain less antigen than the supernatant of the macrophage cell culture or sera from the infected mice.

4.4  
Cox (1966) has shown an immunogenic effect against P.berghei could be obtained in rats by the injection of soluble antigens extracted from the plasma of monkeys during acute P.knowlesi infection. So, we can assume that soluble antigens are produced during treatment and these antigens are necessary for the provoking of the immune response of the animal. However, Collins et al. (1977) attempted to immunize monkeys against P.knowlesi infections by serum-soluble S-antigens. According to them the protection obtained by this method was incomplete and that the antibodies to the soluble antigens had only a limited role in protection of the host against malarial infection. But it has been suggested by Wilson (1974)

that S-antigens are of host origin. According to him, the soluble antigens circulate for a week after chemotherapy and induced homologous antibody.

The exact mechanism of resistance to reinfection in malaria infection is not known. Rank and Weidanz (1976), have shown that after curative treatment of P.gallinaceum, bursaless chickens resisted a challenge infection with the same parasite and they suggested that non-sensitizing immunity is B-cell independent. Gravely and Kreier (1976) prepared T and B lymphocytes from spleens of rats immune to P.berghei and transferred the cells to non-immune rats to test their ability to control a challenge infection. According to them, a B-cell enriched fraction prepared on nylon wool columns was more protective than immune T-cells but less protective than a combined T and B cell population. They suggested that a differentiated B-type lymphocyte, which lacked both theta antigen and the complement receptor, probably an antibody-producing cell, was responsible for conferring immunity to malaria.

Jayawardena (1975) showed that immune spleen cells were highly effective in transferring immunity to normal and T-cell deprived recipients. Treatment of these cells with anti-theta antiserum did not remove their protective effect, so they suggested that the effector cells in the immune spleen cell population were not T-cells.

McDonald and Phillips (1975) demonstrated that when unfractionated spleen cells from mice immune to P.chabaudi infection were transferred to donor mice, they conferred resistance to reinfection, T-cells alone were less effective

and immune macrophages transferred little or no protection. According to them the resistance to reinfection was due to the co-operation of T-cells with other cells, probably radiosensitive cells. They were supported by the work of Brown et al. (1976), who demonstrated that unfractionated spleen cells and T-cells from P.berghei-infected rats could transfer protection to syngenic recipients as early as 11 days after infection of cell donors. They also showed that protection conferred by T-cells increased with the duration of the infection in the donors, at least up to 100 days, and the additional presence of B-cells in transferred lymphocyte populations enhanced the protective capacity shown by T-cells alone. The authors suggested that the protective immunity is likely to be due to the T-cells exerting their protective action mainly through specific co-operation with B-cells. Roberts et al. (1977) also demonstrated by a series of experiments in Nu/Nu mice infected with P.yoelii that T-cells play a crucial role in the control of malaria infection by their helper function in the production of antibody. In my preliminary studies, the chloroquine cured P.chabaudi infected T-cell deprived mice were not as resistant to reinfection as intact treated controls. Probably this indicates that T-cells play a major role in the resistance to reinfection, though some other cells may play some part as well.

However, my experiments showed that duration of infection was as important as density of parasitaemia for development of immunity after chemotherapy.

E. Drug treatment of P.berghei in intact  
and T-cell deprived mice.

1. The effect of early treatment with chloroquine

a. Suppressive treatment with chloroquine for 4 days from  
the day of infection (day 0)

T-cell deprivation for this experiment was by adult thymectomy, irradiation and bone marrow reconstitution. Three groups of mice, T-cell deprived, sham thymectomized and intact (normal), were infected with P.berghei and treated with  $10 \text{ mg kg}^{-1} \text{ day}^{-1}$  of chloroquine on days 0-3 inclusive. Control groups were infected but not drug treated.

The pattern of parasitaemia (table XVIII B) of the three untreated groups were similar. The parasitaemia was below 1.5% on day 3 and rose to 18-25% on day 8. Terminal parasitaemia ranged from 30-65%, and intact and sham operated mice began to die on day 7, while the first death of the deprived control mice was on the 32nd day. The mean survival time for the intact group was  $16.2 \pm 8.7$  days, for the sham group was  $16.2 \pm 5.5$  days and for the deprived group was  $36.2 \pm 3$  days.

Mice in the treated groups remained aparasitaemic for at least 8 days on blood film examination (fig 24). 5 out of 10 intact and treated mice became blood film positive and died with a mean survival time of  $28.2 \pm 5.6$  days. Only 2 out of 10 mice from each of the sham and T-cell deprived groups became parasitaemic and died with mean survival times



Table XVIII A Statistical analysis of the data in Fig 24. (n=10)

Group	Day														
	Date	3	5	8	10	12	15	17	19	22	26	29	33	35	37
Deprived treated	Mean	0.0	0.0	0.0	0.02	6.9	9.3	14.1	18.4	14.5	14.3	2.1	24.8	15.3	12.0
	SD	-	-	-	0.01	3.2	4.2	8.8	6.6	5.7	3.8	12.7	5.3	6.8	2.7
Intact treated	Mean	0.0	0.0	0.0	0.01	0.7	11.5	3.7	4.0	20.7	24.6	19.8	1.1	5.0	4.0
	SD	-	-	-	-	0.8	14.7	1.1	2.3	4.1	9.3	30.6	0.0	0.0	0.0
Significance	P <	NS	NS	NS	NS	0.001	NS	0.01	0.001	0.05	0.02	NS	0.001	0.001	0.001
Deprived treated	Mean	0.0	0.0	0.0	0.02	6.9	9.3	14.1	18.4	14.5	14.3	2.1	24.8	15.3	12.0
	SD	-	-	-	0.01	3.2	4.2	8.8	6.6	5.7	3.8	12.7	5.3	6.8	2.7
Sham treated	Mean	0.0	0.0	0.0	0.02	0.3	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD	-	-	-	-	0.4	0.9	-	-	-	-	-	-	-	-
Significance	P <	NS	NS	NS	NS	0.001	NS	0.001	0.001	0.001	0.001	NS	0.001	0.001	0.001

The figures correspond to the mean percentage parasitaemia and SDs of the recrudescences (2 in both sham and deprived treated group and 5 in intact group).

For untreated controls see Table XVIII B

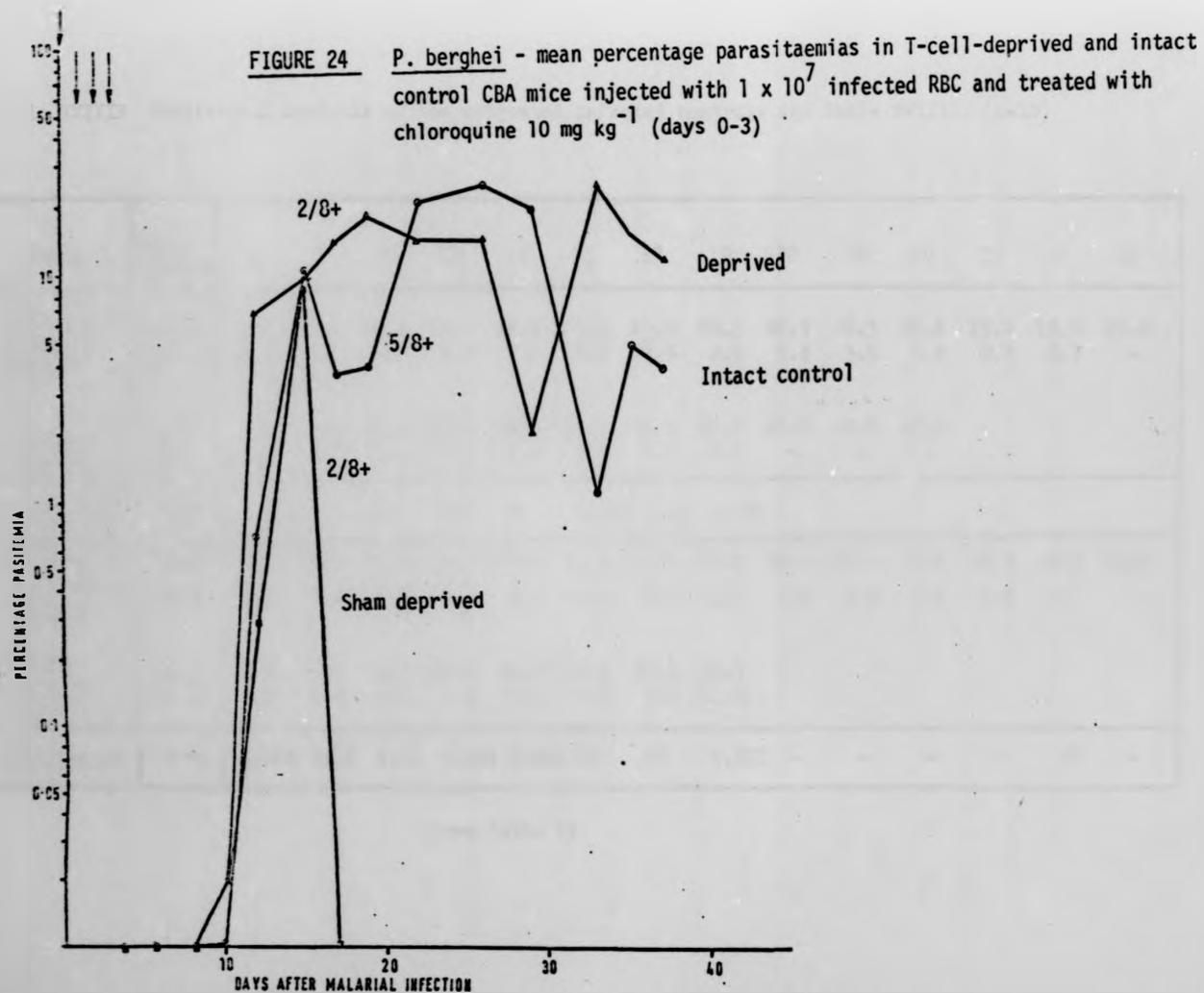


Table XVIII B Statistical analysis of the untreated infected controls for Table XVIII A. (n=10)

Group	Day Data	3	5	8	10	12	15	17	19	22	26	29	33	35	37
		Deprived infected control	Mean SD †	1.5 0.3	10.9 2.1	25.4 6.0	19.6 5.0	24.6 2.0	23.4 3.6	29.5 7.5	20.9 4.5	28.5 8.4	26.3 3.5	28.6 3.9	19.0 0.7
Intact infected control	Mean SD †	0.7 0.3	8.8 2.8	23.2 6.2	16.2 9.5	19.1 1.2	31.5 3.4	36.7 3.1	62.7 2.5	65.0 -	47.0 -	47.0 -			
Significance	P <	0.001	NS	NS	NS	NS	0.001	0.02	0.001						
Deprived infected control	Mean SD †	1.5 0.3	10.9 2.1	25.4 6.0	19.6 5.0	24.6 2.0	23.4 3.6	29.5 7.5	20.9 4.5	28.5 8.4	26.3 3.5	28.6 3.9	19.0 0.7	19.0 0.7	25.0 -
Sham infected control	Mean SD †	0.7 0.1	9.1 1.2	18.2 4.4	11.3 1.6	15.6 0.8	18.6 7.6	26.9 2.0	37.3 11.0						
Significance	P <	0.001	0.05	0.01	0.001	0.001	NS	NS	0.001	-	-	-	-	-	-

\*see table VI

of 17 days and  $34.3 \pm 6.2$  days respectively.

The recrudescence parasites were stabilised on day 15 for drug sensitivity testing.

b. Treatment with chloroquine for 7 days from day 2 of infection

T-cell deprivation in this experiment was by adult thymectomy and ATS (1) treatment. From day 2, treatment with chloroquine at  $10 \text{ mg kg}^{-1}$  daily for 7 days was given to groups of deprived, sham deprived and intact mice. The parasitaemia was measured every 2-3 days (fig 25). The patterns of parasitaemia of the three untreated control groups were again similar. The parasitaemia was about 1% when treatment was started and reached 30-40% on the 14th day. Terminal parasitaemia was 50-65%. The deaths in the intact untreated group started on the 9th day and in the T-cell deprived animals on the 23rd day. The mean survival time for the intact group was  $18.1 \pm 6.4$  days, sham  $20.4 \pm 2.7$  and the deprived group  $25.8 \pm 3.5$  days. The treated groups became aparasitaemic after 3 days of treatment and remained so until day 16 of the infection in intact and day 18 in deprived and sham deprived mice. In the intact group, 7 out of 8 mice showed a recrudescence of infection, and died with a mean survival time of  $32 \pm 17.1$  days. 4 out of 8 in the T-cell deprived group showed a recrudescence starting from day 18 of the infection. Only 1 died from the T-cell deprived group on day 62 of the infection. The rest recovered. The parasites were stabilised on day 21 as before (fig 25).



Table XIXA Statistical analysis of the data in Fig 25a. (n=8)

Group	Day Data	2	4	7	9	11	14	16	18	21	23	25	31	33	37	41	43	48	52	55	60
Deprived treated	Mean	1.4	0.01	0.0	0.0	0.0	0.0	0.6	0.03	0.9	0.8	1.6	1.6	1.2	0.2	0.4	0.2	0.1	1.0	0.4	0.4
	SD †	1.0	0.1	-	-	-	-	0.02	0.01	0.5	0.3	0.8	0.6	0.3	0.1	0.2	0.03	0.04	0.03	0.1	0.2
Intact treated	Mean	1.1	0.02	0.0	0.0	0.0	0.0	0.06	2.4	5.8	4.2	9.0	27.0	32.0	29.0	32.0	34.0	15.0	28.0	32.0	31.0
	SD †	0.33	0.02	-	-	-	-	0.06	1.5	2.1	1.8	3.1	6.1	7.1	3.8	9.8	12.0	6.2	10.5	13.0	14.0
Significance	P <	NS	-	NS	NS	NS	NS	NS	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

- see table XIXB for untreated controls and table VI for abbreviations

- The figures correspond to the mean parasitaemias of recrudescences only (7/8 + in intact, 4/8 + in deprived mice after treatment.)

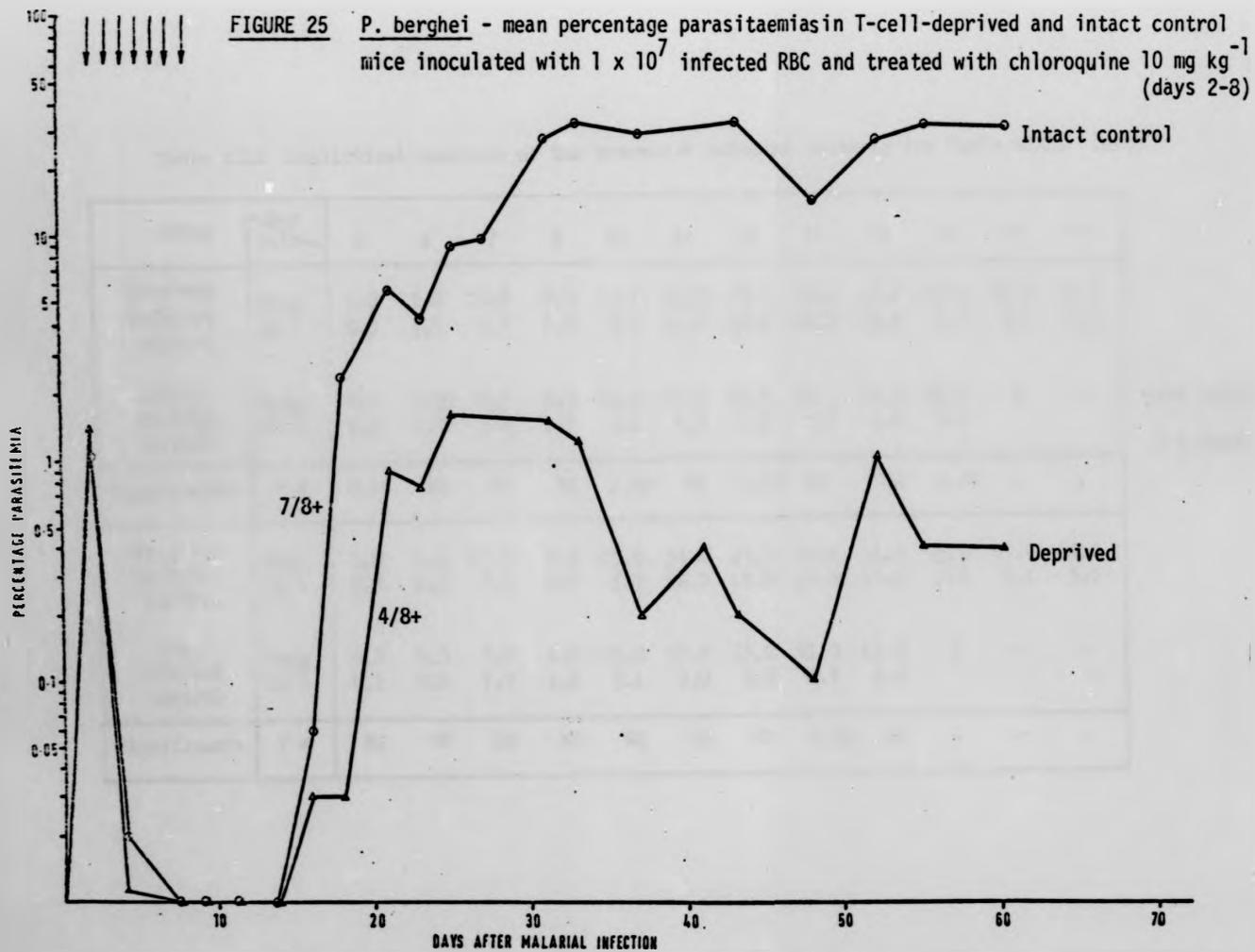


Table XIXB Statistical analysis of the untreated infected controls for Table XIXA. (n=9)

Group	Day Data	2	4	7	9	11	14	16	18	21	23	25	27
Deprived infected control	Mean	1.0	6.0	10.0	6.3	13.0	34.0	43.5	43.4	53.0	63.0	65.0	62.0
	SD	0.3	1.1	5.3	2.0	3.9	12.8	12.8	10.8	19.9	6.8	9.1	5.0
Intact infected control	Mean	0.7	4.03	9.5	8.0	18.0	36.0	56.0	52.0	67.0	57.0	D	-
	SD	0.2	2.7	3.2	1.7	3.1	8.2	6.3	5.7	4.6	3.5		
Significance	P <	0.05	NS	NS	NS	0.02	NS	0.05	NS	NS	0.05	-	-
Deprived infected control	Mean	1.0	6.0	10.0	6.3	13.0	34.0	43.5	43.4	53.0	63.0	65.0	62.0
	SD	0.3	1.1	5.3	2.0	3.9	12.8	12.8	10.8	19.9	6.8	9.1	5.0
Sham infected control	Mean	0.8	5.0	7.0	6.0	15.0	37.0	53.0	61.0	63.0	D	-	-
	SD	0.2	0.8	1.7	1.6	3.1	9.0	8.6	4.2	4.2			
Significance	P <	NS	NS	NS	NS	NS	NS	NS	0.001	NS	-	-	-

-see table VI

D = death

1. The first part of the report  
 2. The second part of the report  
 3. The third part of the report  
 4. The fourth part of the report  
 5. The fifth part of the report  
 6. The sixth part of the report  
 7. The seventh part of the report  
 8. The eighth part of the report  
 9. The ninth part of the report  
 10. The tenth part of the report

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

The following table shows the results of the experiment. The first column shows the number of trials, and the second column shows the number of successes. The third column shows the percentage of successes. The fourth column shows the standard deviation. The fifth column shows the confidence interval.

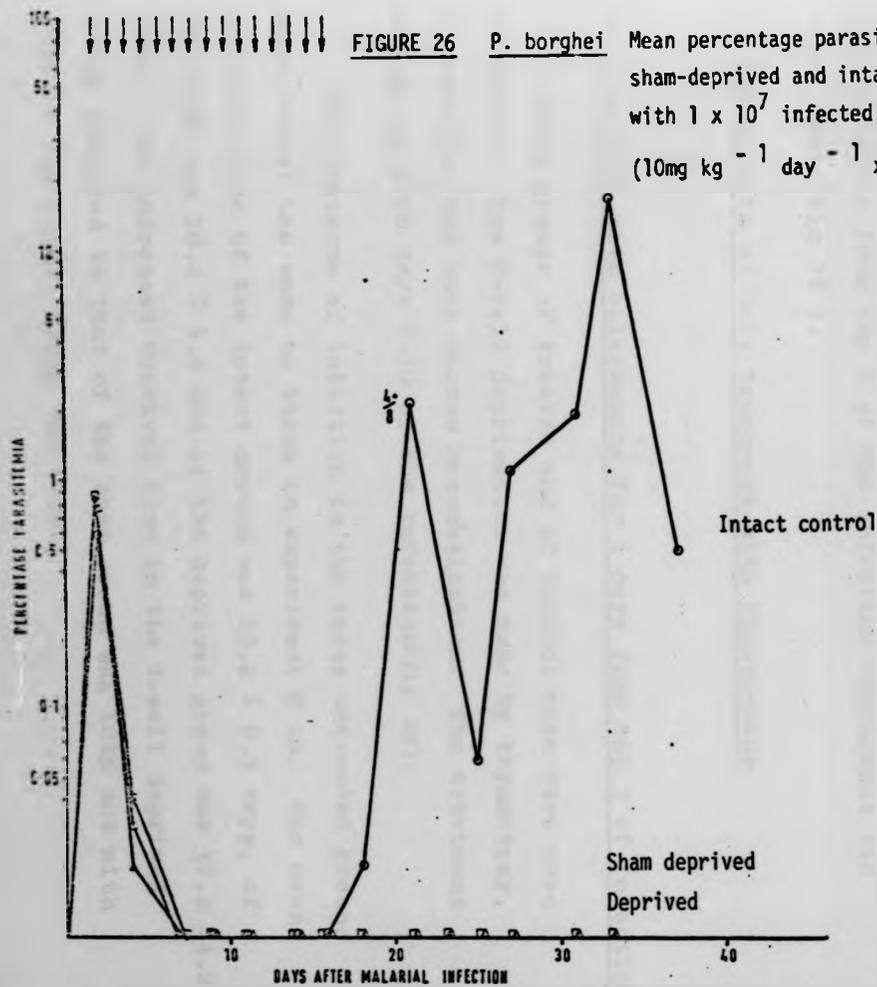


Table XX Statistical analysis of the data in Fig 26. (n=8)

Group	Day Data	2	4	7	9	11	14	16	18	21	23	25	27	31	33	37
Deprived treated	Mean	0.8	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD <sup>+</sup>	0.2	0.01													
Intact treated	Mean	0.6	0.03	0.0	0.0	0.0	0.0	0.0	0.02	2.0	0.3	0.06	1.1	1.9	17.0	0.5
	SD <sup>-</sup>	0.3	0.2						0.01	1.0	0.1	0.03	0.5	0.7	6.0	0.1
Significance	P <	NS	NS	NS	NS	NS	NS	NS	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Deprived treated	Mean	0.8	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD <sup>+</sup>	0.4	0.01													
Sham treated	Mean	0.8	0.04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD <sup>+</sup>	0.4	0.02													
Significance	P <	NS	0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

- Figures after day 16 correspond to mean percentage parasitaemias of recrudescences only. (4/8 recrudesced in intact group).

see table XIXB for uninfected controls and table VI for abbreviations



c. Treatment with chloroquine for 14 days from day 2 of infection

This experiment was similar to b (above) except the period of treatment was from day 2 to day 15.

The treated intact and sham groups and deprived groups became aparasitaemic from day 3 of the infection. Four out of 8 of the intact group became parasitaemic again on day 18 of the infection and reached a maximum mean parasitaemia of 17%. The infection resolved later and all the mice survived. The deprived and sham deprived treated groups remained aparasitaemic from day 4 of the infection throughout the experiment (fig 26 ).

2. The effects of late treatment with chloroquine

a. Treatment with chloroquine for 4 days from day 7 of infection

Three groups of treated and of control mice were used as before. The T-cell deprivation was done by thymectomy, irradiation and bone marrow reconstitution. The treatment was given from days 7-10 of the infection (fig 27).

The patterns of infection in the three untreated groups were almost the same as those in experiment E 1a. The mean survival time of the intact groups was  $15.2 \pm 8.7$  days, of the sham was  $18.4 \pm 5.6$  and of the deprived group was  $37.2 \pm 4.2$  days. The increased survival time in the T-cell deprived group compared to that of the sham group was 101% and with that of the intact group was 145%.

Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
1900	10	15	20	25	30	35	40	45	50	55	60	65
1901	12	18	23	28	33	38	43	48	53	58	63	68
1902	14	20	25	30	35	40	45	50	55	60	65	70
1903	16	22	27	32	37	42	47	52	57	62	67	72
1904	18	24	29	34	39	44	49	54	59	64	69	74
1905	20	26	31	36	41	46	51	56	61	66	71	76
1906	22	28	33	38	43	48	53	58	63	68	73	78
1907	24	30	35	40	45	50	55	60	65	70	75	80
1908	26	32	37	42	47	52	57	62	67	72	77	82
1909	28	34	39	44	49	54	59	64	69	74	79	84
1910	30	36	41	46	51	56	61	66	71	76	81	86

1900  
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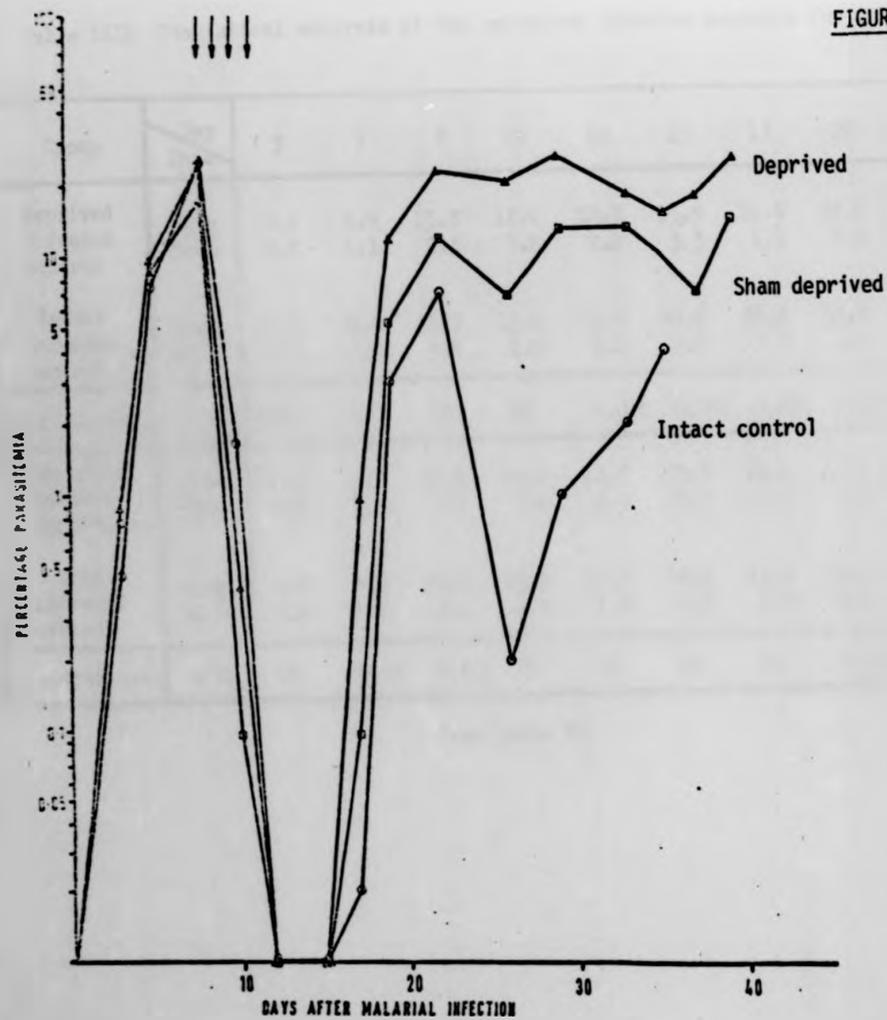
1900  
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Table XXIA Statistical analysis of the data in Fig 27. (n=10)

Group	Day Data	3	5	8	10	12	15	17	19	22	26	29	33	35	37	39
Deprived treated	Mean	0.8	9.8	24.0	0.4	0.0	0.0	0.9	11.5	22.6	20.6	25.7	18.2	15.0	18.0	25.0
	SD	0.4	2.1	8.1	0.4	-	-	2.5	7.1	7.02	8.2	4.0	1.5	2.2	1.1	7.0
Intact treated	Mean	0.5	7.6	25.0	1.6	0.0	0.0	0.02	3.0	7.0	0.2	1.0	2.0	4.0	0.0	0.0
	SD	0.6	2.4	11.6	4.0	-	-	0.01	1.9	3.7	0.01	1.0	0.9	4.0	-	-
Significance	P <	NS	0.05	NS	NS	-	-	NS	0.01	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Deprived treated	Mean	0.8	9.8	24.0	0.4	0.0	0.0	0.9	11.5	22.6	20.6	25.7	18.2	15.0	18.0	25.0
	SD	0.4	2.1	8.1	0.4	-	-	2.5	7.1	7.0	8.2	4.0	1.5	2.2	1.1	7.0
Sham treated	Mean	0.7	8.9	16.6	0.1	0.0	0.0	0.1	5.3	12.0	6.9	12.4	13.0	10.0	7.0	14.0
	SD	0.2	1.1	5.8	0.1	-	-	0.1	1.5	2.4	5.0	4.0	3.0	-	-	-
Significance	P <	NS	NS	0.05	NS	NS	NS	NS	0.02	0.001	0.001	0.001	0.001	-	-	-

- for untreated controls see table XXIB

\*see table VI



**FIGURE 27** *P. berghei* - mean percentage parasitaemias in T-cell-deprived and intact control CBA mice inoculated with  $1 \times 10^7$  infected RBC and treated with chloroquine  $10 \text{ mg kg}^{-1}$  (days 7-10)

Table XXIB Statistical analysis of the untreated infected controls for table XXIA. (n=10)

Group	Day Data	3	5	8	10	12	15	17	19	22	26	29	33	35	37	39
		Deprived infected control	Mean SD ±	0.6 0.3	6.9 1.1	13.3 2.3	14.0 5.6	12.7 2.0	15.9 3.3	21.6 1.9	22.6 6.5	25.2 3.0	27.9 5.8	21.8 3.8	19.3 3.8	26.6 14.0
Intact infected control	Mean SD ±	0.8 0.7	8.8 3.5	15.7 5.8	11.0 2.6	19.0 3.2	30.0 9.9	32.8 9.0	59.0 4.0	32.0	52.0	60.0	33.0	60.0	63.0	-
Significance	P <	NS	NS	NS	NS	0.001	0.001	0.001	0.001	-	-	-	-	-	-	-
Deprived infected control	Mean SD ±	0.6 0.3	6.9 1.1	13.3 2.3	14.0 5.6	12.7 2.0	15.9 3.3	21.6 1.9	22.6 6.5	25.2 3.0	27.9 5.8	21.8 3.8	19.3 3.8	26.3 14.0	41.0 18.8	- -
Sham infected control	Mean SD ±	0.8 0.2	8.3 1.2	21.8 3.1	10.9 4.4	11.9 1.8	16.2 0.3	24.6 7.7	36.3 6.6	44.5 0.7	25.0 2.0	-	-	-	-	-
Significance	P <	NS	0.02	0.001	NS	NS	NS	NS	0.001	0.001	NS	-	-	-	-	-

\*see table VI

All the treated groups became blood film negative after 4 days of treatment. The infections were patent again on day 17 and the parasitaemic patterns were all similar. The mean survival time in the treated deprived group was  $36.4 \pm 5$  days, in the sham deprived group  $22.3 \pm 2.1$  days and in the intact group was  $20.8 \pm 5.2$  days. The mean survival times were not significantly different from those of the corresponding untreated controls (table XXIB).

The parasites were stablited after recrudescence on day 22.

b. Treatment with chloroquine for 7 days from day 7 of the infection

Deprivation was done by thymectomy and ALS (1) treatment but grouping was done as before.

Mice of the experimental groups were treated on days 7-13 after infection at a dose rate of  $10 \text{ mg kg}^{-1} \text{ day}^{-1}$ . All of the treated groups became aparasitaemic after 3 days of treatment, and infections started to recur on day 21. 5 out of 8 from both the deprived and intact groups recrudesced and 4 out of 8 from the sham group (fig 28 ). There was one death in the sham group on day 63 and one in the deprived group on day 31, otherwise the infections resolved. (see table XIXB for untreated controls).

c. Treatment with chloroquine for 14 days from day 7 of infection

Deprivation was done by thymectomy and ALS (1) treatment. The chloroquine treated groups became negative by blood film

The following table shows the results of the analysis of the soil samples collected during the field work. The data are given in the form of a table with the following columns: Date, Location, Sample No., Moisture, pH, Organic Matter, Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sodium, Chloride, Sulfate, and Total Solids.

Date	Location	Sample No.	Moisture	pH	Organic Matter	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sodium	Chloride	Sulfate	Total Solids
10/15/19	Field 1	101	15.2	6.8	2.1	0.15	0.05	0.12	0.08	0.04	0.01	0.02	0.03	18.5
10/16/19	Field 2	102	18.5	7.2	2.5	0.18	0.06	0.15	0.10	0.05	0.02	0.03	0.04	20.0
10/17/19	Field 3	103	12.8	6.5	1.8	0.12	0.04	0.10	0.07	0.03	0.01	0.02	0.03	16.5
10/18/19	Field 4	104	16.0	7.0	2.2	0.16	0.05	0.13	0.09	0.04	0.01	0.02	0.03	19.0
10/19/19	Field 5	105	14.5	6.7	2.0	0.14	0.04	0.11	0.08	0.03	0.01	0.02	0.03	17.5
10/20/19	Field 6	106	17.0	7.1	2.4	0.17	0.06	0.14	0.11	0.05	0.02	0.03	0.04	20.5
10/21/19	Field 7	107	13.5	6.6	1.9	0.13	0.04	0.10	0.07	0.03	0.01	0.02	0.03	17.0
10/22/19	Field 8	108	15.8	6.9	2.1	0.15	0.05	0.12	0.08	0.04	0.01	0.02	0.03	18.5
10/23/19	Field 9	109	16.5	7.0	2.3	0.16	0.05	0.13	0.09	0.04	0.01	0.02	0.03	19.5
10/24/19	Field 10	110	14.0	6.7	1.9	0.13	0.04	0.11	0.07	0.03	0.01	0.02	0.03	17.0

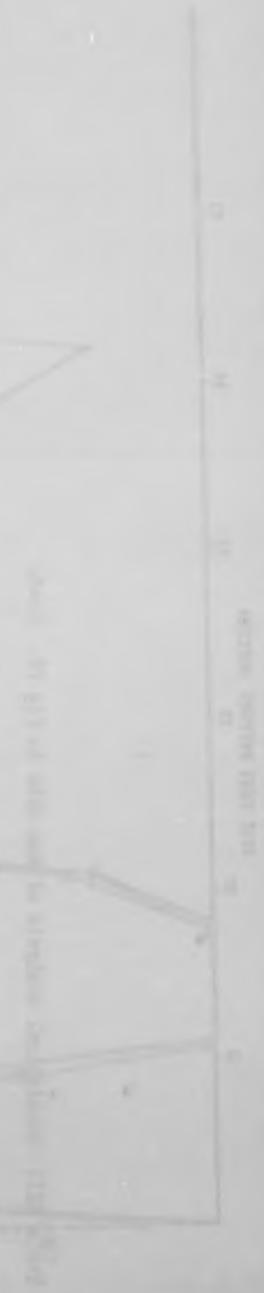


Table XXII Statistical analysis of the data in Fig 28. (n=8)

Group	Day Data	2	4	7	9	11	14	16	18	21	23	25	27	31	33	37	41	43	48	52	55
Deprived treated	Mean	0.9	6.0	8.0	0.2	0.0	0.0	0.0	0.0	0.03	0.8	2.8	4.2	4.8	13.0	22.0	19.0	22.0	0.1	0.03	2.0
	SD $\pm$	0.3	2.0	3.2	0.1						0.1	0.5	0.7	2.0	3.3	6.1	7.2	2.3	4.1	0.02	0.01
Intact treated	Mean	0.6	4.3	6.0	1.1	0.0	0.0	0.0	0.0	0.03	0.4	2.6	1.6	5.0	1.5	0.6	3.4	3.5	0.2	0.08	0.4
	SD $\pm$	0.3	2.1	3.1	0.03						0.01	0.02	1.3	0.5	1.2	0.3	0.2	1.2	0.8	0.1	0.03
Significance	P <	NS	NS	NS	0.001	NS	NS	NS	NS	NS	0.05	NS	0.01	NS	0.001	0.001	0.001	0.001	0.02	0.02	0.01
Deprived treated	Mean	0.9	6.0	8.0	0.2	0.0	0.0	0.0	0.0	0.03	0.8	2.8	4.2	4.8	13.0	22.0	19.0	22.0	0.1	0.03	2.0
	SD $\pm$	0.3	2.0	3.2	0.1						0.01	0.5	0.7	2.0	3.3	6.1	7.2	2.3	4.1	0.02	0.01
Sham treated	Mean	0.5	4.7	7.2	0.6	0.0	0.0	0.0	0.0	0.04	2.0	2.7	3.0	12.0	17.0	8.0	4.0	32.0	28.0	29.0	20.1
	SD $\pm$	0.2	2.1	3.2	0.3						0.02	1.1	1.2	0.9	2.5	4.1	3.1	1.1	8.2	6.4	5.1
Significance	P <	0.01	NS	NS	0.01	NS	NS	NS	NS	NS	0.02	NS	0.001	0.001	NS	0.001	0.001	0.01	0.001	0.01	0.01

see table XIXB for uninfected controls, and table VI for abbreviations

- figures after day 18 correspond to the mean parasitaemia ( $\pm$  SD) of the recrudescences only  
(5 mice recrudesced in both intact and deprived groups, 4 mice in sham group).

see table LIXB for uninfected controls, and table VI for abbreviations

- figures after day 18 correspond to the mean parasitaemia ( $\pm$  SD) of the recrudescences only (5 mice recrudesced in both intact and deprived groups, 4 mice in sham group).

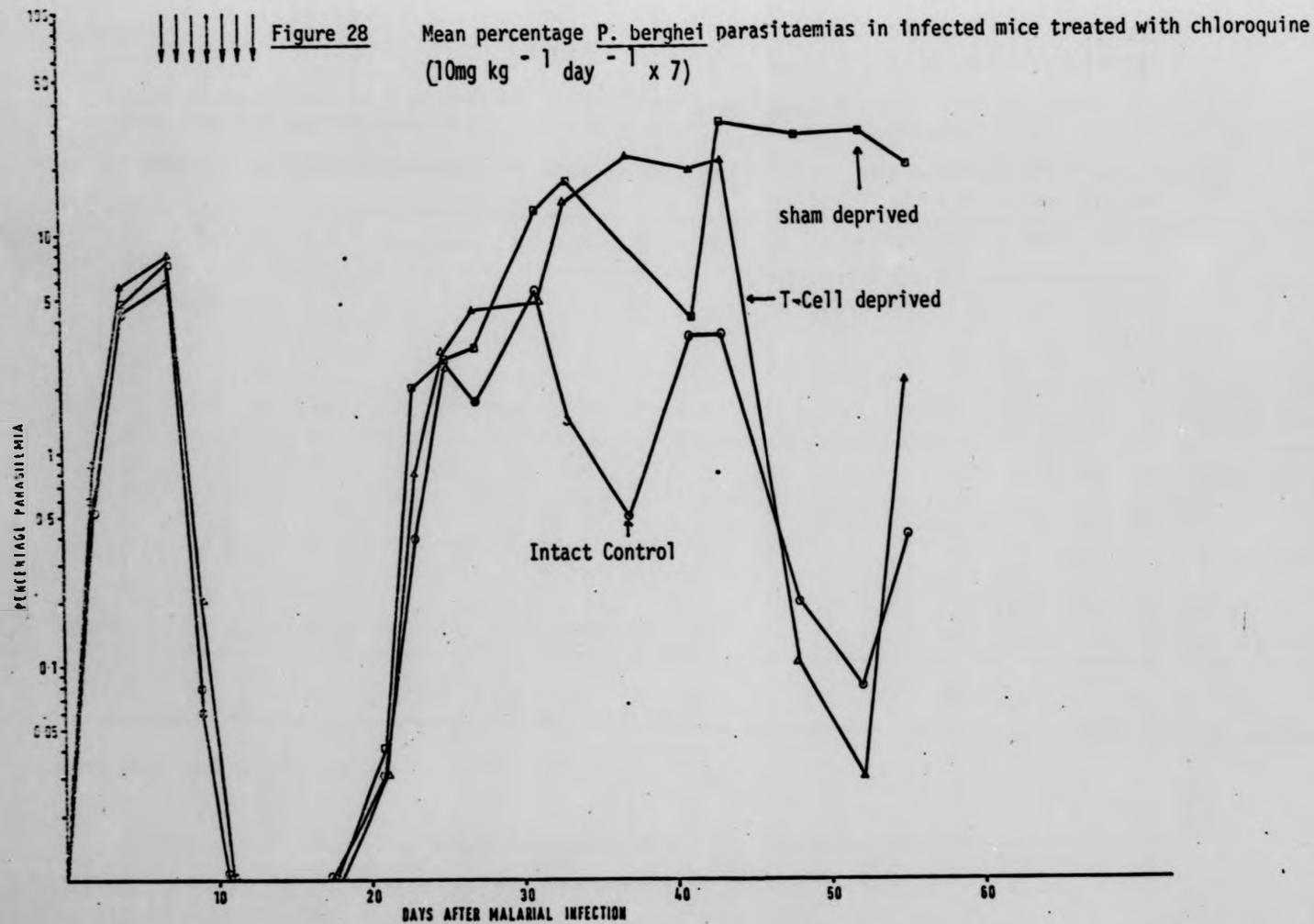


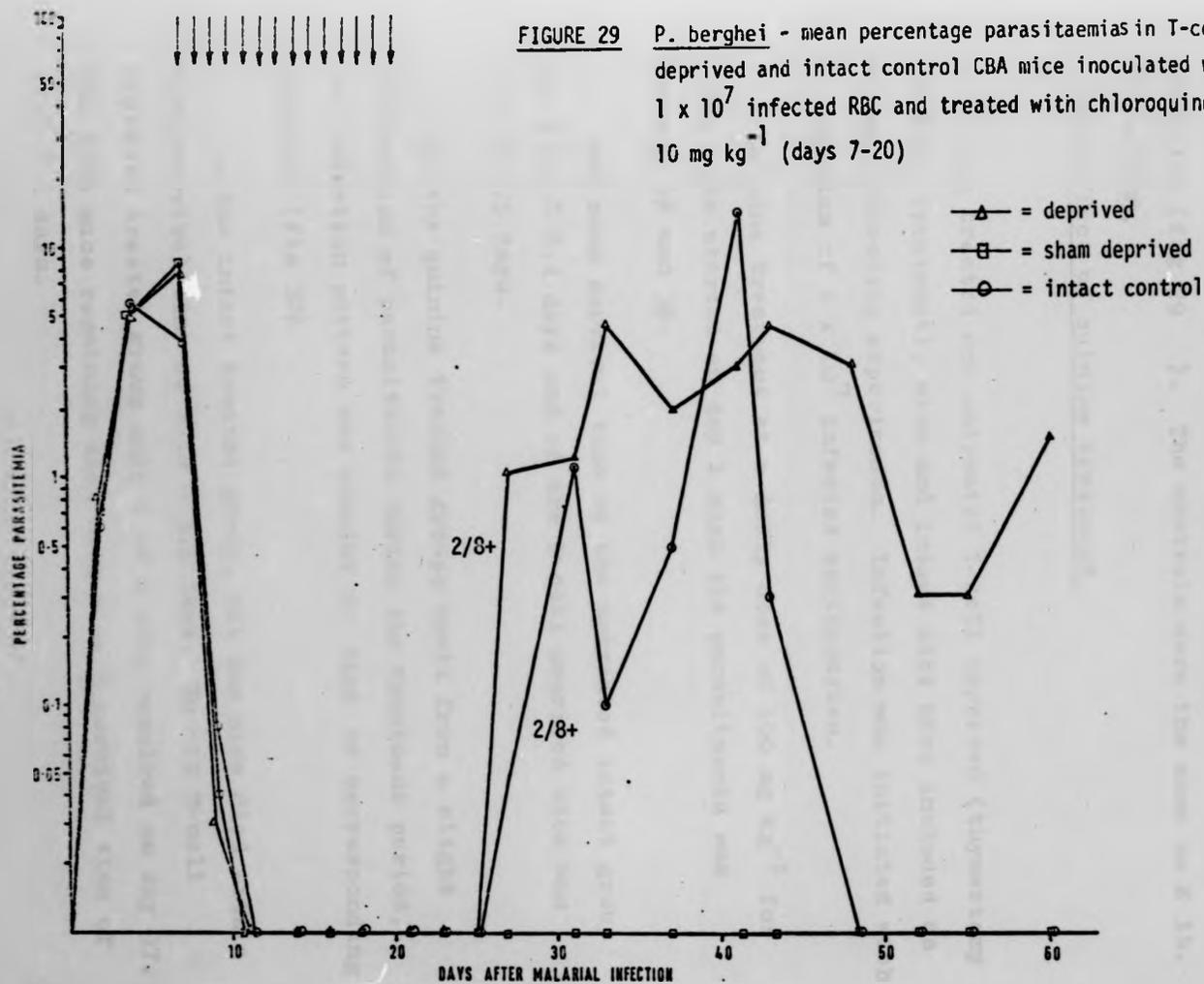


Table XXIII Statistical analysis of the data in Fig 29. (n=8)

Group	Day Data	2	4	7	9	11	14	16	18	21	23	25	27	31	33	37	41	43	48	52	55	60
Deprived treated	Mean	0.8	5.0	7.8	0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.2	4.5	2.1	3.0	4.5	3.0	0.3	0.3	1.5
	SD $\pm$	0.3	1.0	2.0	0.01									0.6	0.7	1.3	0.3	1.0	1.3	0.9	0.1	0.1
Intact treated	Mean	0.6	5.8	4.0	0.08	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	1.3	0.1	0.5	14.0	0.3	0.0	0.0	0.0	0.0
	SD $\pm$	0.4	2.1	1.1	0.02									0.02	0.7	0.02	0.2	5.0	0.1			
Significance	P<	NS	NS	0.001	0.001	NS	0.001	NS	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001						
Deprived treated	Mean	0.8	5.0	7.8	0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.2	4.5	2.1	3.0	4.5	3.0	0.3	0.3	1.5
	SD $\pm$	0.3	1.0	2.0	0.01									0.6	0.7	1.3	0.3	1.0	1.3	0.9	0.1	0.1
Sham treated	Mean	0.7	5.0	8.5	0.04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	AD	-	-	-	-	-	-	-	-	-
	SD $\pm$	0.2	1.2	3.2	0.01																	
Significance	P<	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	-	-	-	-	-	-	-	-	-

see table XIXR for untreated controls and table VI for abbreviations. AD=accidental death due to drowning

figures after day 25 correspond to the mean parasitaemias ( $\pm$  SD) of recrudescences only.  
(2 mice recrudesced in both deprived and intact groups).



examination after the 3rd day of infection and remained so for 16 days. 2 out of 8 mice from both the deprived and the intact groups subsequently showed recrudescence of infection (fig 29 ). The controls were the same as E lb. ( table XIXB).

### 3. The effect of quinine treatment

Drug treated and untreated T-cell deprived (thymectomy and ALS<sub>2</sub> treatment), sham and intact mice were included as in the preceding experiments. Infection was initiated with an inoculum of  $1 \times 10^7$  infected erythrocytes.

Quinine treatment at a daily dose of  $100 \text{ mg kg}^{-1}$  for 7 days was started on day 1 when the parasitaemia was between 1% and 3%.

The mean survival time of the untreated intact group was  $17.4 \pm 8.4$  days and of the T-cell deprived mice was  $33.3 \pm 6.5$  days.

In the quinine treated groups apart from a slight suppression of parasitaemia during the treatment period, the infection pattern was similar to that of corresponding controls (fig 30)

In the intact treated group, all the mice died with a mean survival time of  $27.8 \pm 3.4$  days. In the T-cell deprived treated group only 1 of 6 mice resolved on day 37. The five mice remaining died with a mean survival time of  $37.8 \pm 1$  days.

TABLE 1. Data for the 1950-1951 season.  
 (a) General data for the season. (b) 1950-1951 season. (c) 1951-1952 season. (d) 1952-1953 season. (e) 1953-1954 season. (f) 1954-1955 season. (g) 1955-1956 season. (h) 1956-1957 season. (i) 1957-1958 season. (j) 1958-1959 season. (k) 1959-1960 season. (l) 1960-1961 season. (m) 1961-1962 season. (n) 1962-1963 season. (o) 1963-1964 season. (p) 1964-1965 season. (q) 1965-1966 season. (r) 1966-1967 season. (s) 1967-1968 season. (t) 1968-1969 season. (u) 1969-1970 season. (v) 1970-1971 season. (w) 1971-1972 season. (x) 1972-1973 season. (y) 1973-1974 season. (z) 1974-1975 season. (aa) 1975-1976 season. (ab) 1976-1977 season. (ac) 1977-1978 season. (ad) 1978-1979 season. (ae) 1979-1980 season. (af) 1980-1981 season. (ag) 1981-1982 season. (ah) 1982-1983 season. (ai) 1983-1984 season. (aj) 1984-1985 season. (ak) 1985-1986 season. (al) 1986-1987 season. (am) 1987-1988 season. (an) 1988-1989 season. (ao) 1989-1990 season. (ap) 1990-1991 season. (aq) 1991-1992 season. (ar) 1992-1993 season. (as) 1993-1994 season. (at) 1994-1995 season. (au) 1995-1996 season. (av) 1996-1997 season. (aw) 1997-1998 season. (ax) 1998-1999 season. (ay) 1999-2000 season. (az) 2000-2001 season. (ba) 2001-2002 season. (bb) 2002-2003 season. (bc) 2003-2004 season. (bd) 2004-2005 season. (be) 2005-2006 season. (bf) 2006-2007 season. (bg) 2007-2008 season. (bh) 2008-2009 season. (bi) 2009-2010 season. (bj) 2010-2011 season. (bk) 2011-2012 season. (bl) 2012-2013 season. (bm) 2013-2014 season. (bn) 2014-2015 season. (bo) 2015-2016 season. (bp) 2016-2017 season. (bq) 2017-2018 season. (br) 2018-2019 season. (bs) 2019-2020 season. (bt) 2020-2021 season. (bu) 2021-2022 season. (bv) 2022-2023 season. (bv) 2023-2024 season. (bv) 2024-2025 season.



Year	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025																																																																																																																											
Total	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150	155	160	165	170	175	180	185	190	195	200	205	210	215	220	225	230	235	240	245	250	255	260	265	270	275	280	285	290	295	300	305	310	315	320	325	330	335	340	345	350	355	360	365	370	375	380	385	390	395	400	405	410	415	420	425	430	435	440	445	450	455	460	465	470	475	480	485	490	495	500	505	510	515	520	525	530	535	540	545	550	555	560	565	570	575	580	585	590	595	600	605	610	615	620	625	630	635	640	645	650	655	660	665	670	675	680	685	690	695	700	705	710	715	720	725	730	735	740	745	750	755	760	765	770	775	780	785	790	795	800	805	810	815	820	825	830	835	840	845	850	855	860	865	870	875	880	885	890	895	900	905	910	915	920	925	930	935	940	945	950	955	960	965	970	975	980	985	990	995	1000
Net	5	8	12	18	25	32	40	48	55	62	70	78	85	92	100	108	115	122	130	138	145	152	160	168	175	182	190	198	205	212	220	228	235	242	250	258	265	272	280	288	295	302	310	318	325	332	340	348	355	362	370	378	385	392	400	408	415	422	430	438	445	452	460	468	475	482	490	498	505	512	520	528	535	542	550	558	565	572	580	588	595	602	610	618	625	632	640	648	655	662	670	678	685	692	700	708	715	722	730	738	745	752	760	768	775	782	790	798	805	812	820	828	835	842	850	858	865	872	880	888	895	902	910	918	925	932	940	948	955	962	970	978	985	992	1000																																																																

(b) Net (shaded) for the 1950-1951 season. (c) Net (shaded) for the 1951-1952 season. (d) Net (shaded) for the 1952-1953 season. (e) Net (shaded) for the 1953-1954 season. (f) Net (shaded) for the 1954-1955 season. (g) Net (shaded) for the 1955-1956 season. (h) Net (shaded) for the 1956-1957 season. (i) Net (shaded) for the 1957-1958 season. (j) Net (shaded) for the 1958-1959 season. (k) Net (shaded) for the 1959-1960 season. (l) Net (shaded) for the 1960-1961 season. (m) Net (shaded) for the 1961-1962 season. (n) Net (shaded) for the 1962-1963 season. (o) Net (shaded) for the 1963-1964 season. (p) Net (shaded) for the 1964-1965 season. (q) Net (shaded) for the 1965-1966 season. (r) Net (shaded) for the 1966-1967 season. (s) Net (shaded) for the 1967-1968 season. (t) Net (shaded) for the 1968-1969 season. (u) Net (shaded) for the 1969-1970 season. (v) Net (shaded) for the 1970-1971 season. (w) Net (shaded) for the 1971-1972 season. (x) Net (shaded) for the 1972-1973 season. (y) Net (shaded) for the 1973-1974 season. (z) Net (shaded) for the 1974-1975 season. (aa) Net (shaded) for the 1975-1976 season. (ab) Net (shaded) for the 1976-1977 season. (ac) Net (shaded) for the 1977-1978 season. (ad) Net (shaded) for the 1978-1979 season. (ae) Net (shaded) for the 1979-1980 season. (af) Net (shaded) for the 1980-1981 season. (ag) Net (shaded) for the 1981-1982 season. (ah) Net (shaded) for the 1982-1983 season. (ai) Net (shaded) for the 1983-1984 season. (aj) Net (shaded) for the 1984-1985 season. (ak) Net (shaded) for the 1985-1986 season. (al) Net (shaded) for the 1986-1987 season. (am) Net (shaded) for the 1987-1988 season. (an) Net (shaded) for the 1988-1989 season. (ao) Net (shaded) for the 1989-1990 season. (ap) Net (shaded) for the 1990-1991 season. (aq) Net (shaded) for the 1991-1992 season. (ar) Net (shaded) for the 1992-1993 season. (as) Net (shaded) for the 1993-1994 season. (at) Net (shaded) for the 1994-1995 season. (au) Net (shaded) for the 1995-1996 season. (av) Net (shaded) for the 1996-1997 season. (aw) Net (shaded) for the 1997-1998 season. (ax) Net (shaded) for the 1998-1999 season. (ay) Net (shaded) for the 1999-2000 season. (az) Net (shaded) for the 2000-2001 season. (ba) Net (shaded) for the 2001-2002 season. (bb) Net (shaded) for the 2002-2003 season. (bc) Net (shaded) for the 2003-2004 season. (bd) Net (shaded) for the 2004-2005 season. (be) Net (shaded) for the 2005-2006 season. (bf) Net (shaded) for the 2006-2007 season. (bg) Net (shaded) for the 2007-2008 season. (bh) Net (shaded) for the 2008-2009 season. (bi) Net (shaded) for the 2009-2010 season. (bj) Net (shaded) for the 2010-2011 season. (bk) Net (shaded) for the 2011-2012 season. (bl) Net (shaded) for the 2012-2013 season. (bm) Net (shaded) for the 2013-2014 season. (bn) Net (shaded) for the 2014-2015 season. (bo) Net (shaded) for the 2015-2016 season. (bp) Net (shaded) for the 2016-2017 season. (bq) Net (shaded) for the 2017-2018 season. (br) Net (shaded) for the 2018-2019 season. (bs) Net (shaded) for the 2019-2020 season. (bt) Net (shaded) for the 2020-2021 season. (bu) Net (shaded) for the 2021-2022 season. (bv) Net (shaded) for the 2022-2023 season. (bv) Net (shaded) for the 2023-2024 season. (bv) Net (shaded) for the 2024-2025 season.

Table XXIV Statistical analysis of the data in Fig 30 and it's untreated infected controls (n=6)

Group	Day Data	1	2	3	7	10	12	14	17	21	23	27	30	33	35	40	44	47	53	57
Deprived treated	Mean	1.3	3.0	3.7	3.8	7.0	15.3	15.6	28.0	33.0	31.0	25.0	38.0	61.5	1.0	0.0	0.0	0.03	3.5	0.1
	SD <sup>+</sup>	0.2	0.4	1.5	1.3	1.7	4.0	0.6	11.3	5.1	27.0	30.0	34.0	4.9	0.3					
Intact treated	Mean	1.0	3.3	2.8	1.3	6.0	7.3	16.5	26.0	51.0	58.0	63.0	D							
	SD <sup>+</sup>	0.3	1.0	1.1	0.9	3.4	7.4	4.7	5.7	12.5	5.7	0.7								
Significance	P <	NS	NS	NS	0.01	NS	0.05	NS	NS	0.01	0.05	0.01	-	-	-	-	-	-	-	-
Deprived infected control	Mean	0.6	1.4	3.0	5.5	13.0	15.0	19.3	19.0	29.0	47.0	57.0	49.0							
	SD <sup>+</sup>	0.5	1.5	2.7	3.0	6.6	7.6	10.7	7.2	5.0	4.0	11.7	19.0							
Intact infected control	Mean	1.07	3.4	6.7	23.6	22.0	21.0	15.0	23.0	51.0	62.0	D								
	SD <sup>+</sup>	0.6	2.2	1.9	23.7	12.0	14.0	3.5	5.3	21.0	-									
Significance	P <	NS	NS	0.02	NS	NS	NS	NS	NS	0.05	-	-	-							

\*see table VI

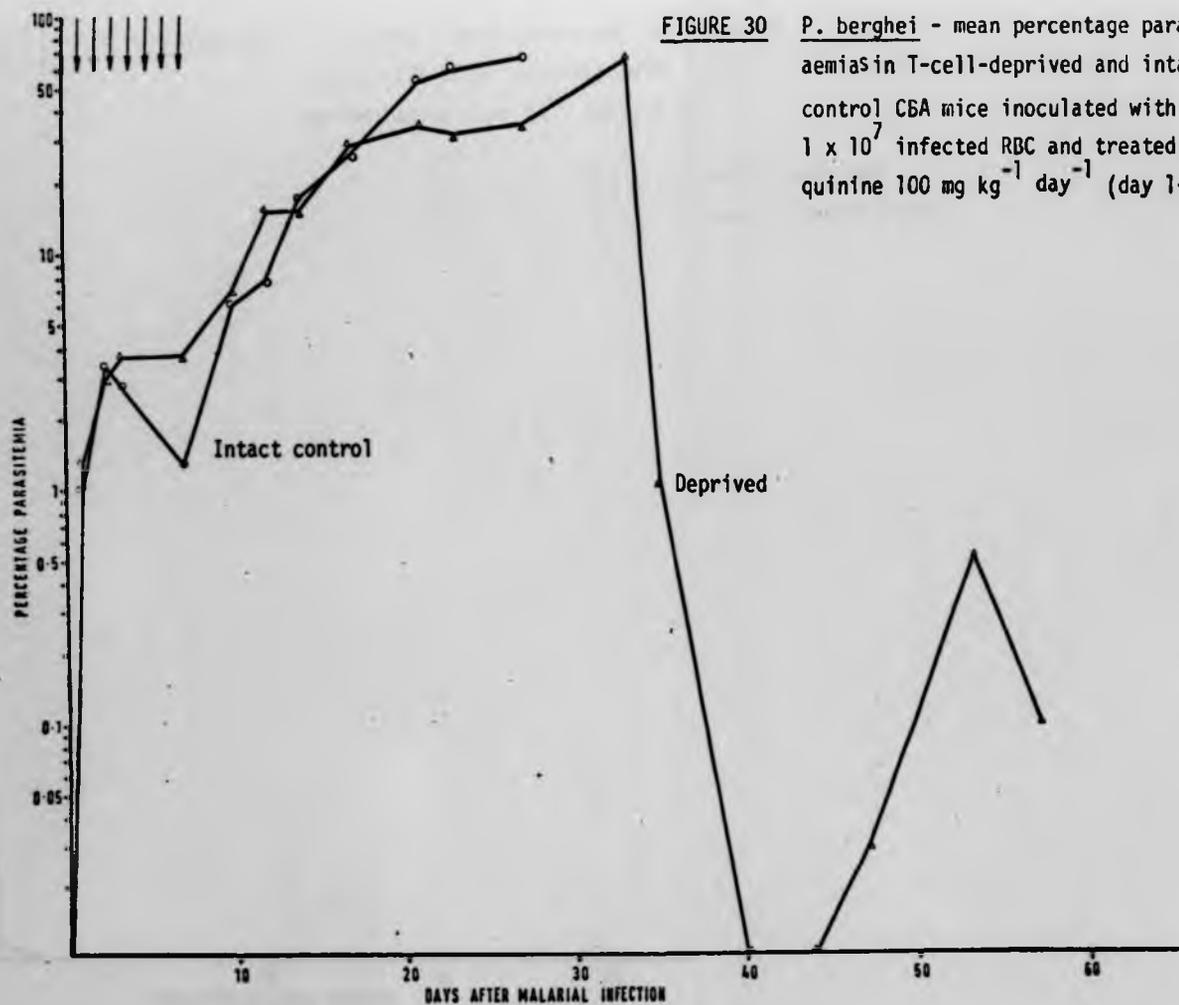
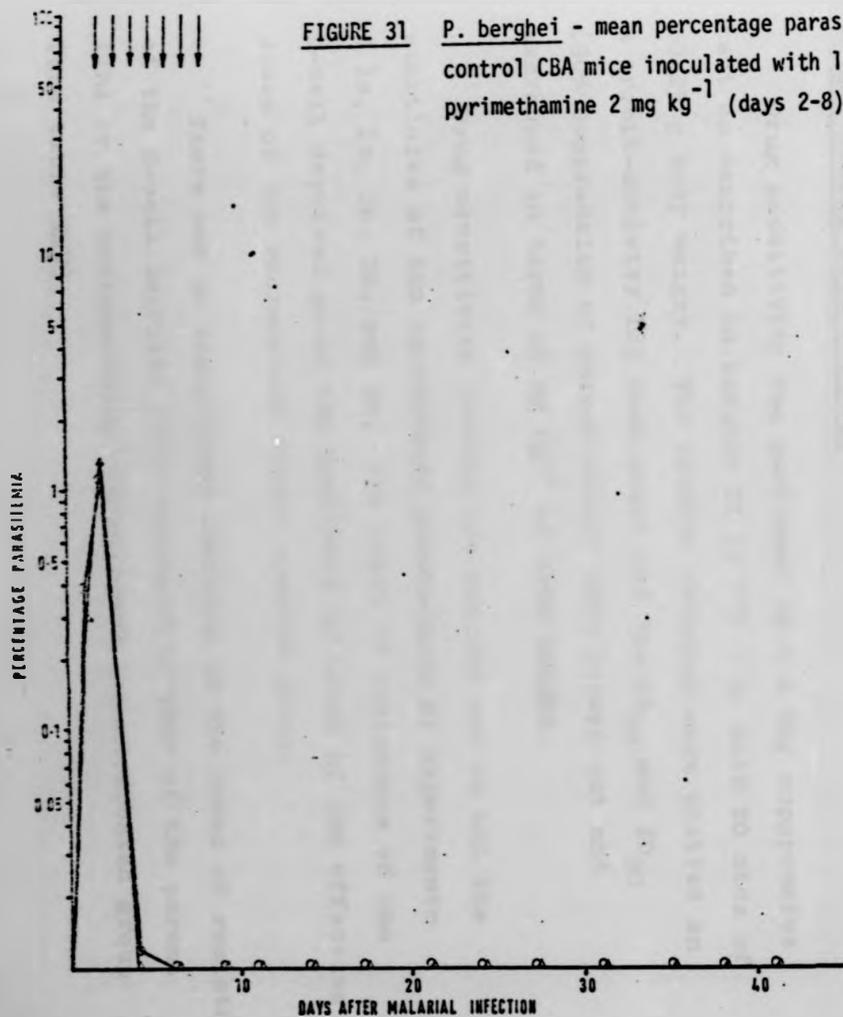


FIGURE 30 *P. berghei* - mean percentage parasit-  
aemia in T-cell-deprived and intact  
control CBA mice inoculated with  
 $1 \times 10^7$  infected RBC and treated with  
quinine  $100 \text{ mg kg}^{-1} \text{ day}^{-1}$  (day 1-7)



#### 4. The effect of pyrimethamine treatment

T-cell deprivation was done by thymectomy and ALS<sub>2</sub> treatment and the mice were grouped as before. Pyrimethamine was given at a dose rate of 2 mg kg<sup>-1</sup> daily for 7 days from day 2 when the parasitaemia was between 1.2% ± 0.7 and 1.5% ± 0.4. The parasitaemia was detectable up to day 3 and then undetectable from day 7 onwards. In all cases subinoculation of blood from two mice of each group to uninfected mice did not produce a parasitaemia (fig 31).

#### 5. Drug sensitivity of P.berghei after chemotherapy in intact and T-cell deprived mice

Drug sensitivity was performed by a 4 day suppressive test as described in chapter II (p 117) in male TO mice of 20-25 g body weight. The results obtained were plotted on a probit-activity log dose paper and the ED<sub>50</sub> and ED<sub>90</sub> (90% suppression of parasitaemia) were worked out and expressed in terms of mg kg<sup>-1</sup> of body weight.

Drug sensitivity testing was carried out on all the stabilates of the recrudesced populations of experiments E 1a, 1b, 2a, 2b, and 2c. The index of resistance of the T-cell deprived group was monitored in terms of the effective doses of the recrudesced intact treated group.

There was no significant increase in the index of resistance of the T-cell deprived group compared to that of the parent line or the corresponding recrudescent intact treated group (see table XXV).

Table XXV Suppressive test of *P. berghei* infection using the recrudescence population after treatment of intact and T-cell deprived mice.

Stabilate tested	Intact treated group		Deprived treated group		Index of resistance	
	ED <sub>50</sub> ± SE	ED <sub>90</sub> ± SE	ED <sub>50</sub> ± SE	ED <sub>90</sub> ± SE	I <sub>50</sub>	I <sub>90</sub>
Original parasite	1.62±0.6	2.92±0.7	-	-	-	-
Exp. E 1a (chloroquine 10 mg kg <sup>-1</sup> x 4 days)	1.6 ±0.4	2.9 ±0.8	1.7 ±0.5	3.0 ±1.0	1.06	1.03
Exp. E 1b (chloroquine 10 mg kg <sup>-1</sup> x 7 days)	1.8 ±0.2	3.2 ±0.4	1.9 ±0.3	3.2 ±0.9	1.06	1.00
Exp. E 2a (chloroquine 10 mg kg <sup>-1</sup> x 4 days)	1.75±0.5	3.2 ±0.3	1.8 ±0.3	3.1 ±0.8	1.03	0.97
Exp. E 2b (chloroquine 10 mg kg <sup>-1</sup> x 7 days)	1.9 ±0.5	3.1 ±0.7	1.7 ±0.5	3.3 ±0.7	0.89	1.06
Exp. E 2c (chloroquine 10 mg kg <sup>-1</sup> x 14 days)	1.87±0.6	3.17±0.8	1.78±0.07	3.1 ±0.6	0.95	0.98

-all doses (mg kg<sup>-1</sup>) are single daily doses of chloroquine given on consecutive days. ED<sub>50</sub> and ED<sub>90</sub> are computed graphically as described. I (index of resistance) =  $\frac{\text{ED}_{50} \text{ or } \text{ED}_{90} \text{ of deprived treated group}}{\text{ED}_{50} \text{ or } \text{ED}_{90} \text{ of intact treated group}}$

-SE was computed graphically as described by (Peters, 1965b).

DISCUSSION

The parasitaemias in the P.berghei infected intact, sham- and T-cell deprived mice were similar, the increased mean survival time in the T-cell deprived group being the main difference (Fig 9a and 9b ). Chemotherapy of P.berghei infections in mice has been much studied. Peters (1970a) has compiled details of the effective doses of different antimalarials for P.berghei but, according to him, factors like the strain of mouse used, sex, age, concomitant infections, environmental factors, strains of parasites and the technique used may all vary the effective dose. In carrying out experiments the size of the infective inoculum, the time spent in preparation of the inocula, the route of drug administration, the timing of the first drug dose, the frequency and duration of administration, timing of termination of experiments and the technique of reading blood films are important factors that must be studied. Different PABA concentrations in the diet can also change the drug dosage required to control the infection (Carter, 1972; Kirakosyan, 1970).

In my experiments, I tried to minimize these variables by using the same diet, mice, inoculum and techniques for all experiments. There were slight variations of the temperature between 17°C-22°C and humidity between 42% to 61%. The intraperitoneal route was chosen for the drug administration because it is easy and it is a more sure way of knowing that the drug is absorbed than the oral route. Thurston (1953) and Hawking (1966) used the intraperitoneal route for drug administration and Peters (1973) also used the

same route for his treatment with pyrimethamine.

In my preliminary studies on chloroquine treatment of P.berghei infection in mice, the duration of treatment was found to be more important for curing all of the infected mice than using a high dose for a short time. Chloroquine given in high doses for a short time ( $40 \text{ mg kg}^{-1}$  for 4 days) produced a higher recrudescence rate than when given at a lower level for a longer period ( $10 \text{ mg kg}^{-1}$  x 14 days). It is very difficult to get a 100% cure rate of P.berghei infection in mice with chloroquine. Box *et al.* (1954) used an average daily dose of  $183.9 \text{ mg kg}^{-1}$  of hydroxychloroquine in the diet given from days 3-6 of the P.berghei infection but failed to cure any mice.

Suppressive chloroquine given on days 0-3 was more effective than the therapeutic treatment from days 7-10 in all groups of mice. Since chloroquine was given intraperitoneally, the same route as the infection, and because it was given within 3 hours of infection, the drug might have acted before the parasites had entered the blood stream. Also, the chloroquine may have killed a high proportion of parasites before they began to multiply. So after suppressive chemotherapy, there were fewer recrudescences and less mortality in the intact, sham and deprived groups than after therapeutic treatment.

When early chloroquine treatment of P.berghei was compared among the infected intact, sham and T-cell deprived groups, the drug seemed to be more effective in the T-cell deprived group. In suppressive chloroquine chemotherapy (treated 0-3 days) only 2 mice relapsed and died in the

T-cell deprived group though 5 relapsed and died in the intact control group. When treated with chloroquine for 7 days (days 2-7), 2 out of 8 mice relapsed with one death in the deprived group but 5 out of 8 mice relapsed and died in the intact group. Also when treated with chloroquine for 14 days (days 2-15) 4 out of 8 mice recrudesced in the intact group and none relapsed in the T-cell deprived group. Though the numbers of animals used in each experiment were not very large, chloroquine was shown to be more effective in T-cell deprived groups than the intact controls in all the experiments.

Also, when comparing late treatment of P.berghei among the intact control, sham and the T-cell deprived groups, the effect seemed to depend on the drug dosage schedule. Treatment with  $10 \text{ mg kg}^{-1}$  for either 7 or 14 days did not produce any significant difference in recrudescence rate or mortality in all the groups. However, when treated for 4 days all mice showed a recrudescence but 4 deprived mice survived this. This shows that treatment with chloroquine can be effective in T-cell deprived and intact groups but, if a lower dose schedule of chemotherapy was given, T-cell deprived groups survived more than intact control groups.

These results are the opposite of those obtained with P.chabaudi (discussed on p 275 ). There, chemotherapy was shown to be more effective in the intact mice. The exact reason for why T-cell deprived treated mice survived better is not known. It is generally assumed and was shown with P.chabaudi that immune mechanisms are helpful in drug treatment. But mice (Jayawardena et al., 1975) and hamsters

(Wright, 1968; Wright et al., 1971) survive P.berghei infection better if they are T-cell deprived. Though the exact mechanism is not known, Jayawardena (1975) tried to explain this in terms of the infection inducing T-suppressor cells which suppress the immune mechanisms and cause earlier deaths in intact animals. Wright (1968) and Wright et al. (1971) showed that the formation of immune complexes in P.berghei infected intact hamsters caused the animals to die earlier whereas in the ALS treated or neonatally thymectomised hamsters they survived longer. So if the treatment is not effective (chloroquine 10 mg kg<sup>-1</sup> for 4 days) and if the infection recrudesces, the deprived mice would be expected to be better off. There might be a better immune response in T-cell deprived mice because of the absence of T-suppressor cells.

The occurrence of any immune response in intact mice infected with P.berghei is a controversial matter. Since P.berghei invariably kills the mice, the immunity if present is not effective. But, on the other hand suppression of the parasitaemia in P.berghei infections by drugs or by using a milk diet in mice has been shown to induce some immunity (Gilbertson et al., 1970; Carrescia and Arcoleo, 1957). Jayawardena et al. (1975) have shown that P.berghei in mice elicits only limited T-cell activity (eg. to PHA) and a generalised failure of T-cell function (assayed on day 15). Also, Krettli and Nussenzweig (1974) have shown that T-cells (⊕ positive cells) as well as B-cells (complement receptor positive cells) were reduced in numbers in P.berghei infection in mice but a third cell population, without complement receptors or ⊕ antigen, increased in size from day 14 of the infection. According to Gravelly and Kreier

(1976) neither  $\Theta$  positive T-cells nor complement receptor positive B-cells conferred immunity to P.berghei in passive transfer experiments in rats. According to them, the cells which lack both  $\Theta$  antigen and complement receptor cells (probably antibody-producing cells, B-cells) were concerned with immunity. So, P.berghei infection in mice reduces the number as well as the activities of T-cells and of some B-cells (complement receptor positive) but induced the increased production of other B-cells which, in rats seem to be important in the development of immunity. However, Brown *et al.* (1976) showed that protective immunity to P.berghei in rats was due to T-cell activity through co-operation with B-cells. So if T-cell activities are necessary there may not be effective immunity in intact mice infected with P.berghei.

There is an indication that late treatment of P.berghei infection in intact mice leads to less recrudescence and lower mortality than early treatment. Since the number of animals (8 mice) used in these experiments was small, the experiments need to be repeated before definite conclusions can be drawn.

Quinine does not cure P.berghei infections of mice. Hill (1950) and Thurston (1950) just managed to reduce the parasitaemia of P.berghei infection in mice by suppressive treatment. Peters (1970a) and Pinders (1973) in their reviews on the "Rane Test" reported that quinine has definite activity against P.berghei but there were no survivors even with the maximum dosage of the drug.

My preliminary experiments showed that quinine could be given intraperitoneally to CBA mice up to a maximum of 150 mg kg<sup>-1</sup> without killing them. A dose of 100 mg kg<sup>-1</sup> had no effect on the parasitaemia of a P.berghei infection in both intact and T-cell deprived mice, but mean survival time was increased from 17.4 ± 8.4 to 27.8 ± 3.4 days in the intact treated group. In the T-cell deprived treated group 1 out of 6 mice survived and the rest died with a mean survival time of 37.8 ± 1 day

Pyrimethamine was found to be a very effective drug in curing P.berghei infection in CBA mice. Most et al. (1967) obtained a radical cure of P.berghei infection in mice by giving 3 mg kg<sup>-1</sup> of pyrimethamine daily for 4 days 2 mg kg<sup>-1</sup> daily for 7 days completely cured P.berghei infection in my experiments in both normal and T-cell deprived mice.

The recrudescences of the P.berghei or P.chabaudi (Exp.F p244) infections in T-cell deprived mice after various dosages of drug treatment did not show any significant increase in drug resistance, compared with that of the recrudescences from intact treated mice or of the original line of parasite. Drug resistant strains of malaria parasites are easily produced in experimental conditions by suppressing or avoiding the immune response of the host (Peters, 1970a; Warhurst, 1965 ; and Hawking, 1966). Peters (1965a) succeeded in developing drug resistant lines of P.berghei by treating infected mice with increasing doses of chloroquine. Also Rosario (1976) succeeded in developing a stable line of drug resistant P.chabaudi by treating with

chloroquine at  $2 \text{ mg kg}^{-1}$  for 5 days and passaging 5 times. But the P.chabaudi resistant line was only resistant to 6 daily doses of  $3 \text{ mg kg}^{-1}$  of chloroquine. In my experiments, since I used only one course of treatment, the resistance if it developed may be very low.

F. Drug treatment of P.chabaudi in  
intact and T-cell deprived mice

1. The effect of early treatment with chloroquine

a. Treatment with 2 mg kg<sup>-1</sup> chloroquine for 7 days from day  
5 of the infection

Three groups of mice, intact, sham deprived and T-cell deprived (thymectomy and ALS<sub>1</sub> treatment) were treated and there was a corresponding untreated control group for each.  $1 \times 10^7$  P.chabaudi infected erythrocytes were inoculated on day 0. The chloroquine i.p. injections of 2 mg kg<sup>-1</sup> daily were given to mice of the experimental group from days 5 to 11. The parasitaemia was monitored at 3 to 4 day intervals. (fig 32a ).

The parasitaemia in the intact and sham-deprived treated groups went down from the initial mean level of 13 to 14% to 0 on day 11 and remained aparasitaemic until day 18 (intact mice) or day 23 (sham-deprived). After recrudescence, maximum parasitaemias were  $0.8\% \pm 0.6$  in the intact mice and  $0.4\% \pm 0.3$  in the sham-deprived group. Parasitaemia in the T-cell deprived treated group was  $16\% \pm 0.3$  at the onset of treatment and remained patent with a minimum mean parasitaemia of  $0.1\% \pm 0.1$  on day 11 and a subsequent maximum parasitaemia of  $20\% \pm 10$  on day 54.

Parasites from recrudescent infections of the treated groups were stablilated on day 23 for later drug sensitivity testings. In the untreated control groups of intact and sham deprived mice, the first wave of parasitaemia was resolved on days 15 and 18 respectively. The second peak

1. The quantity of water in the soil is a function of the depth of the soil and the amount of water that has infiltrated into the soil. The amount of water that has infiltrated into the soil is a function of the amount of water that has fallen on the soil and the amount of water that has evaporated from the soil.



Fig. 1. Hydrological diagram showing the relationship between precipitation, infiltration, and evaporation over time.

Table XXVIA Statistical analysis of the data in Fig 32.a (n=8)

Group	Day Data	5	7	11	13	15	18	23	26	29	33	36	40	43	46	52	54	58	61	71	
Deprived treated	Mean	16.0	5.0	0.1	0.7	1.7	5.2	5.7	1.2	1.1	3.7	7.0	3.0	7.0	7.3	6.8	20.0	4.5	23.8	9.0	
	SD ±	0.3	4.0	0.1	1.1	4.7	4.7	5.7	2.1	1.7	4.7	8.0	0.4	16.0	8.4	7.4	10.0	1.3	12.0	3.0	
Intact treated	Mean	14.0	5.0	0.01	0.0	0.0	0.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	SD ±	5.3	2.0	0.01			0.1	0.6													
Significance	P <	NS	NS	0.05	NS	NS	0.02	0.05	NS	NS	NS	0.05	0.001	NS	0.05	0.05	0.001	0.001	0.001	0.001	
Sham treated	Mean	13.0	0.5	0.0	0.0	0.0	0.0	0.2	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.0	0.05	0.0
	SD ±	6.0	0.3					0.1	0.3	0.5								0.01		0.01	
Intact treated	Mean	14.0	5.0	0.01	0.0	0.0	0.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	SD ±	5.3	2.0	0.01			0.1	0.6													
Significance	P <	NS	0.001	0.02	NS	NS	0.001	0.02	0.01	NS	NS	NS	NS	NS	NS	NS	NS	0.02	NS	0.001	NS

- for untreated control see table XXVIB and table VI for abbreviations

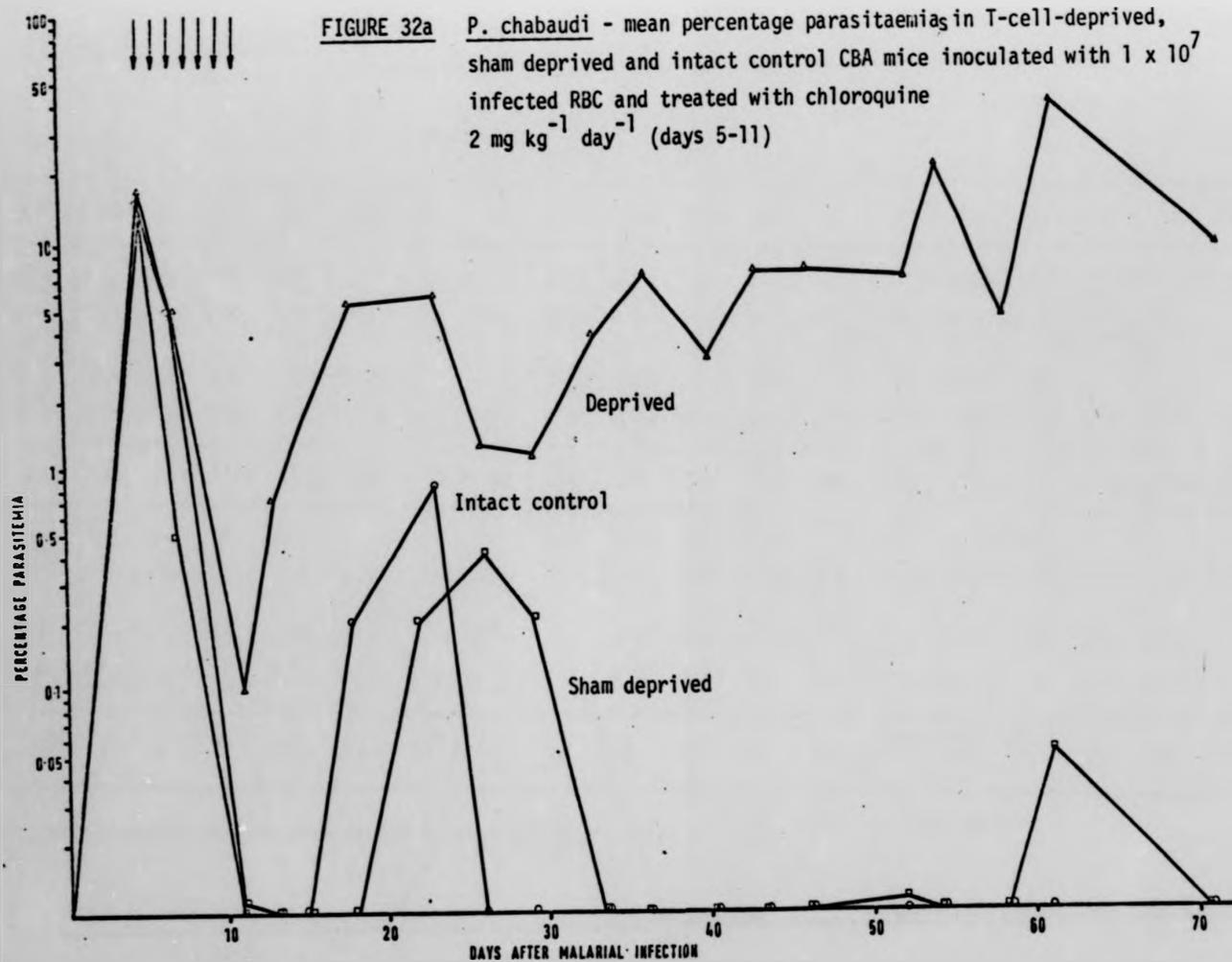


FIGURE 10. *Escherichia coli* O157:H7 in ground beef (percentage of total) versus time (days) for control and experimental groups. The control group was not treated with any antimicrobial agent. The experimental group was treated with a natural antimicrobial agent.

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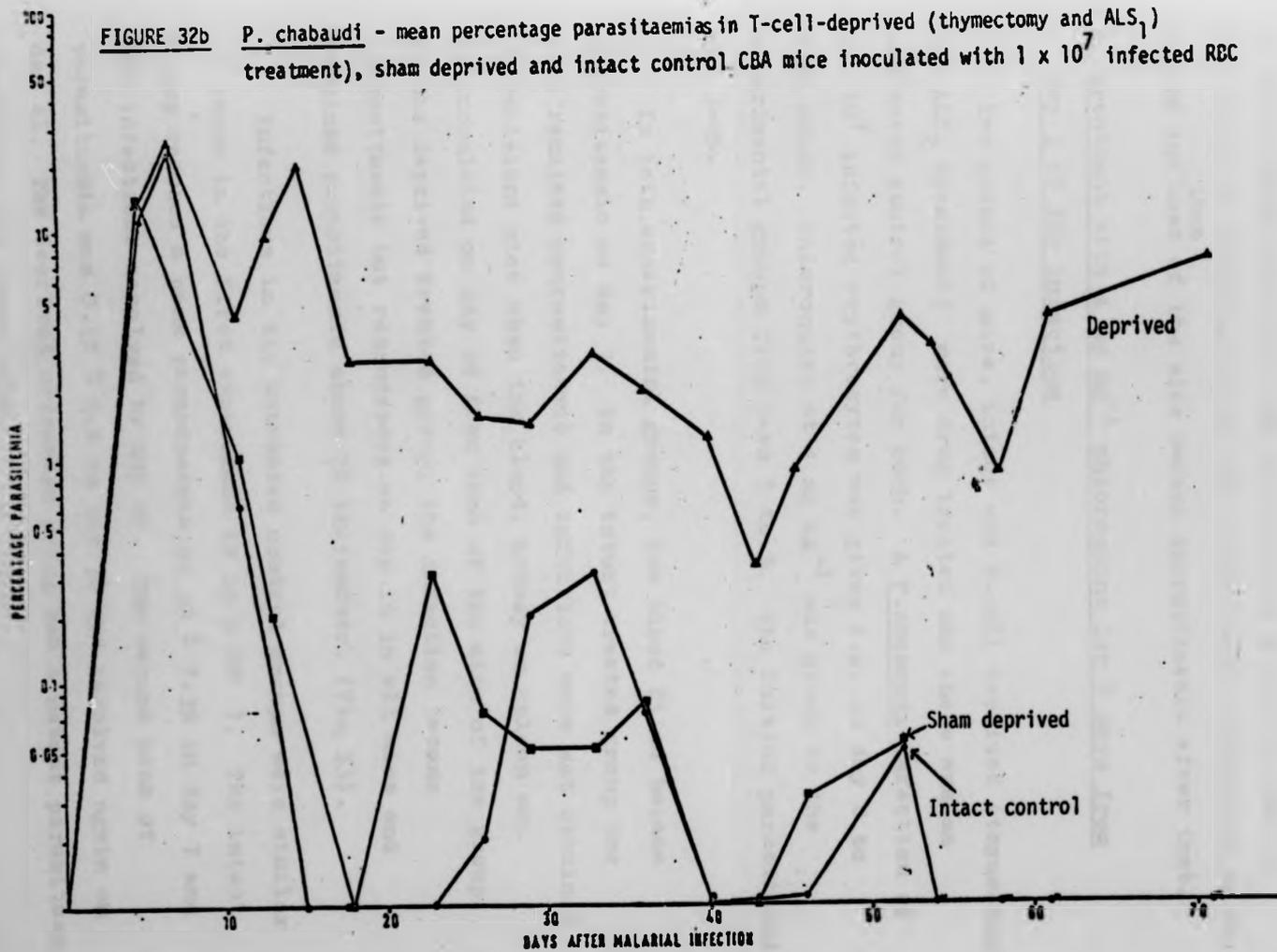
a

Table XXVIB Statistical analysis of the data in Fig 32L. (n=8)

Group	Day Data	5	7	11	13	15	18	23	26	29	33	36	40	43	46	52	54	58	61	71
Deprived infected control	Mean	13.0	26.0	5.2	10.0	20.3	2.7	2.7	1.7	1.4	2.9	2.0	1.4	0.5	9.0	4.0	5.0	0.3	5.0	7.0
	SD $\pm$	3.8	3.9	4.6	3.6	19.6	1.6	3.6	2.6	2.6	4.0	1.9	2.5	0.9	1.5	3.3	3.8	0.2	3.5	2.4
Intact infected control	Mean	11.0	22.6	0.6	0.1	0.0	0.0	0.0	0.02	0.2	0.3	0.07	0.0	0.0	0.01	0.05	0.0	0.0	0.0	0.0
	SD $\pm$	6.6	8.5	0.3	0.1				0.01	0.03	0.2	0.07			0.01	0.04				
Significance	P <	NS	NS	0.02	0.001	0.02	0.001	NS	NS	NS	NS	0.02	NS	NS	0.001	0.01	0.001	0.001	0.001	0.001
Sham infected control	Mean	15.0	21.0	1.0	0.2	0.02	0.0	0.0	0.07	0.05	0.0	0.08	0.0	0.0	0.03	0.05	0.0	0.0	0.0	0.0
	SD $\pm$	5.4	5.3	1.5	0.2	0.01			0.05	0.02		0.03			0.04	0.03				
Intact infected control	Mean	11.0	22.6	0.6	0.1	0.0	0.0	0.0	0.02	0.2	0.3	0.07	0.0	0.0	0.01	0.05	0.0	0.0	0.0	0.0
	SD $\pm$	6.6	8.5	0.3	0.1				0.01	0.03	0.2	0.07			0.01	0.04				
Significance	P <	NS	NS	NS	NS	0.001	NS	NS	0.02	0.001	0.001	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*see table VI

FIGURE 32b *P. chabaudi* - mean percentage parasitaemias in T-cell-deprived (thymectomy and ALS<sub>1</sub>) treatment), sham deprived and intact control CBA mice inoculated with  $1 \times 10^7$  infected RBC



of parasitaemia (0.3% and 0.08%) was seen on days 33 and 36 and a third peak of 0.05% in 5 out of 8 mice on day 52. The deprived untreated group was parasitaemic throughout up to then day 86 and/most of the mice became aparasitaemic after that.

b. Treatment with 4 mg kg<sup>-1</sup> chloroquine for 7 days from day 3 of the infection

Two groups of mice, intact and T-cell deprived (thymectomy and ALS<sub>2</sub> treatment) were drug treated and there was an untreated control group for each. A P.chabaudi infection of  $1 \times 10^7$  infected erythrocytes was given i.p. on day 0 to each mouse. Chloroquine at 4 mg kg<sup>-1</sup> was given to the experimental groups from days 3 to 9. The initial parasitaemia was 3-4%.

In both experimental groups, the blood films became aparasitaemic on day 7. In the intact treated group the mice remained aparasitaemic and infections were not obtained in recipient mice when the blood, kidney or spleen was subinoculated on day 26 from each of two mice of the group. In the deprived treated group, the infection became aparasitaemic but recrudesced on day 19 in all mice and remained parasitaemic above 7% thereafter. (fig 33).

Infections in the untreated control groups were similar to those in the first experiment (F 1a p 252 ). The intact group reached a peak parasitaemia of  $32 \pm 7.3\%$  on day 7 and the infections resolved by day 22. The second peak of parasitaemia was  $0.4\% \pm 0.4$  on day 30 and resolved again on day 41. The deprived untreated group had a patent parasitaemia throughout the experiment.



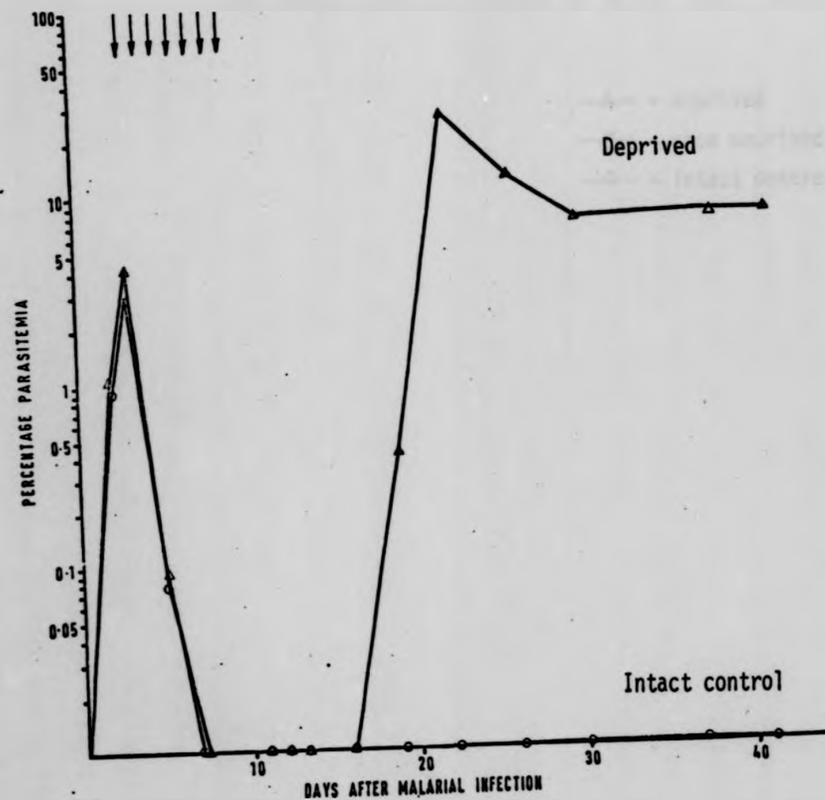
Table XXVII Statistical analysis of the data in Fig 33 . (n=6)

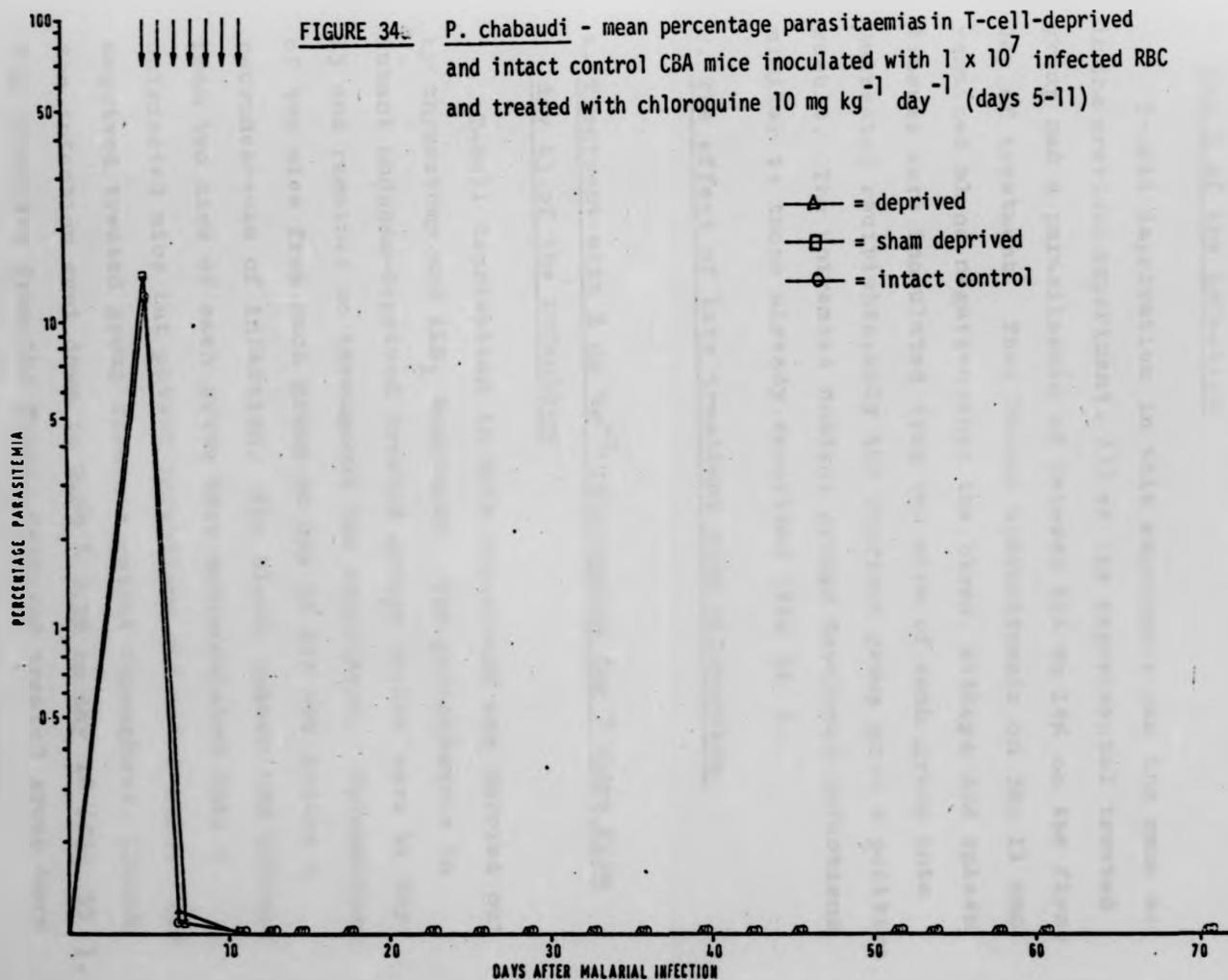
Group	Day Data	2	3	5	7	11	12	16	19	22	26	30	37	41
		Deprived treated	Mean SD <sup>+</sup>	1.1 0.2	4.3 1.0	0.1 0.05	0.0 -	0.0 -	0.0 -	0.0 -	0.4 0.1	26.0 6.2	10.2 7.2	7.0 2.8
Intact treated	Mean SD <sup>+</sup>	0.9 0.5	3.0 1.7	0.1 0.03	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -
Significance	P <	NS	NS	NS	NS	NS	NS	NS	0.001	0.001	0.01	0.001	0.01	0.01

(for untreated controls see table XXXI p )

\*see table VI

**FIGURE 33** *P. chabaudi* - mean percentage parasitaemias in T-cell-deprived and intact control CBA mice inoculated with  $1 \times 10^7$  infected RBC and treated with chloroquine  $4 \text{ mg kg}^{-1} \text{ day}^{-1}$  (days 3-9)





c. Treatment with 10 mg kg<sup>-1</sup> chloroquine for 7 days from day 5 of the infection

T-cell deprivation in this experiment was the same as in the previous experiment. All of the experimental treated group had a parasitaemia of between 12% to 14% on the first day of treatment. They became aparasitaemic on day 11 and remained blood negative; when the blood, kidneys and spleen tissues were inoculated from two mice of each group into untreated recipients, only the deprived group gave a positive result. The untreated control groups developed infections similar to those already described (fig 34 ).

2. The effect of late treatment with chloroquine

a. Treatment with 2 mg kg<sup>-1</sup> chloroquine for 7 days from day 13 of the infection

T-cell deprivation in this experiment was carried out by thymectomy and ALS<sub>1</sub> treatment. The parasitaemia in intact and sham-deprived treated groups became zero by day 15 and remained so throughout the experiment. Splenectomy of two mice from each group on day 36 did not induce a recrudescence of infection. The blood, spleen and kidneys from two mice of each group were subinoculated into 6 uninfected mice but patent infections did not result. The deprived treated group remained patent throughout, though the infection went down to 0.09 ± 0.1% on day 18 (fig 35 ). The parasites from the T-cell deprived treated group were stabilised on day 33 for drug sensitivity testing.



Table XXVIII Statistical analysis of the data in Fig 35. (n=8)

Group	Day Data	5	7	11	13	15	18	23	26	29	33	40	52	54	58	61	71
Deprived treated	Mean	14.6	24.0	5.1	1.3	0.1	0.09	1.9	10.7	5.8	1.4	0.04	3.6	0.6	4.5	2.0	4.1
	SD ±	8.2	9.1	6.1	1.2	0.1	0.1	2.9	6.7	5.2	1.8	0.02	4.9	0.5	6.3	2.8	5.5
Intact treated	Mean	14.0	25.0	3.1	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD ±	3.4	5.3	0.2	0.01												
Significance	P <	NS	NS	NS	0.01	0.02	0.05	NS	0.001	0.01	0.05	0.001	NS	0.01	NS	NS	NS
Sham treated	Mean	9.4	18.3	1.5	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD ±	7.6	6.6	1.6	0.01												
Intact treated	Mean	14.0	25.0	0.3	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD ±	3.4	5.3	0.2	0.01												
Significance	P <	NS	0.05	NS	NS	-	-	-	-	-	-	-	-	-	-	-	-

\*see table XXVI for untreated control and table VI for abbreviations

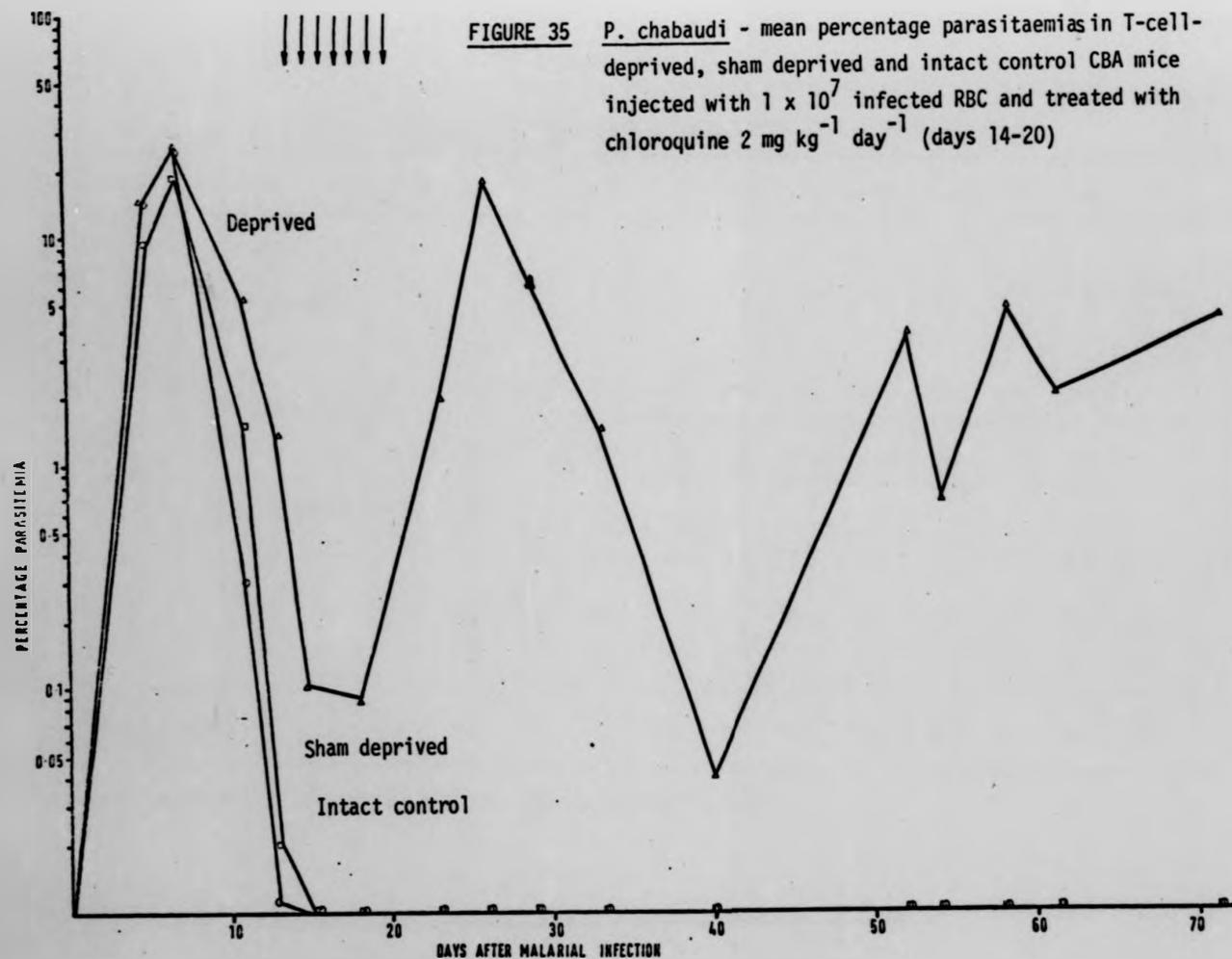




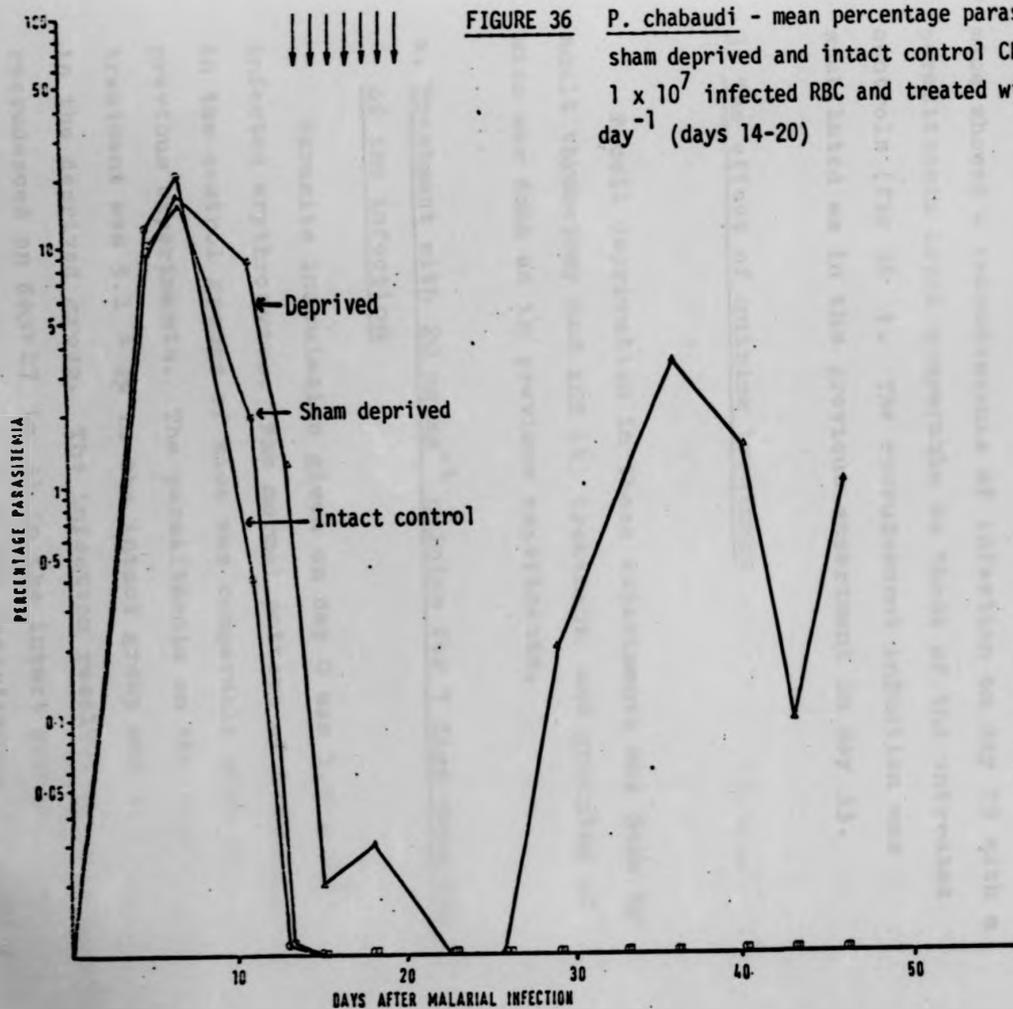
Table XXIX Statistical analysis of the data in Fig 36. (n=8)

Group	Day Data	5	7	11	13	15	18	23	26	29	33	36	40	43	46
Deprived treated	Mean	9.3	16.3	8.5	1.2	0.02	0.03	0.0	0.0	0.2	1.0	3.3	1.4	0.1	1.0
	SD $\pm$	9.1	9.4	1.5	0.8	0.01	0.01			0.2	0.5	5.8	2.4	0.2	1.4
Intact treated	Mean	12.0	20.1	0.4	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD $\pm$	6.0	5.6	0.3	0.01										
Significance	P <	NS	NS	0.001	0.001	0.001	0.001	NS	NS	0.02	0.001	NS	NS	NS	NS
Sham treated	Mean	10.0	15.4	1.9	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD $\pm$	8.9	5.6	1.7	0.01										
Intact treated	Mean	12.0	20.1	0.4	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD $\pm$	6.0	5.6	0.3	0.01										
Significance	P <	NS	NS	0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*see table XXVIB for untreated control and table VI for abbreviations

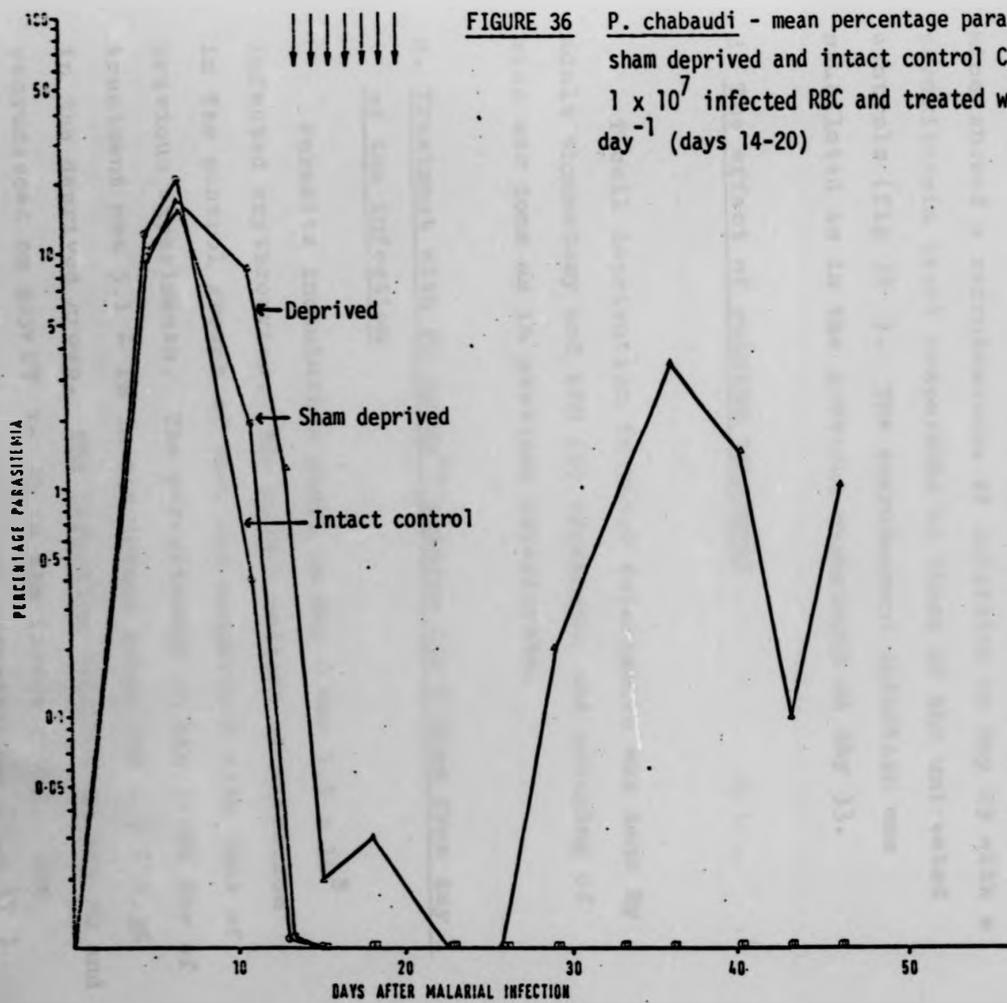
figures after day 26 correspond to the mean parasitaemias of the recrudescences ( $\pm$  SD)  
(4/8 recrudesced in the deprived group)

figures after day 26 correspond to the mean parasitaemias of the recrudescences ( $\pm$  SD)  
(4/8 recrudesced in the deprived group)



\*see table XXVIB for untreated control and table VI for chloroquine

figures after day 26 correspond to the mean parasitaemias of the recrudescences ( $\pm$  SD)  
(4/8 recrudesced in the deprived group)



b. Treatment with 10 mg kg<sup>-1</sup> chloroquine for 7 days from day 13 of the infection

The design and T-cell deprivation of the experiment was the same as that for the previous experiment. The drug treated groups of intact and sham-deprived mice became blood film negative on day 15 and remained aparasitaemic to the end of the experiment. The T-cell deprived treated group became blood film negative on day 23 and then 4 out of 8 mice showed a recrudescence of infection on day 29 with a parasitaemia level comparable to those of the untreated controls (fig 36 ). The recrudescence infection was stabilised as in the previous experiment on day 33.

3. The effect of quinine treatment

T-cell deprivation in these experiments was done by adult thymectomy and ALS (2) treatment. and grouping of mice was done as in previous experiments.

a. Treatment with 20 mg kg<sup>-1</sup> quinine for 7 days from day 1 of the infection

Parasite inoculation given on day 0 was  $1.5 \times 10^8$  infected erythrocytes. The normal pattern of infection in the control groups of mice was comparable with that of previous experiments. The parasitaemia on the first day of treatment was  $5.1 \pm 1\%$  in the intact group and  $4.5 \pm 1.3\%$  in the deprived group. The infection resolved by day 20 and recrudesced on days 27 to 31 in the intact group. The deprived group showed a continuous parasitaemia (Fig 37 )

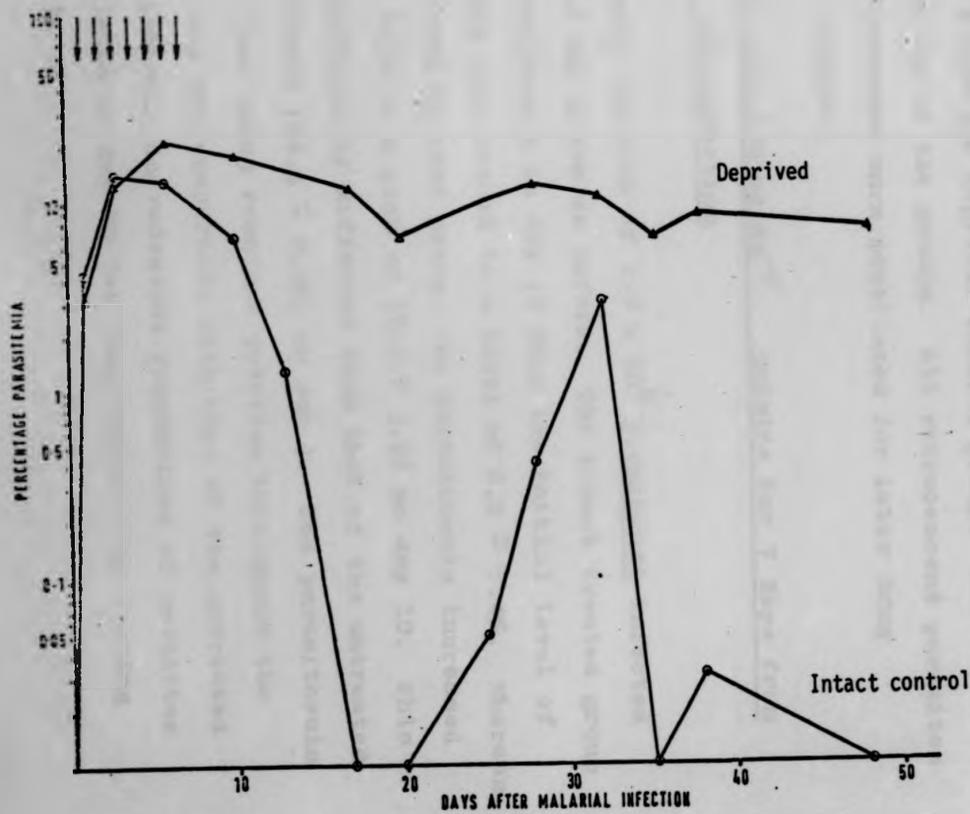


Table XXX Statistical analysis of the data in Fig 37 and its untreated infected controls. (n=6)

Group	Day Data	1	3	6	10	13	17	20	24	27	31	34	38	48
		Deprived treated	Mean SD -	3.4 0.6	12.3 5.2	20.3 7.3	18.0 9.0	14.3 5.0	11.5 5.3	6.6 6.6	9.6 9.2	12.0 7.0	10.0 5.9	7.1 8.6
Intact treated	Mean SD -	4.3 0.5	14.5 7.1	13.0 6.9	6.8 2.4	1.3 1.3	0.0 -	0.0 -	0.05 0.04	0.4 0.6	3.0 3.8	0.0 -	0.03 0.02	0.0 -
Significance	P <	0.02	NS	NS	0.05	0.001	0.001	0.05	0.05	0.01	0.05	NS	0.05	0.05
Deprived controls	Mean SD -	4.5 1.3	24.3 8.6	23.0 7.3	2.6 2.7	9.5 2.5	7.4 5.5	7.0 4.8	6.8 5.4	3.0 4.4	6.3 4.9	7.5 5.0	-	11.5 0.7
Intact controls	Mean SD -	5.1 1.0	27.8 1.7	6.3 2.6	0.8 0.8	0.7 0.4	0.4 0.3	0.0 -	0.0 -	0.01 0.03	0.1 0.01	0.0 -	0.0 -	0.0 -
Significance	P <	NS	NS	0.001	NS	0.001	0.02	0.01	0.02	NS	0.02	0.01	-	0.001

\*see table VI

FIGURE 37 *P. chabaudi* - mean percentage parasitaemias in T-cell-deprived and intact control CBA mice inoculated with  $1.5 \times 10^8$  infected RBC and treated with quinine  $20 \text{ mg kg}^{-1}$  (days 1-7)



In the intact drug treated group of mice the peak was seen on day 3 with a parasitaemia of  $14.5 \pm 7.1\%$  compared to that of the untreated intact control of  $27.8 \pm 1.7\%$ . The infection became negative on day 17 and recrudesced on day 27. In the deprived treated group the maximum parasitaemia was  $20.3 \pm 7.3\%$  compared with that of the untreated deprived group of  $24.3 \pm 8.6\%$ . The infection persisted at a level above 2.6% in the deprived control group. There were no deaths in any of the groups. All recrudescent parasites after drug treatment were stabilised for later drug sensitivity testing.

b. Treatment with  $100 \text{ mg kg}^{-1}$  quinine for 7 days from day 1 of the infection

The heavy inoculum of  $1.5 \times 10^8$  P.chabaudi infected erythrocytes was given as before. The intact treated group became aparasitaemic on day 10 from the initial level of  $3.5 \pm 1\%$  then recrudesced to a level of  $8.2 \pm 5.4\%$ , whereas, in the deprived treated group, the parasitaemia increased from  $4.8 \pm 0.2\%$  to a peak of  $17.3 \pm 3.9\%$  on day 10. This was not significantly different from that of the untreated deprived control ( $24.3 \pm 8.6\%$ ) on day 3. The parasitaemia in the deprived group remained positive throughout the experiment and was comparable with that of the untreated deprived control. Recrudescent populations of parasites were stabilised on day 20 for drug sensitivity testing (fig 38 ).

TABLE 2. *Staphylococcus aureus* (strain 101) and *Staphylococcus aureus* (strain 102) in the presence of 10% formalin and 10% formalin with 10% glycerol.

Time (hr)	10% formalin	10% formalin + 10% glycerol	10% formalin + 10% glycerol + 10% formalin	10% formalin + 10% glycerol + 10% formalin + 10% glycerol
0	100	100	100	100
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	100	100	100
5	100	100	100	100
6	100	100	100	100
7	100	100	100	100
8	100	100	100	100
9	100	100	100	100
10	100	100	100	100
11	100	100	100	100
12	100	100	100	100
13	100	100	100	100
14	100	100	100	100
15	100	100	100	100
16	100	100	100	100
17	100	100	100	100
18	100	100	100	100
19	100	100	100	100
20	100	100	100	100
21	100	100	100	100
22	100	100	100	100
23	100	100	100	100
24	100	100	100	100
25	100	100	100	100
26	100	100	100	100
27	100	100	100	100
28	100	100	100	100
29	100	100	100	100
30	100	100	100	100
31	100	100	100	100
32	100	100	100	100
33	100	100	100	100
34	100	100	100	100
35	100	100	100	100
36	100	100	100	100
37	100	100	100	100
38	100	100	100	100
39	100	100	100	100
40	100	100	100	100
41	100	100	100	100
42	100	100	100	100
43	100	100	100	100
44	100	100	100	100
45	100	100	100	100
46	100	100	100	100
47	100	100	100	100
48	100	100	100	100
49	100	100	100	100
50	100	100	100	100

10% formalin + 10% glycerol + 10% formalin + 10% glycerol

Table XXXI Statistical analysis of the data in Fig 38. (n=6)

Group	Day Data	1	3	6	10	13	17	20	25	29	32	35	38	48
		Deprived treated	Mean SD ±	4.8 0.2	7.9 4.9	12.8 13.7	17.3 3.9	16.0 3.2	7.4 6.0	13.5 8.8	11.7 9.0	4.5 5.8	9.3 9.5	6.0 5.4
Intact treated	Mean SD ±	3.5 1.0	4.1 1.5	0.01 0.01	0.0	0.08 0.2	8.2 5.4	4.2 5.5	0.0	0.0	0.0	0.0	0.0	0.0
Significance	P <	0.01	NS	0.05	0.001	0.001	NS	0.05	0.01	NS	0.05	0.02	0.001	0.01
Deprived infected control	Mean SD ±	4.5 1.3	24.3 8.6	23.0 7.3	2.6 2.7	9.5 2.5	7.4 5.5	7.0 4.8	6.8 5.4	3.0 4.4	6.3 4.9	7.5 5.0	-	11.5 0.7
Intact infected control	Mean SD ±	5.1 1.0	27.8 1.7	6.3 2.6	0.8 0.8	0.7 0.4	0.2 0.3	0.0	0.0	0.01 0.03	0.1 0.05	0.0	0.0	0.0
Significance	P <	NS	NS	0.001	NS	0.001	0.01	0.01	0.01	NS	0.01	0.01		0.001

\*see table VI

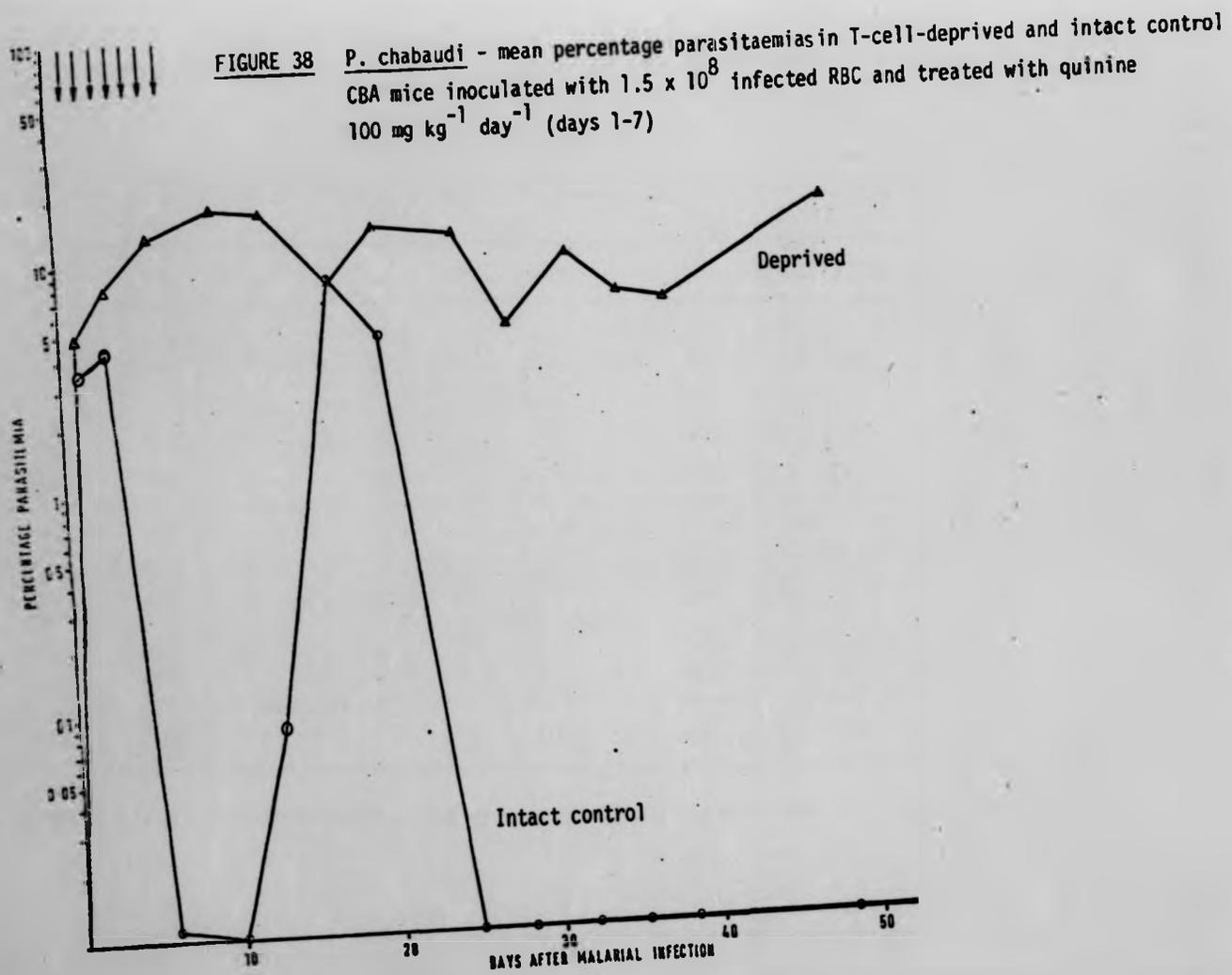




Table XXIII Statistical analysis of the data in Fig 39 and its untreated infected controls (n=6).

Group	Day Data	2	4	5	7	11	12	16	19	22	26	30	37	41
Deprived treated	Mean	1.1	22.0	22.3	2.8	8.0	8.6	27.0	12.3	21.0	10.2	18.8	17.0	0.0
	SD	0.3	6.7	6.2	3.6	6.2	5.2	4.5	2.7	7.0	5.5	5.2	2.1	0.0
Intact treated	Mean	1.1	16.0	14.4	0.5	0.1	0.06	0.1	0.2	0.5	0.1	0.0	0.0	0.0
	SD	0.3	3.4	3.4	0.1	0.1	0.05	0.1	0.2	0.5	0.3	-	-	6.0
Significance	P <	NS	NS	0.05	NS	0.02	0.01	0.001	0.001	0.001	0.001	0.001	0.001	NS
Deprived infected control	Mean	0.3	15.8	33.8	44.6	10.0	18.0	12.0	12.3	13.0	8.2	6.2	6.5	6.7
	SD	0.4	7.5	9.8	7.7	4.2	1.6	3.0	1.5	1.5	2.3	4.5	0.7	1.2
Intact infected control	Mean	0.9	11.3	26.0	32.0	1.2	0.3	0.1	0.04	0.0	0.2	0.4	0.02	0.0
	SD	0.3	4.6	5.8	7.3	0.7	0.3	0.1	0.02	-	0.2	0.4	0.04	-
Significance	P <	NS	NS	NS	0.02	0.001	0.001	0.001	0.001	0.001	0.001	0.02	0.001	0.001

\*see table VI

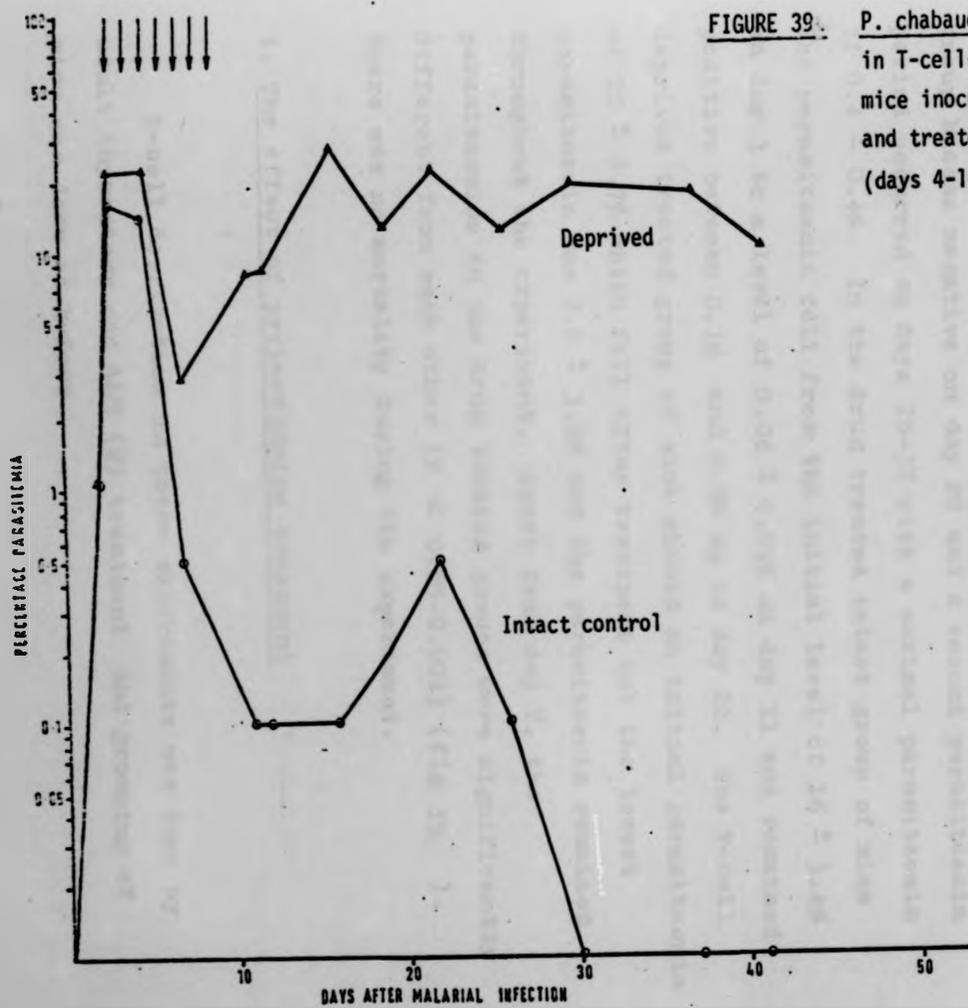


FIGURE 39. *P. chabaudi* - mean percentage parasitaemias in T-cell-deprived and intact control CBA mice inoculated with  $1 \times 10^7$  infected RBC and treated with quinine  $100 \text{ mg kg}^{-1} \text{ day}^{-1}$  (days 4-10)

c. Treatment with 100 mg kg<sup>-1</sup> quinine for 7 days starting from day 4 of the infection

A. P. chabaudi infection of  $1 \times 10^7$  infected erythrocytes was given on day 0. The intact and the T-cell deprived untreated group showed a parasitaemia of  $0.8 \pm 0.4\%$  and  $0.9 \pm 0.3\%$  on day 2 and a peak of  $44.6 \pm 7.7\%$  and  $32 \pm 7.3\%$  respectively on day 7. The blood films from the intact group became negative on day 22 and a second parasitaemia period occurred on days 26-37 with a maximal parasitaemia of  $0.4 \pm 0.4\%$ . In the drug treated intact group of mice the parasitaemia fell from the initial level of  $16 \pm 3.4\%$  on day 3 to a level of  $0.06 \pm 0.05\%$  on day 11 and remained positive between 0.1% and 0.5% up to day 22. The T-cell deprived treated group of mice showed an initial parasitaemia of  $22 \pm 6.7\%$  which fell after treatment but the lowest parasitaemia was  $2.8 \pm 3.6\%$  and the parasitaemia remained throughout the experiment. Apart from day 7, the parasitaemias in the drug treated groups were significantly different from each other ( $P < 0.05-0.001$ ) (fig 39 ). There was no mortality during the experiment.

4. The effect of pyrimethamine treatment

T-cell deprivation in these experiments was done by adult thymectomy and ALS (2) treatment and grouping of mice was done as before.



Time Interval	Series 1 (%)	Series 2 (%)
1	100	60
2	40	20
3	60	40
4	20	20
5	40	40
6	20	20
7	40	40
8	20	20
9	40	40
10	20	20

(Caption text describing the graph data)

d

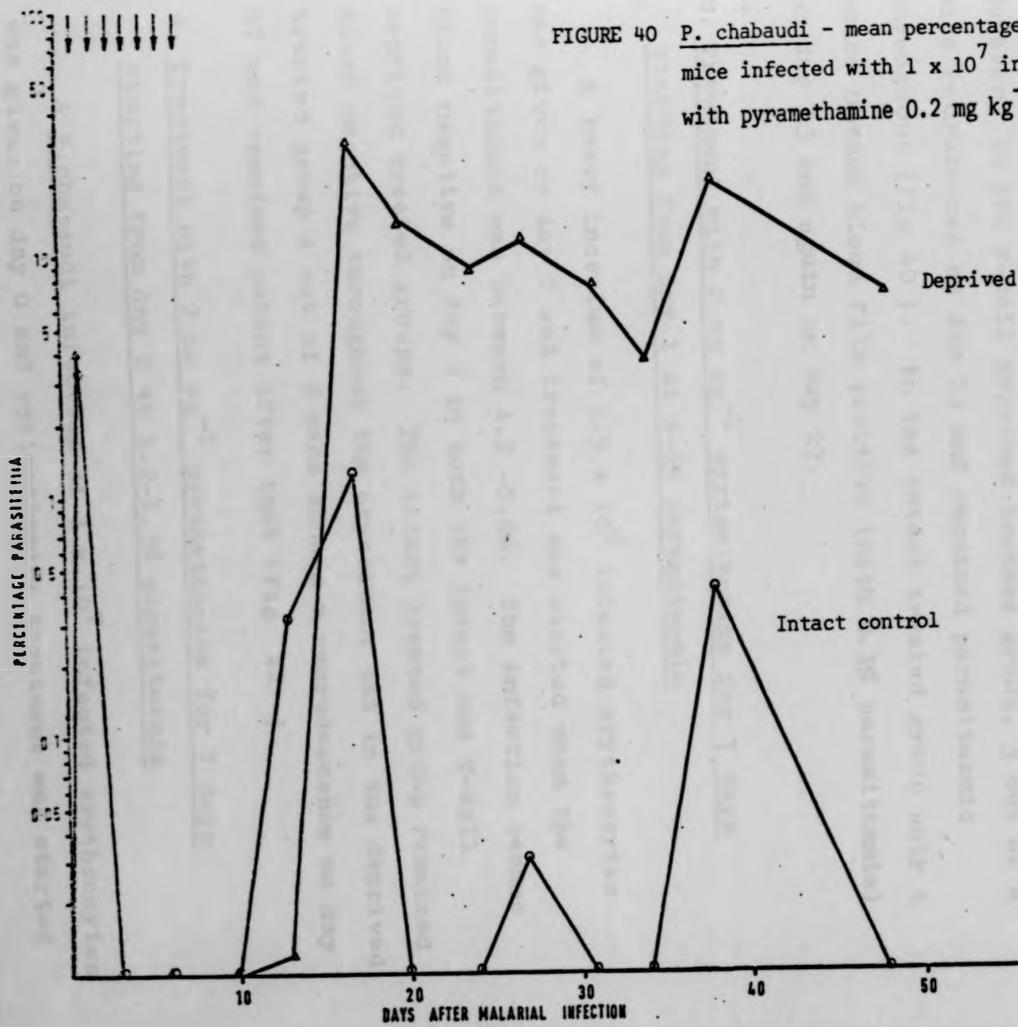
Table XXXIII Statistical analysis of the data in Fig 40. (n=6)

Group	Day Data	1	3	6	10	13	17	20	24	27	31	34	38	48
Deprived treated	Mean $\pm$	4.0	0.04	0.0	0.0	0.01	28.5	13.3	7.5	10.5	7.0	3.5	18.5	6.6
	SD $\pm$	2.7	0.1	-	-	0.01	9.2	16.6	6.4	2.2	1.4	0.7	10.6	7.7
Intact treated	Mean $\pm$	3.7	0.0	0.0	0.0	0.3	1.2	0.0	0.0	0.03	0.0	0.0	0.4	0.0
	SD $\pm$	0.9	-	-	-	-	-	-	-	-	-	-	0.0	-

(for untreated controls see table XX X p 259)

The figure corresponds to the mean of the recrudescences only. (3 recrudesced in deprived group and 1 in intact group).

\*see table VI



a. Treatment with  $0.2 \text{ mg kg}^{-1}$  pyrimethamine for 7 days starting from day 1 of the infection

$1.5 \times 10^8$  P.chabaudi-infected erythrocytes were inoculated on day 0. Infections in the untreated intact and T-cell deprived mice were like those already described for earlier experiments. Both the treated groups remained blood negative starting from the 3rd day after treatment. However, in the T-cell deprived treated group, 3 out of 6 mice recrudesced on day 13 and remained parasitaemic throughout (fig 40). In the intact treated group only 1 mouse became blood film positive (with 0.3% parasitaemia) on day 13 and again on day 27.

b. Treatment with  $2 \text{ mg kg}^{-1}$  pyrimethamine for 7 days starting from day 1 at 4-6% parasitaemia

A heavy inoculum of  $1.5 \times 10^8$  infected erythrocytes was given on day 0 and treatment was started when the parasitaemia was between 4.2 - 5.6%. The infection became blood negative on day 3 in both the intact and T-cell deprived treated groups. The intact treated group remained blood negative throughout the experiment but in the deprived treated group 4 out of 6 mice showed a recrudescence on day 17 and remained patent after that (fig 41).

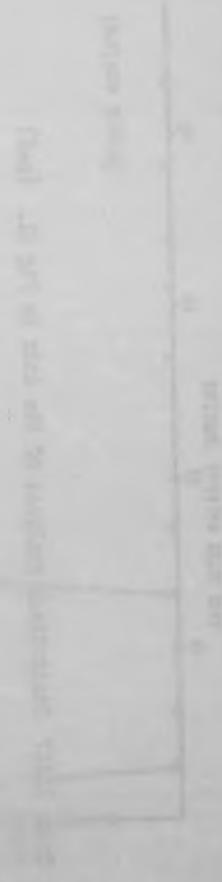
c. Treatment with  $2 \text{ mg kg}^{-1}$  pyrimethamine for 7 days starting from day 2 at 1.2-1.3% parasitaemia

A P.chabaudi infection of  $1 \times 10^7$  infected erythrocytes was given on day 0 and pyrimethamine treatment was started on day 2 as before.

TABLE 1. The results of the analysis of variance for the effect of the treatment on the yield of the crop. The results are given in the following table.

The results of the analysis of variance for the effect of the treatment on the yield of the crop are given in the following table.

Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100																																																																																																																																																																																																																																						
Yield	1.2	1.5	1.8	2.1	2.4	2.7	3.0	3.3	3.6	3.9	4.2	4.5	4.8	5.1	5.4	5.7	6.0	6.3	6.6	6.9	7.2	7.5	7.8	8.1	8.4	8.7	9.0	9.3	9.6	9.9	10.2	10.5	10.8	11.1	11.4	11.7	12.0	12.3	12.6	12.9	13.2	13.5	13.8	14.1	14.4	14.7	15.0	15.3	15.6	15.9	16.2	16.5	16.8	17.1	17.4	17.7	18.0	18.3	18.6	18.9	19.2	19.5	19.8	20.1	20.4	20.7	21.0	21.3	21.6	21.9	22.2	22.5	22.8	23.1	23.4	23.7	24.0	24.3	24.6	24.9	25.2	25.5	25.8	26.1	26.4	26.7	27.0	27.3	27.6	27.9	28.2	28.5	28.8	29.1	29.4	29.7	30.0	30.3	30.6	30.9	31.2	31.5	31.8	32.1	32.4	32.7	33.0	33.3	33.6	33.9	34.2	34.5	34.8	35.1	35.4	35.7	36.0	36.3	36.6	36.9	37.2	37.5	37.8	38.1	38.4	38.7	39.0	39.3	39.6	39.9	40.2	40.5	40.8	41.1	41.4	41.7	42.0	42.3	42.6	42.9	43.2	43.5	43.8	44.1	44.4	44.7	45.0	45.3	45.6	45.9	46.2	46.5	46.8	47.1	47.4	47.7	48.0	48.3	48.6	48.9	49.2	49.5	49.8	50.1	50.4	50.7	51.0	51.3	51.6	51.9	52.2	52.5	52.8	53.1	53.4	53.7	54.0	54.3	54.6	54.9	55.2	55.5	55.8	56.1	56.4	56.7	57.0	57.3	57.6	57.9	58.2	58.5	58.8	59.1	59.4	59.7	60.0	60.3	60.6	60.9	61.2	61.5	61.8	62.1	62.4	62.7	63.0	63.3	63.6	63.9	64.2	64.5	64.8	65.1	65.4	65.7	66.0	66.3	66.6	66.9	67.2	67.5	67.8	68.1	68.4	68.7	69.0	69.3	69.6	69.9	70.2	70.5	70.8	71.1	71.4	71.7	72.0	72.3	72.6	72.9	73.2	73.5	73.8	74.1	74.4	74.7	75.0	75.3	75.6	75.9	76.2	76.5	76.8	77.1	77.4	77.7	78.0	78.3	78.6	78.9	79.2	79.5	79.8	80.1	80.4	80.7	81.0	81.3	81.6	81.9	82.2	82.5	82.8	83.1	83.4	83.7	84.0	84.3	84.6	84.9	85.2	85.5	85.8	86.1	86.4	86.7	87.0	87.3	87.6	87.9	88.2	88.5	88.8	89.1	89.4	89.7	90.0	90.3	90.6	90.9	91.2	91.5	91.8	92.1	92.4	92.7	93.0	93.3	93.6	93.9	94.2	94.5	94.8	95.1	95.4	95.7	96.0	96.3	96.6	96.9	97.2	97.5	97.8	98.1	98.4	98.7	99.0	99.3	99.6	99.9



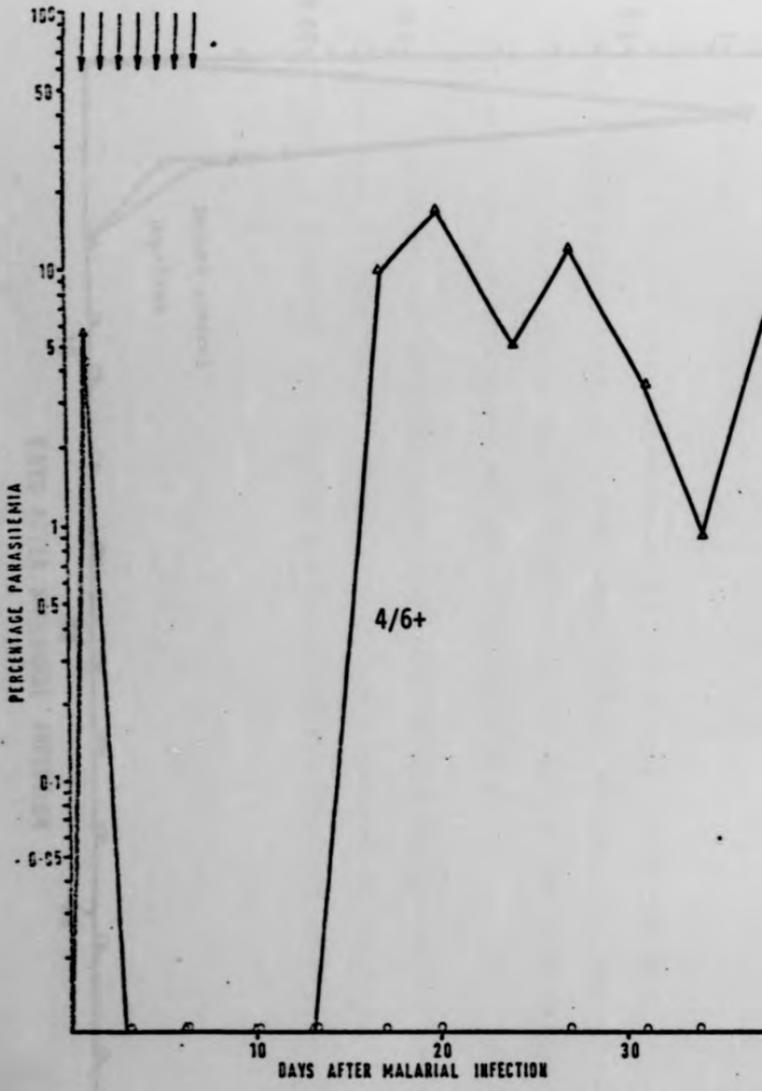
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Table XXIV Statistical analysis of the data in Fig 41. (n=6)

Group	Day Date	1	3	6	10	13	17	20	24	27	31	34	38	48
Deprived treated	Mean	3.6	0.0	0.0	0.0	0.0	10.0	16.8	5.0	11.7	3.5	0.9	9.7	7.5
	SD $\pm$	1.1	-	-	-	-	1.1	14.5	6.1	10.4	5.6	1.4	6.0	2.1
Intact treated	Mean	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD $\pm$	1.3	-	-	-	-	-	-	-	-	-	-	-	-
Significance	P <	NS	NS	NS	NS	NS	0.001	0.02	NS	0.02	NS	NS	0.01	0.001

(for untreated controls see table XX X p259 )

In the deprived group, the figures correspond to the mean of 3 recrudescences (after treatment)



**FIGURE 4.1** *P. chabaudi* - mean percentage parasitaemias  
in T-cell-deprived and intact control CBA  
mice inoculated with  $1.5 \times 10^8$  infected  
RBC and treated with pyrimethamine  
 $2 \text{ mg kg}^{-1}$  (days 1-7)

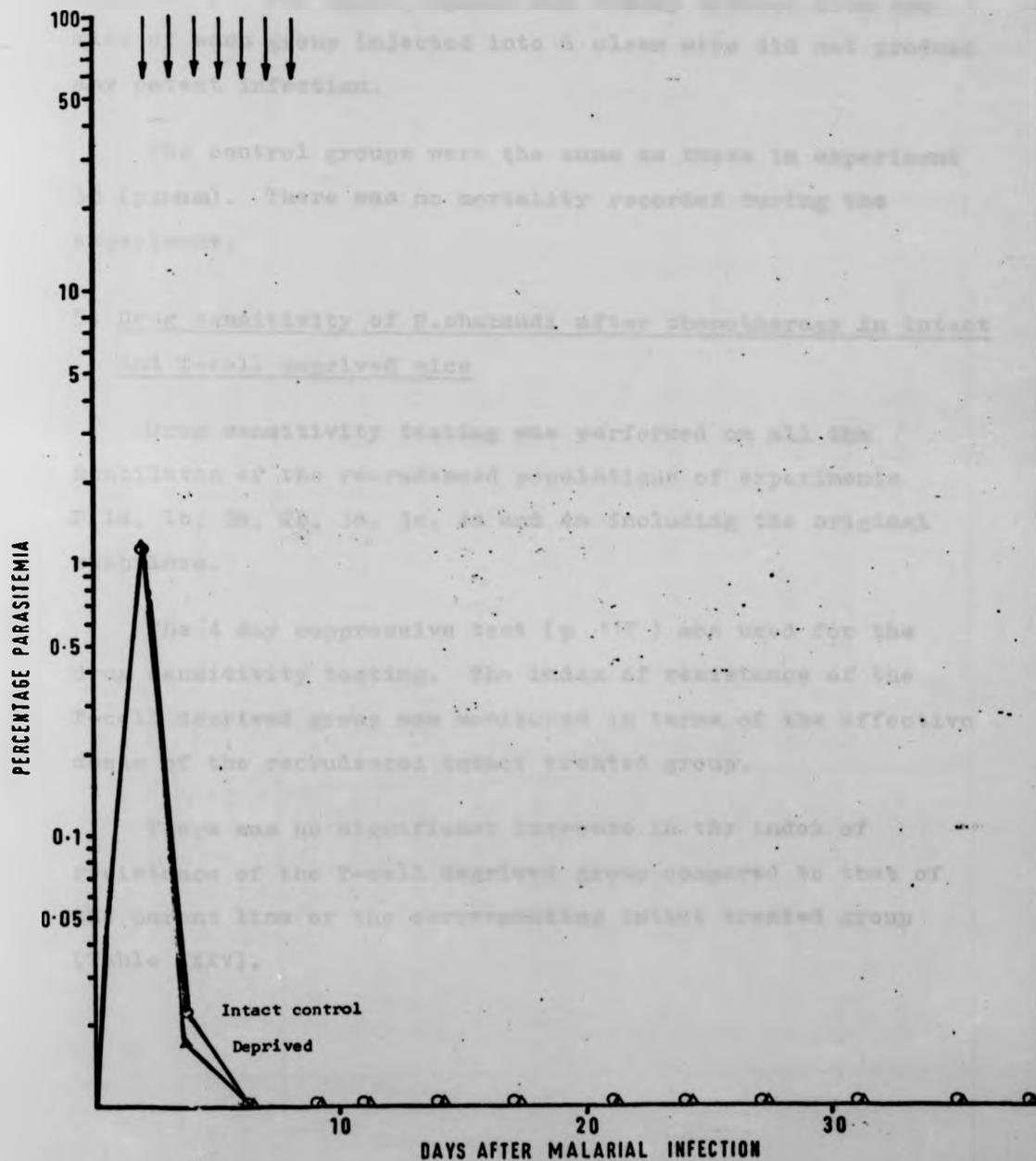


Deprived

Intact control



FIGURE 42 - *P. chabaudi* - mean percentage parasitaemias in CBA mice infected with  $1 \times 10^7$  infected RBC and treated with pyramethamine  $2 \text{ mg kg}^{-1} \text{ day}^{-1}$  (days 2-8).



Parasitaemia in both the treated groups became negative on day 6 and remained as such throughout the experiment (fig 42 ). The blood, spleen and kidney tissues from two mice of each group injected into 6 clean mice did not produce any patent infection.

The control groups were the same as those in experiment 3c (p 266). There was no mortality recorded during the experiment.

5. Drug sensitivity of P.chabaudi after chemotherapy in intact and T-cell deprived mice

Drug sensitivity testing was performed on all the stabilates of the recrudesced populations of experiments F 1a, 1b, 2a, 2b, 3a, 3c, 4a and 4a including the original stabilate.

The 4 day suppressive test (p 117 ) was used for the drug sensitivity testing. The index of resistance of the T-cell deprived group was monitored in terms of the effective doses of the recrudesced intact treated group.

There was no significant increase in the index of resistance of the T-cell deprived group compared to that of the parent line or the corresponding intact treated group (Table XXXV).

Table XXXV Suppressive test of *P.chabaudi* infection using the recrudescence population after treatment of intact and T-cell deprived mice.

Drug tested	Stabilate tested	Intact treated group		Deprived treated group		Index of resistance	
		ED <sub>50</sub> ± SE	ED <sub>90</sub> ± SE	ED <sub>50</sub> ± SE	ED <sub>90</sub> ± SE	I <sub>50</sub>	I <sub>90</sub>
Chloroquine	Original stabilate	1.6 ± 0.4	2.5 ± 0.8	-	-	-	-
	Exp. F 1a (chloroquine 2 mg kg <sup>-1</sup> x 7 days)	1.5 ± 0.5	2.73 ± 1.2	1.5 ± 0.7	2.4 ± 0.8	1.0	0.88
	Exp. F 1b (chloroquine 10 mg kg <sup>-1</sup> x 7 days)	-	-	2.1 ± 0.3	2.9 ± 0.9	-	-
	Exp. F 2a (chloroquine 2 mg kg <sup>-1</sup> x 7 days)	1.4 ± 0.3	2.3 ± 0.6	1.75 ± 0.7	3.04 ± 0.4	1.25	1.32
	Exp. F 2b (chloroquine 10 mg kg <sup>-1</sup> x 7 days)	-	-	1.4 ± 0.4	2.4 ± 0.5	-	-
Quinine	Original stabilate	39.0 ± 7.0	68.0 ± 18.0	-	-	-	-
	Exp. F 3a (quinine 20 mg kg <sup>-1</sup> x 7 days)	38.0 ± 8.0	72.0 ± 21.0	39.0 ± 7.0	77.0 ± 18.0	1.03	1.07
	Exp. F 3c (quinine 100 mg kg <sup>-1</sup> x 7 days)	42.0 ± 12.0	75.0 ± 17.0	38.0 ± 9.0	83.0 ± 25.0	0.9	1.11
Pyri-methamine	Original stabilate	0025 ± 0.01	0035 ± 0.02	-	-	-	-
	Exp. F 4a (pyrimethamine 0.2 mg kg <sup>-1</sup> x 7 days)	0028 ± 0.01	0042 ± 0.02	0.02 ± 0.01	0.05 ± 0.03	0.71	1.19
	Exp. F 4b (pyrimethamine 2 mg kg <sup>-1</sup> x 7 days)	-	-	0.03 ± 0.02	0045 ± 0.02	-	-

see table XXV

DISCUSSION

The line of P.chabaudi used in the experiments is sensitive to chloroquine and pyrimethamine (Walliker, personal communication). According to Rosario (1976), infection in C<sub>57</sub>/Black mice is sensitive to 6 daily oral doses of 3 mg kg<sup>-1</sup> of chloroquine. In my preliminary tests, intraperitoneal injections of 7 daily doses of 4 mg kg<sup>-1</sup> of chloroquine was found to cure 100% of CBA mice without recrudescence. When 7 daily doses of 2 mg kg<sup>-1</sup> of chloroquine were given to the P.chabaudi infected mice, 100% recrudescence occurred. I used 3 different drug regimes against P.chabaudi, 2 mg kg<sup>-1</sup> (subcurative dose), 4 mg kg<sup>-1</sup> (minimum curative dose) and 10 mg kg<sup>-1</sup> (above curative dose), with the drug dosages given intraperitoneally for 7 days.

Chemotherapy of blood induced P.chabaudi infection in intact mice depends not only on the drug dosage but also on the time of treatment. This effect can be clearly seen with a low drug dosage (2 mg kg<sup>-1</sup>) schedule. When given from days -1 to 20, the parasitaemia was maintained at a low level (0.4%) but went up to 1.3% after treatment. The infection lasted for 36 days. However, when administered on days 5 to 11 the mice became aparasitaemic by day 11. The infection recrudesced 5 days earlier than the control group with a slightly higher parasitaemia of 0.8% ± 0.6. When the same dose of chloroquine was given on days 13-19, the infection resolved completely. This indicates that a drug dose which is subcurative when given early in the infection was more effective when used in the later part of the infection. When a higher dose of chloroquine (10 mg kg<sup>-1</sup>) was used all

of the mice were cured whether treatment was started early or late. In these respects the chemotherapeutic effect of chloroquine against P.chabaudi infection is comparable with that against P.berghei infection.

However, early chloroquine treatment of P.chabaudi infected T-cell deprived mice was more effective than late treatment, though not with low doses of chloroquine. In both the early and late treatment, 7 daily doses of chloroquine at  $2 \text{ mg kg}^{-1}$  just reduced the parasitaemia to about the 1% level but this rose again to the original level as soon as the chemotherapy was stopped. However, early institution of 7 daily doses of chloroquine at  $10 \text{ mg kg}^{-1}$  cleared parasites from the blood of the T-cell deprived group, though 4 out of 8 mice recrudesced. The reason why chloroquine is less effective in late treatment of T-cell deprived mice is not known but the results agree with those obtained with treatment of P.berghei in T-cell deprived mice. A similar result has also been obtained by Smith and Wood (1965a) in treatment of pneumococcal infection with penicillin in intact and irradiated (650 rads) mice. According to these authors penicillin was equally effective when given 9 hours after infection but less effective in the irradiated mice when the treatment was delayed until 24 hours. These authors found penicillin was more effective when the organisms were rapidly multiplying (9 hours) but less so when the infection was established. They proposed that at that stage the curative action was not due to the bactericidal action of penicillin alone but a combined effect with the cellular defences of the host. The reason why chloroquine acts more effectively early in the infection in T-cell deprived mice

is not known. In chemotherapy 3 major factors are involved, drug, host and parasite (Whiteside, 1962).

The drug chloroquine has been shown to be effective independently without the requirement of the immune response of the host (Elko and Cantrell, 1970), and this was confirmed in my experiments where early treatment of T-cell deprived mice led to a 100% cure. But, when treated late, though the parasitaemia was reduced to zero as effectively as in the early treated group, 50% of the mice recrudesced. So this effect was unlikely to be due to that of drug alone. Jayawardena et al. (1977) have shown the persistence of P.yoelii infection in the kidneys of mice up to one month after patency. If something similar occurred with P.chabaudi it might explain the recrudescence after chemotherapy late in the infection as proposed by Jennings et al. (1977) for T.brucei. In the immunologically intact mice, the immune response would remove these parasites but this might not occur in immune deprived mice, where T-cells are absent.

The recrudescence population of P.chabaudi from the drug treated T-cell deprived mice did not show any significant increase of drug resistance when tested with the 4 day suppressive test and compared with the original stock. It may be that the 4 day suppressive test is not sensitive enough to show slight variations in drug resistance of the parasite because chloroquine resistant strains of P.chabaudi have been obtained by treating infected mice with oral doses of chloroquine ( $2 \text{ mg kg}^{-1}$  daily for 5 days) and by passaging the infection and repeating the treatment 5 times (Rosario, 1976). However, before drawing any conclusions, it will be

necessary to repeat the same type of experiments with other antimalarials to test the drug sensitivity with other available methods like the Rane test and the 2% bioassay test.

The treatment of P.chabaudi in immunologically intact mice is more effective than that in T-cell deprived mice. This effect is the same for both early and late treatments. In early treatment with chloroquine ( $2 \text{ mg kg}^{-1}$  for 7 days) the parasitaemia in the T-cell deprived group stayed above the 0.1% level, though the intact and sham-treated groups fell to zero. The difference was more pronounced after treatment with  $4 \text{ mg kg}^{-1}$  for 7 days, when all the infected intact mice were cured totally but all the T-cell deprived mice recrudesced. All of the mice in the infected intact group were cured by treatment with  $2 \text{ mg kg}^{-1}$  for 7 days while the parasitaemia in the T-cell deprived group was reduced only to  $0.9\% \pm 0.1$ . Using  $10 \text{ mg kg}^{-1}$  daily doses of chloroquine for 7 days the parasitaemia in both the groups went down to zero, 50% of the mice recrudesced in the T-cell deprived group. This showed that with subcurative doses of chloroquine, an effective immune response (T-cell probably) was necessary to bring down the parasitaemia to zero. Also, when an effective dose of chloroquine was given ( $40$  or  $10 \text{ mg kg}^{-1}$  for 7 days) an intact immune response was required for the prevention of recrudescence. Roberts et al. (1977) have also shown that clindamycin treatment of P.yoelii infection in Nu/Nu mice resulted in recrudescences, though the same treatment gave a curative effect in the intact mice.

Taliaferro (1948), Taliaferro and Kelsey (1948) and Taliaferro and Taliaferro (1949) have shown that quinine was more effective in the intact control than the splenectomised chickens infected with P.gallinaceum. Also in other protozoal infections, Jancso and Jancso (1934) and Kolpikow (1926) showed that chemotherapy of T.brucei infection in intact mice was better than in the splenectomised mice. Muntui et al. (1961) have shown that neoarsphenamine was not as effective in lethally irradiated rats infected with T.brucei as in the intact controls. They used a lethal dosage of irradiation which killed the rats in 8-17 days. Irradiation affects the rapidly dividing cells of the body like haemopoietic cells and gastro-intestinal cells together with the lymphoid cells. Though the effects of other factors like lack of nutrients required for the parasite and anaemia etc, might play some role as well, one can see the important role of the immune mechanism in drug treatment.

Quinine treatment of P.chabaudi infection was also more effective in intact mice than in T-cell deprived mice. Though quinine chemotherapy (100 mg kg<sup>-1</sup> for 7 days) in P.chabaudi infection is not curative, the parasitaemia went down to zero in the intact group but invariable recrudesced after the treatment. However, the same dose of quinine did not bring down the parasitaemia as low in any of the experiments with T-cell deprived mice. When quinine treatment was given on day 1 after a heavy inoculum ( $1.5 \times 10^8$ ) the parasitaemia did not fall at all. It fell to zero in the intact group (fig 38 ). However, quinine treatment after an inoculum of  $1 \times 10^7$  infected erythrocytes reduced the parasitaemia in deprived mice but still not as much as in the treated intact group (fig 39 ).

Pyrimethamine chemotherapy of P.chabaudi infection was also more effective in intact mice than in T-cell deprived mice. This effect was most clearly seen where the pyrimethamine treatment ( $0.2 \text{ mg kg}^{-1}$  or  $2 \text{ mg kg}^{-1}$  daily for 7 days) was given after a heavy inoculum ( $1.5 \times 10^8$  infected erythrocytes) of P.chabaudi. 3 mice recrudesced in the T-cell deprived group though  $5/6$  were cured in the intact group (fig 40 ).

The reason for more effective antimalarial chemotherapy in intact P.chabaudi infected mice than in T-cell deprived mice is not known. This effect seems to depend partly on the drug dosage, where a large enough dose of drug was given the chemotherapy was as effective in the T-cell deprived group as in the intact group (Exp. 34 and 42 ). The time at which the chemotherapy was given also seemed to play a role. Though early treatment with chloroquine ( $10 \text{ mg kg}^{-1}$  for 7 days) was equally effective in deprived and intact mice, the same drug dosage schedule given late in the infection was less effective in the T-cell deprived group. The size of inoculum also plays some role. Pyrimethamine ( $2 \text{ mg kg}^{-1}$  for 7 days) was equally effective in both groups infected with an inoculum of  $1 \times 10^7$  infected RBC but less effective in the T-cell deprived group given a higher inoculum ( $1.5 \times 10^8$  infected RBC)

The reduced effectiveness of antimalarial chemotherapy in T-cell deprived mice does not seem to depend on the antibody level. FA titres of T-cell deprived mice in P.yoelii infections were detectable only after day 12 of the infection (Jayawardena, 1975) and so if the effectiveness of anti-

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malariae depended on the antibody titre it should have been more effective in the late treatment than the early treatment but this was not so (see discussion p 275 ) Also, in my preliminary studies I found that, when hyperimmune serum was combined with quinine treatment, the serum and drug given together seem to act better than drug or antiserum given alone. However, the infection still recrudesced, showing that antibody was probably not an important factor in preventing recrudescence. Roberts *et al.* (1977) have shown that recrudescence of P.yoelii infection in Nu/Nu mice after treatment with clindamycin could be prevented by thymus graft or by giving hyperimmune serum. They also showed that B-cell deficient mice (Nu/Nu mice treated from birth with anti  $\mu$  chain serum) were unable to control P.yoelii infection. According to them T-cells play a crucial role in preventing recrudescence malaria infection as a helper function in the production of antibody.

G. Treatment of malaria infection with  
steroids and antimalarials.

1. P.chabaudi infections

a. High single dose of hydrocortisone-acetate combined with  
chloroquine treatment

There were four groups of mice, A given hydrocortisone alone, B hydrocortisone and chloroquine, C chloroquine and D the untreated control group.

A P.chabaudi inoculum of  $1 \times 10^7$  infected erythrocytes was given on day 0. A single dose of hydrocortisone acetate ( $200 \text{ mg kg}^{-1}$ ) was given subcutaneously on day 3 to groups A and B. Daily i.p. injections of chloroquine ( $2 \text{ mg kg}^{-1}$ ) were given to mice of groups B and C on days 3 to 9. The blood films were taken on slides coated with brilliant cresyl blue at 2-3 day intervals, and the parasites as well as the reticulocytes were counted. Mortality was recorded daily.

The parasitaemic patterns in the untreated control group and the group given hydrocortisone alone were comparable up to day 5 with peak parasitaemias of  $47\% \pm 10$  and  $47\% \pm 4.8$  respectively. By day 8 all of the mice treated with hydrocortisone alone had died with a mean survival time of  $7.5 \pm 0.6$  days. In the untreated control group the parasitaemia started to resolve from day 8 and the mice became aparasitaemic on day 19 (fig 43).

In the infected group given chloroquine alone the blood films became negative on days 8 to 13 from the initial

FIGURE 4. *S. choleraesuis* and percentage parasitaemia in CIA mice inoculated at 0, 1 & 10<sup>7</sup> infected and all treated with streptomycin sulfate 1000 mg kg<sup>-1</sup> daily for 7 days

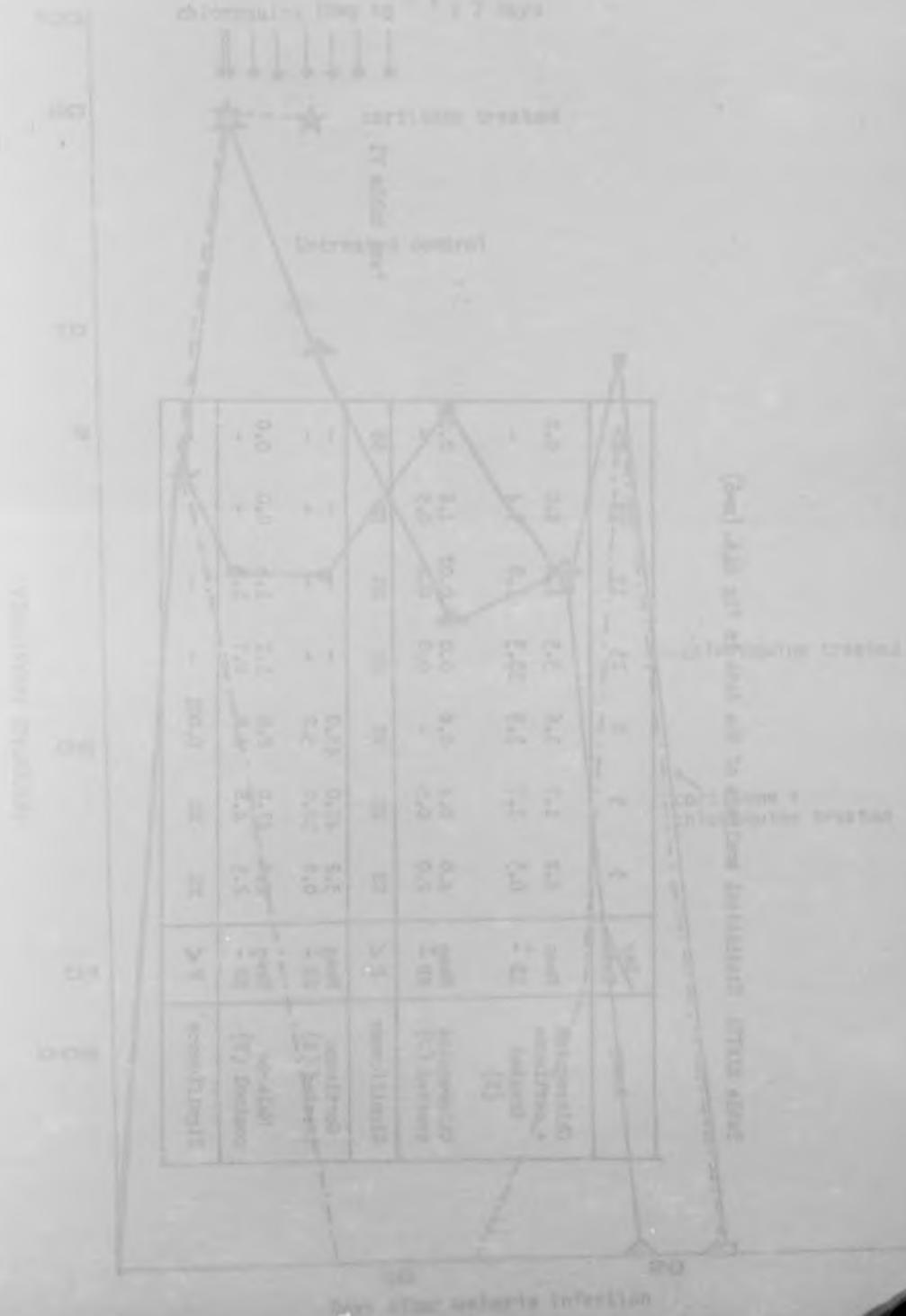


Table XXXVIA Statistical analysis of the data in Fig 43.. (n=6)

Group	Day Data	3	5	8	13	17	19	22
Chloroquine + cortisone treated (B)	Mean	4.2	1.7	1.6	5.5	1.4	8.0	0.0
	SD $\pm$	0.5	1.7	1.9	10.3	1.8	9.4	-
Chloroquine treated (C)	Mean	4.0	0.4	0.0	0.0	0.07	4.6	0.01
	SD $\pm$	2.0	0.5	-	0.0	0.4	0.9	-
Significance	P <	NS	NS	NS	NS	NS	NS	NS
Cortisone treated (A)	Mean	3.2	47.0	43.0	-	-	-	-
	SD $\pm$	0.8	10.0	5.2	-	-	-	-
Malaria control (D)	Mean	4.8	47.0	8.0	1.1	1.6	0.0	0.0
	SD $\pm$	2.5	4.8	4.8	0.7	1.9	-	-
Significance	P <	NS	NS	0.001	-	-	-	-

\*see table VI

FIGURE 43 *P. chaboudi* Mean percentage parasitaemias in CBA mice inoculated with  $1 \times 10^7$  infected RBC and treated with hydrocortisone acetate  $200\text{mg kg}^{-1}$  and/or chloroquine  $10\text{mg kg}^{-1} \times 7$  days

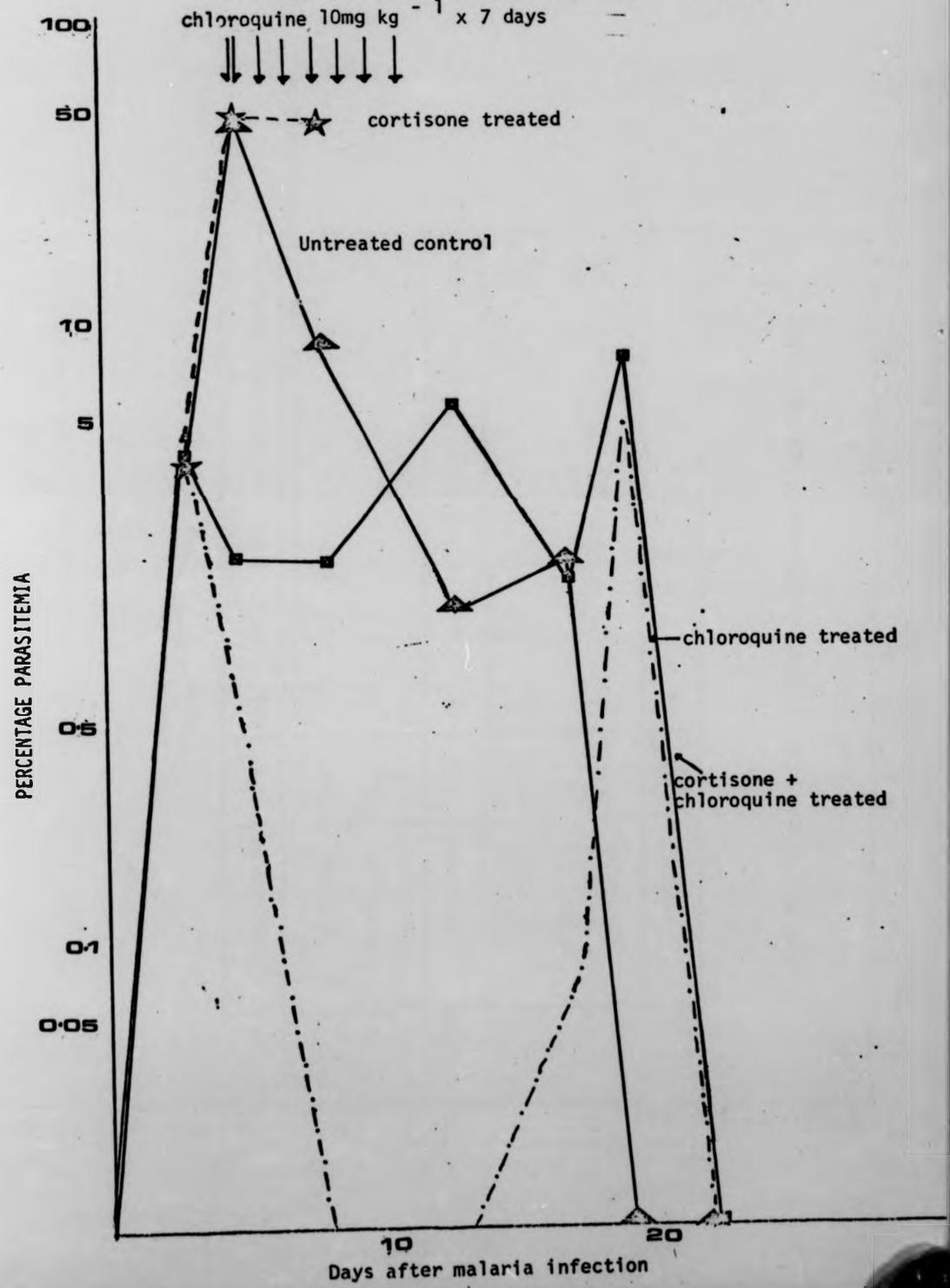
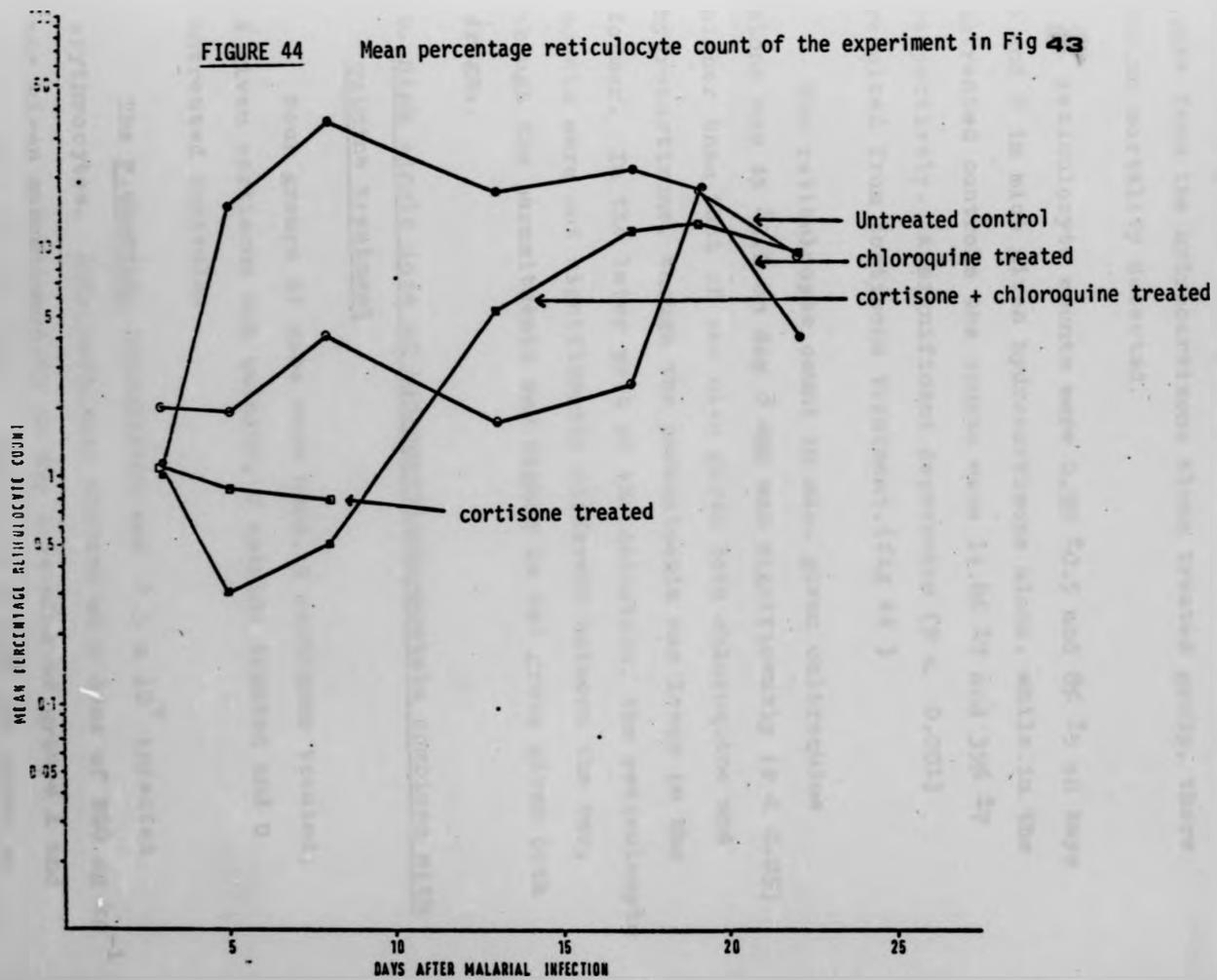




Table XXXVIB Statistical analysis of the data in Fig 44. (n=6)

Group	Day Data	3	5	8	13	17	19	22
Chloroquine + cortisone treated (B)	Mean	1.1	0.3	0.5	5.3	11.3	12.2	9.3
	SD <sup>+</sup> -	0.5	0.08	0.4	0.5	10.6	3.8	5.8
Chloroquine treated (C)	Mean	2.0	1.9	4.0	1.7	2.5	16.4	8.6
	SD <sup>+</sup> -	1.8	1.9	3.2	0.6	0.9	12.1	3.5
Significance	P <	NS	NS	0.05	0.001	NS	NS	NS
Cortisone treated (A)	Mean	1.1	0.9	8.0	all			
	SD <sup>+</sup> -	0.4	0.5	5.0	died			
Malaria control (D)	Mean	1.08	14.8	35.0	17.0	21.8	17.3	4.0
	SD <sup>+</sup> -	0.7	7.04	7.0	4.7	18.0	12.0	1.7
Significance	P <	NS	0.001	0.001	-	-	-	-

\*see table VI



mean parasitaemia of  $4\% \pm 2$ . But in the chloroquine plus hydrocortisone treated group the parasitaemia stayed above  $1.4\% \pm 1.8$  up to day 19 and resolved on day 22. Apart from the hydrocortisone alone treated group, there was no mortality detected.

Reticulocyte counts were  $0.9\% \pm 0.5$  and  $8\% \pm 5$  on days 5 and 8 in mice given hydrocortisone alone, while in the untreated controls the counts were  $14.8\% \pm 7$  and  $35\% \pm 7$  respectively. A significant depression ( $P < 0.001$ ) resulted from cortisone treatment. (fig 44)

The reticulocyte count in mice given chloroquine alone was  $4\% \pm 3.2$  on day 8 and was significantly ( $P < 0.05$ ) higher than that of the mice given both chloroquine and hydrocortisone, though the parasitaemia was lower in the former. In the later part of the infection, the reticulocyte counts were not significantly different between the two, though the parasitaemia was higher in the group given both drugs..

b. High single dose of hydrocortisone-acetate combined with quinine treatment

Four groups of mice were used, A cortisone treated, B given cortisone and quinine, C quinine treated and D untreated controls.

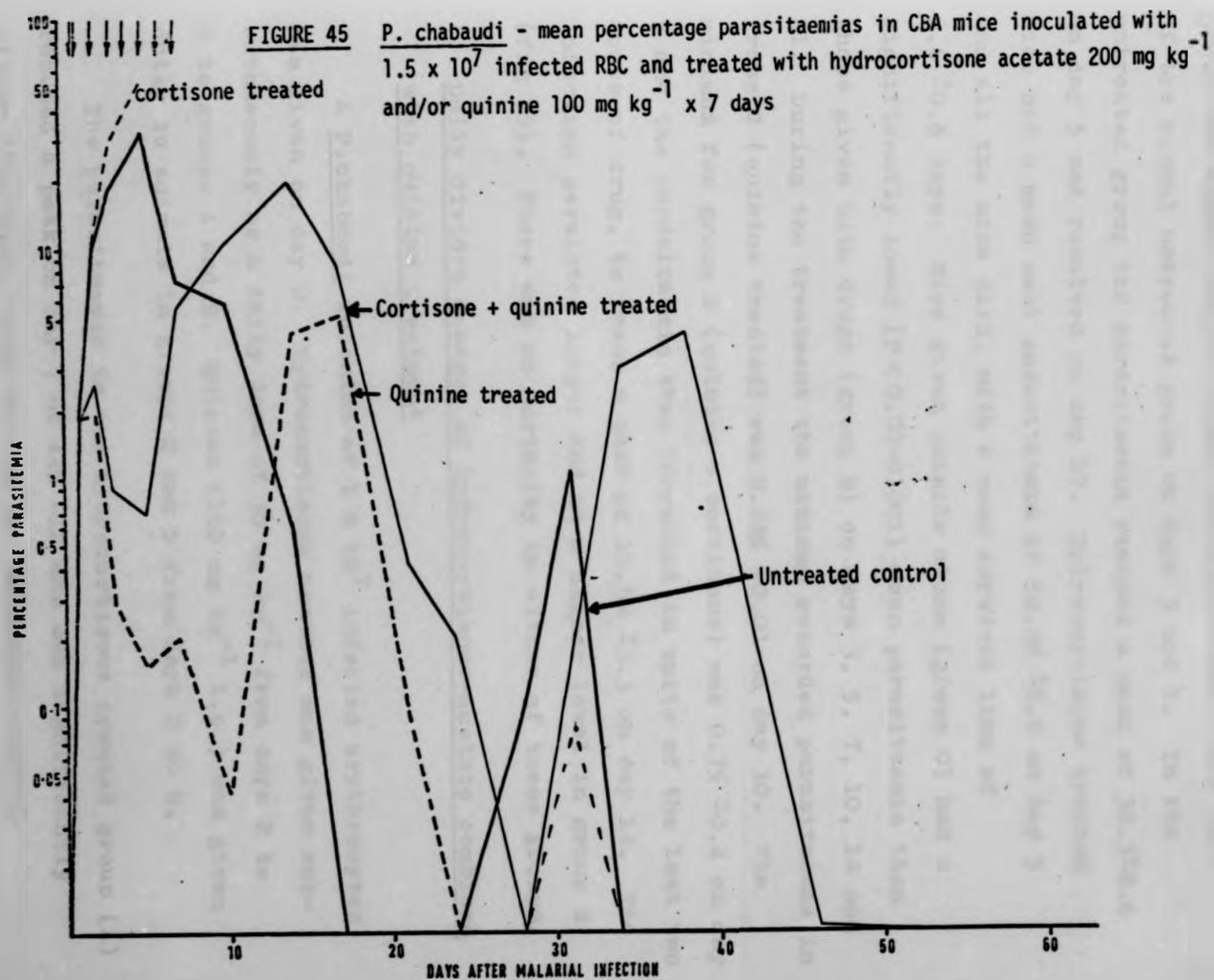
The P.chabaudi inoculation was  $1.5 \times 10^7$  infected erythrocytes. Hydrocortisone acetate at a dose of  $200 \text{ mg kg}^{-1}$  was given subcutaneously on day 1 to mice of groups A and B. Quinine at  $100 \text{ mg kg}^{-1}$  daily for 7 days was given to mice of groups B and C starting 2 hours after the



Table XXVII Statistical analysis of the data in Fig 45. (n=8)

Group	Day Data	1	2	3	5	7	10	14	17	21	24	28	31	34	38	46	52
Quinine + cortisone (B)	Mean	1.9	2.6	0.9	0.7	5.5	10.2	19.5	8.2	0.4	0.2	0.01	0.2	3.0	4.2	0.01	0.0
	SD	0.5	0.7	0.3	0.4	2.4	6.8	3.3	8.2	0.1	0.4	-	0.2	5.4	5.4		
Quinine treated (C)	Mean	1.8	1.9	0.3	0.15	0.2	0.04	4.3	4.9	0.08	0.0	0.0	0.1	0.0	0.0	0.0	0.0
	SD	0.5	0.9	0.2	0.12	0.2	0.01	3.9	4.6	0.1			0.01				
Significance	P <	NS	NS	0.001	0.02	0.001	0.001	0.001	NS	0.001	NS	NS	NS	NS	NS	NS	NS
Cortisone treated (A)	Mean	2.0	10.6	28.4	52.6												
	SD	0.5	3.6	6.0	8.6												
Malaria control (D)	Mean	1.9	9.8	18.6	32.3	7.2	5.8	0.6	0.0	0.0	0.0	0.1	1.1	0.0	0.0	0.0	0.0
	SD	0.6	3.0	3.9	6.6	1.9	0.81	0.5	-	-	-		1.1				
Significance	P <	NS	NS	0.01	0.001	-	-	-	-	-	-	-	-	-	-	-	-

\*see table VI



hydrocortisone treatment.

The mean parasitaemia in the hydrocortisone treated group was significantly higher ( $P < 0.01-0.001$ ) than that of the normal untreated group on days 3 and 5. In the untreated group the parasitaemia reached a peak of  $32.3 \pm 6.6$  on day 5 and resolved on day 17. Hydrocortisone treated mice had a mean peak parasitaemia of  $52.6\% \pm 8.6$  on day 5 and all the mice died, with a mean survival time of  $6.6 \pm 0.6$  days. Mice given quinine alone (group C) had a significantly lower ( $P < 0.02-0.001$ ) mean parasitaemia than those given both drugs (group B) on days 3, 5, 7, 10, 14 and 21. During the treatment the minimum recorded parasitaemia in group C (quinine treated) was  $0.04\% \pm 0.01$  on day 10. The minimum for group B (quinine + cortisone) was  $0.7\% \pm 0.4$  on day 5 and the parasitaemia then increased in spite of the last two doses of drug, to reach a peak of  $19.5\% \pm 3.3$  on day 14. The infection persisted longer and at a higher level in group B (fig 45). There was no mortality in either of these groups.

c. Daily divided dosages of hydrocortisone-acetate combined with quinine treatment

A P.chabaudi inoculum of  $1 \times 10^7$  infected erythrocytes was given on day 0. Hydrocortisone acetate was given subcutaneously as a daily dose of  $30 \text{ mg kg}^{-1}$  from days 2 to 8 to groups A and B. Quinine ( $100 \text{ mg kg}^{-1}$  i.p.) was given daily to animals in groups C and D from days 2 to 8.

The parasitaemia in the hydrocortisone treated group (A) reached a peak on day 7 of  $44\% \pm 12$  and was significantly higher ( $P < 0.05$ ) than that of the untreated control

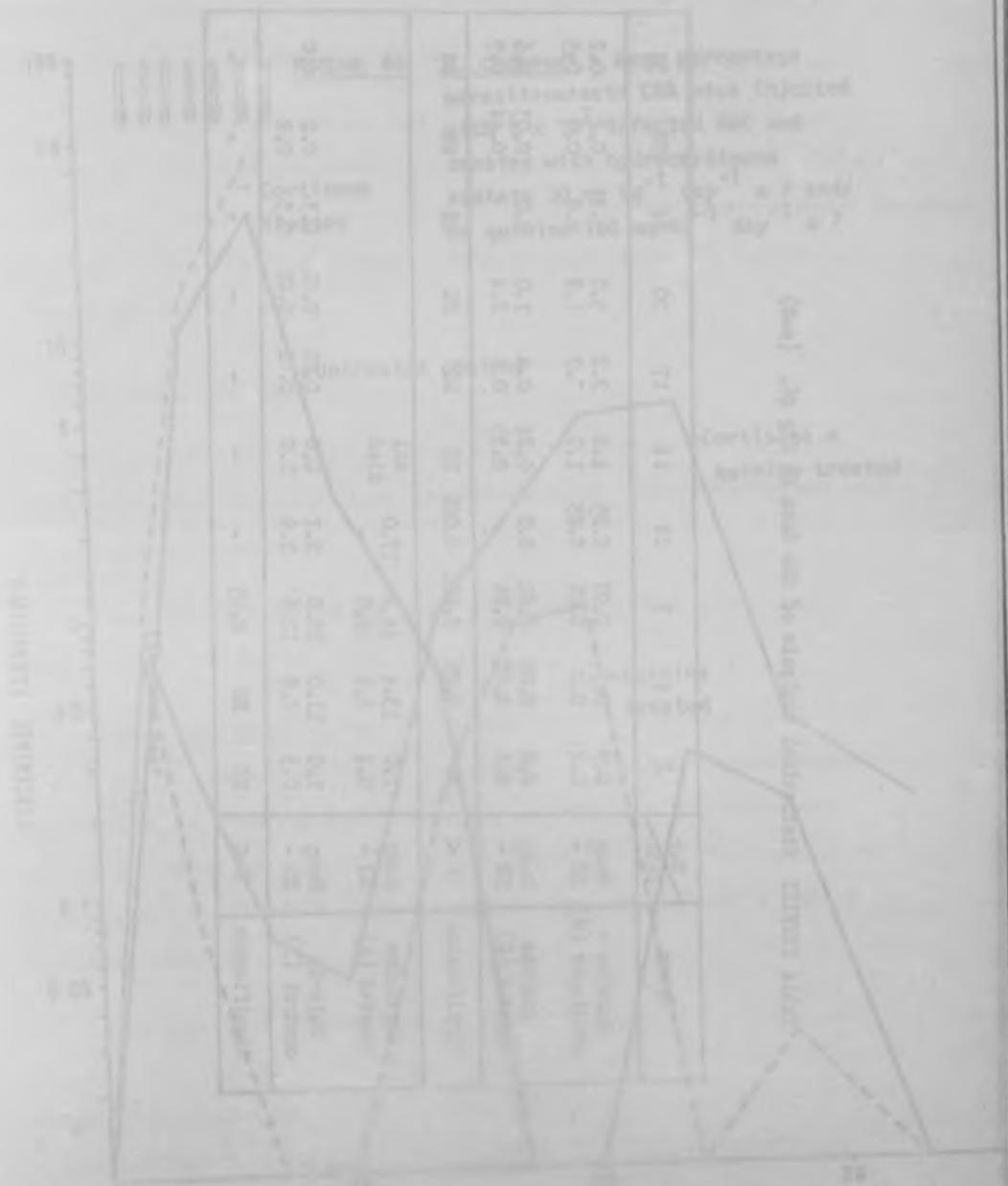
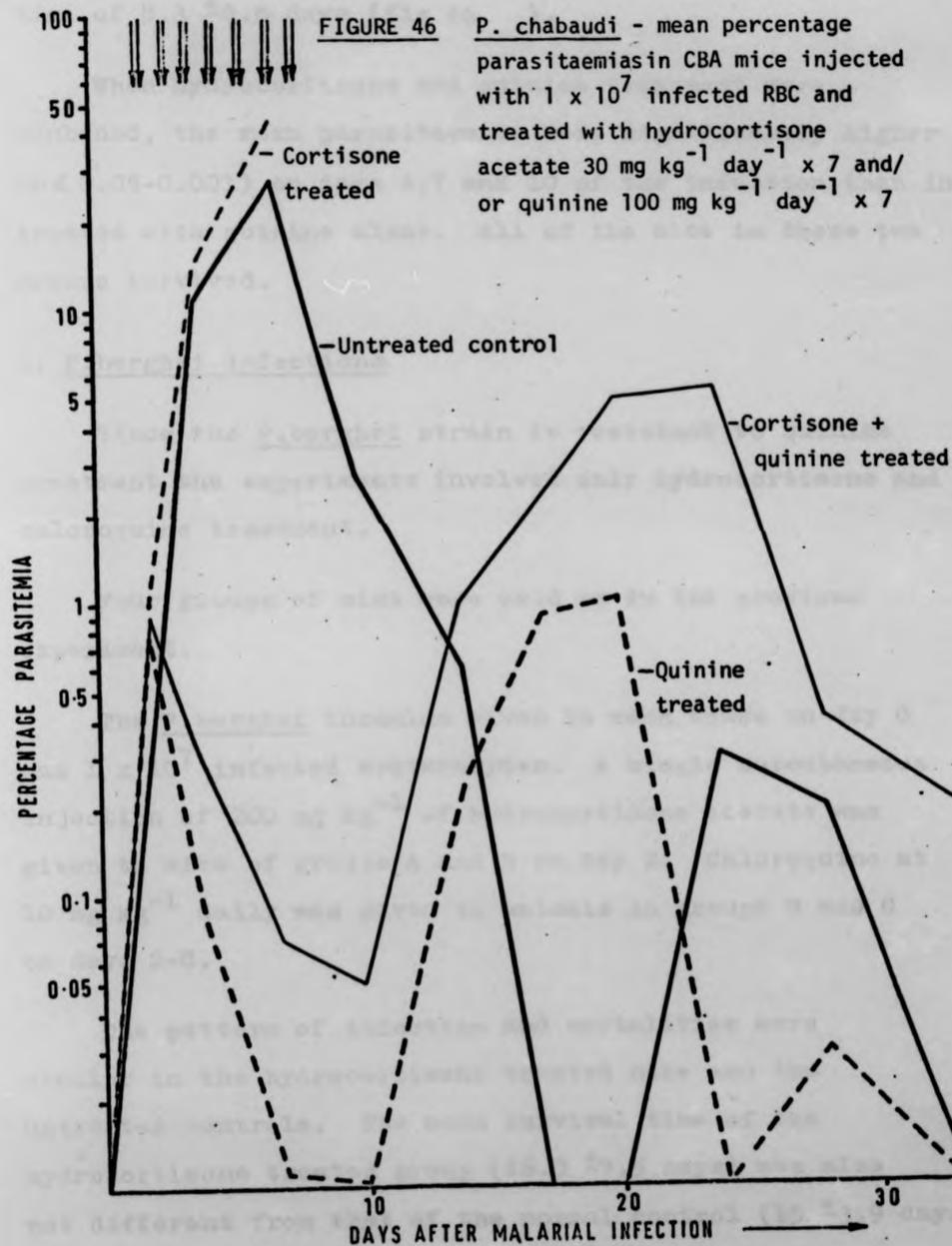


Table XXVIII Statistical analysis of the data in Fig 46. (n=8)

Group	Day Data	2	4	7	10	14	17	20	24	28	33
Quinine + cortisone (B)	Mean	0.9	0.3	0.07	0.05	1.0	2.2	5.0	5.4	0.4	0.2
	SD	0.4	0.2	0.01	0.03	1.7	7.9	5.8	8.8	0.8	0.3
Quinine treated (C)	Mean	0.8	0.08	0.01	0.0	0.24	0.9	1.0	0.0	0.03	0.6
	SD	0.4	0.07	0.01		0.35	0.9	2.4		0.44	0.9
Significance	P <	NS	0.05	0.001	0.001	NS	NS	NS	NS	NS	NS
Cortisone treated (A)	Mean	1.0	13.4	44.0	17.0	all					
	SD	0.4	5.2	12.0	-	died					
Malaria control (D)	Mean	1.0	11.0	28.0	2.7	0.6	0.01	0.01	0.3	0.2	0.0
	SD	0.3	4.8	12.6	1.9	1.2	0.01	0.01	0.3	0.2	
Significance	P <	NS	NS	0.05	-	-	-	-	-	-	-

\*see table VI



( $28\% \pm 12.6$ ). All the control mice survived but the hydrocortisone treated animals died with a mean survival time of  $8.3 \pm 0.8$  days (fig 46 ).

When hydrocortisone and quinine treatment were combined, the mean parasitaemias were significantly higher ( $P < 0.05-0.001$ ) on days 4,7 and 10 of the infection than in mice treated with quinine alone. All of the mice in these two groups survived.

## 2. P.berghei infections

Since the P.berghei strain is resistant to quinine treatment the experiments involved only hydrocortisone and chloroquine treatment.

Four groups of mice were used as in the previous experiment.

The P.berghei inoculum given to each mouse on day 0 was  $1 \times 10^7$  infected erythrocytes. A single subcutaneous injection of  $200 \text{ mg kg}^{-1}$  of hydrocortisone acetate was given to mice of groups A and B on day 2. Chloroquine at  $10 \text{ mg kg}^{-1}$  daily was given to animals in groups B and C on days 2-8.

The pattern of infection and mortalities were similar in the hydrocortisone treated mice and the untreated controls. The mean survival time of the hydrocortisone treated group ( $16.9 \pm 7.5$  days) was also not different from that of the normal control ( $15 \pm 3.9$  days). The chloroquine treated group was also comparable with that given both hydrocortisone and chloroquine (fig 47 p 295 ).



Table XXXIX Statistical analysis of the data in Fig 47. (n=6)

Group	Day Data	2	6	9	11	13	16	19	27	29	35	38
Chloroquine + cortisone treated (B)	Mean	3.2	0.0	0.0	0.0	0.0	0.01	1.5	24.7	26.0	35.0	32.5
	SD	0.5						0.9	10.0	5.3	10.9	0.5
Chloroquine treated (C)	Mean	3.8	0.0	0.0	0.0	0.0	0.0	2.04	2.0	14.0	32.0	40.0
	SD	1.1						1.3				
Significance	P <	NS	NS	NS	NS	NS	NS	NS	-	-	-	-
Cortisone treated (A)	Mean	3.5	25.0	12.7	43.7	29.0	60.0	61.0				
	SD	2.1	17.6	2.1	29.0	8.9						
Malaria control (D)	Mean	3.8	21.2	20.5	27.8	53.0	52.0	72.0				
	SD	1.3	11.0	6.5	4.8	10.1						
Significance	P <	NS	NS	0.01	NS	0.001	-	-				

\*see table VI

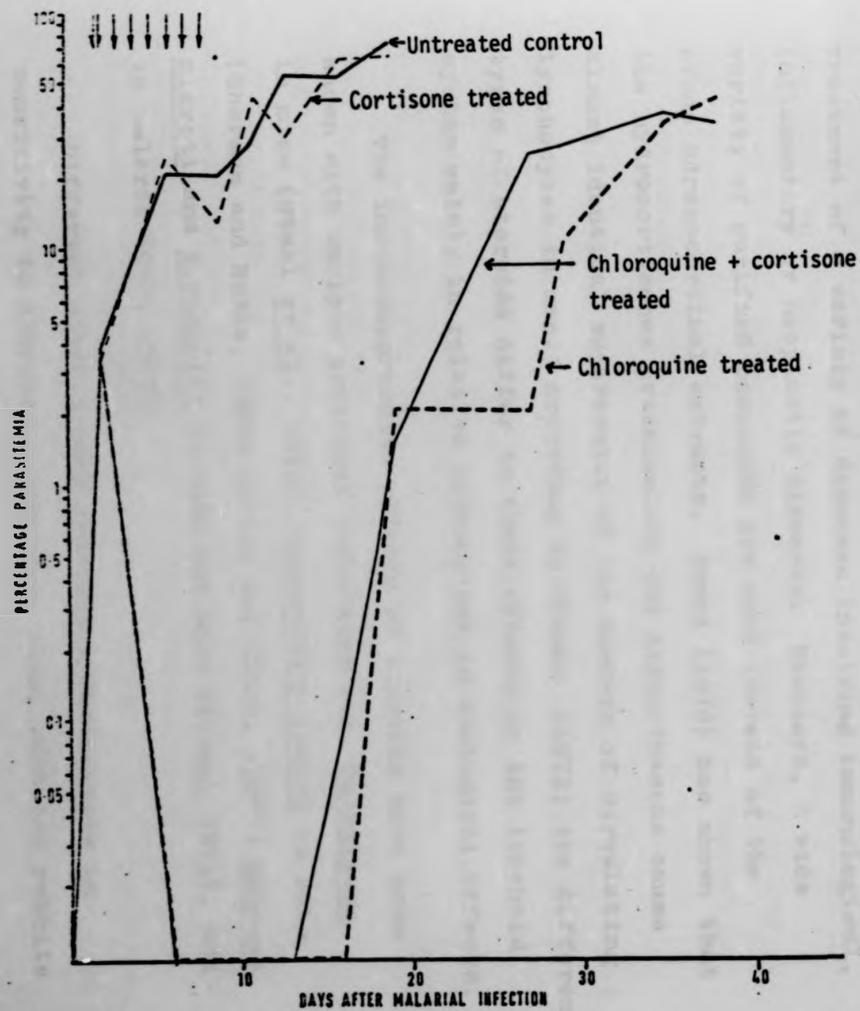


FIGURE 47 *P. berghei* - mean percentage parasitaemias in CBA mice inoculated with  $1 \times 10^7$  infected RBC and treated with hydrocortisone acetate  $200 \text{ mg kg}^{-1} \times 1$  and/or chloroquine  $100 \text{ mg kg}^{-1} \text{ day}^{-1} \times 7$

The infections became blood negative on day 6 in both cases and recrudescent between days 16 and 19. There was no significant difference in parasitaemia and mean survival time for the chloroquine treated group ( $25 \pm 10.1$  days) and the chloroquine and hydrocortisone treated group ( $28.6 \pm 17.2$  days).

#### DISCUSSION

Glucocorticosteroid (steroid) hormones produced in the adrenal cortex have striking pharmacological effects on lymphoid cells and tissues. These effects form part of the basis for the widespread use of these hormones in the treatment of a variety of diseases involving immunological, inflammatory or neoplastic diseases. Nowadays, a wide variety of purified compounds are used instead of the crude adrenocortical extracts. Fauci (1976) has shown that the hydrocortisones prednisolone and dexamethasone cause almost identical suppression of the numbers of circulating lymphocytes in man. According to Claman (1972) the different types of steroids differ in their effects on the lymphoid system mainly in relative potency not in biological effects.

The immunosuppressive effects of steroids have been shown with various protozoal infections e.g. Toxoplasma in mice (Stahl et al., 1966), Trypanosoma lewisi in rats (Sherman and Ruble, 1967; Patton and Clark, 1968), Babesia microti and B. rodhaini in rats and mice (Young, 1973), and in malaria (Cox, 1968).

Different species show remarkable differences in sensitivity to steroids. Hamsters, mice, rats and rabbits

are sensitive to steroids but man, guinea pigs, monkeys and ferrets are relatively resistant to them (Claman, 1972). Steroid treatment in the steroid sensitive species produces profound lymphopenia, atrophy of the thymic cortex, shrinkage of lymph nodes and the spleen and impairment of antibody production (Claman, 1975). Fauci (1976) referring to other workers, stated that the immunosuppressive effects of steroids include decreased migration of cells into inflammatory sites, circulating lymphopenia and monocytopenia, decreased immunoglobulin levels and impaired expression of cutaneous delayed hypersensitivity. Yu et al. (1974) demonstrated that both T and B lymphocytes are affected by prednisolone but that T-cell lymphopenia is more pronounced. However, according to Claman (1975) the suppressive effects of steroids on cell mediated immunity may be due not to any inhibition of lymphocyte function but rather to inhibition of macrophage function..

In malaria infections, immunosuppressant therapy is used in the management of nephrotic syndrome associated with P.malariae (Kibukamusoke, 1968), and in the treatment of cerebral malaria caused by P.falciparum (Woodruff and Dickinson, 1968; Oricello, 1968 and Smithskamp and Wolthuis, 1971). Smithskamp and Wolthuis (1971) suggested that steroids reduce or prevent cerebral and pulmonary oedema by preserving the normal function of capillary endothelium. Migasena and Maegraith (1967) have shown that, in P.knowlesi malaria infection in monkeys, chloroquine as well as cortisone restores the normal net movement of albumin across the blood brain barrier within one hour. On the other hand Reid and Nkrumah (1972) doubted the value of steroids in

the treatment of cerebral malaria. They demonstrated increased levels of fibrin degradation products in cerebral malaria patients and that cerebral intravascular coagulation occurs in severe falciparum malaria. Hall (1977) in his review on the treatment of severe P.falciparum infection, suggested that steroid treatment is not helpful in a diffuse vascular disease such as falciparum malaria. Schneider (1953) did not find any effect of cortisone on parasitaemia or liver function in two patients with P.vivax infections. Schmidt and Squires (1951) showed that cortisone in daily doses of  $10 \text{ mg kg}^{-1}$  during the primary attack of P.cynomolgi infection in monkeys produced a striking increase in parasitaemia; when given during the chronic phase of the infection, severe recrudescence of the infection occurred. There was also marked reduction in the sizes of peripheral lymph nodes and the spleen with lymphopenia in the treated animals. The authors described the effects of cortisone treatment as closely similar to splenectomy.

There are differences of opinion on the action of cortisone in experimental malarias. Findlay and Howard (1952) demonstrated that after intramuscular injection of 5 mg of cortisone acetate 30 min before and 6 hrs and 4 days after inoculations of P.berghei into mice, the parasites appeared early and the mice died quicker than the controls. Tandon and Bhattacharya (1970) showed that when P.berghei infected mice were treated with 1 mg of dexamethasone every 12 hours for 12 days, the parasitaemia increased 4 fold over that of untreated controls. Interestingly this effect, according to the authors, was due to an increase in gametocytes rather than asexual forms. Carrescia (1961)

also showed that mice infected with P.berghei and treated with prednisolone (0.5 mg - 1.25 mg) died more rapidly than the controls.

There are others groups of workers who have obtained quite different results. Roberts (1954) treated P.berghei infections in mice and in hamsters with cortisone acetate. Though he used the same dosage as Findlay and Howard (1952) he did not get the rapid increase of parasitaemia; rather the infection was found to be inhibited. Singer (1954b) treated P.berghei infections in mice with different schedules of cortisone e.g. (i) 0.25 mg daily from 2 days before infection and then throughout the infection, (ii) 5 mg of drug a few minutes before or some days after the infection (iii) 5 mg of cortisone for 8 successive days starting 2 days before or 6 days after infection. The drug decreased the parasitaemia, and reticulocytosis and anaemia were held in check. He thought that the reduced production of reticulocytes which serves as a suitable nidus for the parasite served to limit the parasitaemia. Jackson (1955) proved the same fact by showing that, when intramuscular infections of 5 mg of cortisone acetate were given daily from 2 days before to 2 days after P.berghei infection in rats, there was a two-fold decrease in parasitaemia, a decrease in reticulocyte count, and an increase in survival time. Schneider (1953) reported that cortisone had no effect on P.berghei infection in mice, when they were treated with  $10 \text{ mg kg}^{-1}$  body weight from the day of appearance of the infection until the day of death.

In P.gallinaceum infected chickens, treatment with

50 mg of cortisone for 24 days did not have any effect on the parasitaemia or on the functions of the liver. Cox (1974a) compared the effects of betamethasone on mice infected with P.chabaudi or P.yoelii by treating them at various intervals after the infection. The P.chabaudi infections were more severe in the treated animals and P.yoelii infections were depressed by betamethasone. The author suggested that the effect was due to the depression of reticulocytosis by the drug. The malarial antibody levels in the betamethasone treated animals were lower than the controls.

In my experiments hydrocortisone acetate was used. This is rather insoluble, is slowly absorbed and therefore has a 'depot effect'. If one used soluble compounds, in dosages roughly equivalent to those of insoluble compounds, effects may be difficult to see unless very large amounts or repeated administration were used (Claman, 1972).

I used a single subcutaneous dose of  $200 \text{ mg kg}^{-1}$  (4 mg per mouse) or daily doses of  $30 \text{ mg kg}^{-1}$  (0.6 mg per mouse) for 7 successive days. I wanted to simulate the treatment of the human situation and my drug schedule was started only after the appearance of the parasitaemia. It has been shown that steroids are most effective in depressing the early stages of the immune response but have no effect on the immunity once it has been established in malaria infection (Cox, 1968). Since hydrocortisone treatment was started within 1-3 days of the infections, in all my experiments, the maximal immunosuppressive effect could be expected.

P.chabaudi produces a benign resolving infection in CBA mice, but all of the cortisone treated mice died at the first peak of the parasitaemia, within 8 days. Reticulocyte counts were significantly depressed from day 5 by comparison with those of untreated controls (fig 44 p288). The suppressive effect of cortisone on reticulocytosis is well documented (Singer, 1954; Jackson, 1955; Cox, 1974a). The cause of the death of P.chabaudi-infected mice when treated with cortisone may be due to three reasons. First of all P.chabaudi infection in CBA mice is potentially virulent in nature. The virulent nature of this parasite in mice has been shown by Ott (1969), Cox (1970) and Carter and Walliker (1975). In CBA mice 2 or more blood passages produced a much more virulent infection (Lwin, unpublished data). Secondly, the immunosuppressive effect of cortisone on T-cells, B-cells and on macrophages (Claman, 1975) may play a role. Thirdly, since P.chabaudi favours mature red cells and cortisone suppresses reticulocytosis, when the mature RBC are heavily parasitised (50% parasitaemia) but only a fraction of the normal number of reticulocytes are released, the mouse will naturally develop a severe anaemia and die (fig 44 p288). This final point can be seen more clearly when we compare the results of cortisone treatment with another self-resolving malaria, P.yoelii, in mice. Barker and Powers (1971) showed that hydrocortisone treatment given 1 day before and throughout the infection of P.yoelii in mice, suppressed the initial parasitaemia, but impaired both immunity and recovery. Cox (1974a) proposed that the potentiation of P.chabaudi-infection in betamethasone-treated mice was due to a depression of reticulocytosis. In mice immunosuppressed

by T-cell deprivation but where there is no impaired reticulocytosis, P.yoelii infections are fatal (Jayawardena et al., 1977) while P.chabaudi infections are not. (Exp IV 1. p 137). Thus in cortisone treatment, the effect of impairment of reticulocytosis may be a more decisive factor in the virulent nature of P.chabaudi than its immunodepressive effect on the immune response.

Antimalarial therapy was less effective when combined with hydrocortisone treatment. On the basis of results obtained by drug treatment of T-cell deprived mice subcurative doses of chloroquine and quinine were used in order to bring out any differences in the response to treatment. There were indications that chloroquine treatment also was more effective than hydrocortisone and chloroquine given together though the results were not significant. Parasitaemias in the quinine treated group were significantly different ( $P < 0.02 - 0.001$ ) from those of the hydrocortisone plus quinine treated groups between days 4-10, though the parasitaemias were very low in both.

Corticosteroid causes the pronounced lymphopenia of T-cells as well as B-cells in mice, and macrophage function is also supposed to be inhibited (see this discussion p297). The reduced effectiveness of antimalarial drugs when combined with hydrocortisone is thus similar to results from treatment of T-cell deprived mice. Although the number of reticulocytes was significantly lower on day 8 ( $P < 0.05$ ) in the hydrocortisone/chloroquine treated group, no mortality occurred because the parasitaemia ( $1.6\% \pm 1.9$ ) on that day was low (fig 43 p284).

Hydrocortisone treatment of P.berghei infection did not have a significant effect on parasitaemia or mortality after antimalarial therapy compared with controls. Schneider (1953) and Fulton (1954) demonstrated that cortisone acetate treatment of mice or rats infected with P.berghei did not significantly effect the infection when compared with the controls. Cox (1968) also did not find any significant effect on the normal course of P.vinckei infection and mortality in mice treated with high doses of betamethasone. But, as stated earlier, others have shown and discussed that steroids potentiate P.berghei infection in mice as a result of immunosuppression (Findlay and Howard, 1952) or increased gametogenesis (Tandon and Bhattacharya, 1970). There are still others who have demonstrated that steroids depress the course of P.berghei infection in mice. According to them, this effect is due to the depression of reticulocytosis, without which P.berghei cannot grow very well (Singer, 1954b and Jackson, 1955).

According to Cox (1968) steroids do not have any effect on the parasitaemia of P.vinckei in mice which kill the mice within 6 days; but when the infection normally persisted 6-9 days, steroids enhanced the parasitaemia. Young (1973) demonstrated that steroids had no action on parasitaemias of a virulent strain of B.rodhaini in mice though it enhanced the benign B.microti infection. The same is true with trypanosome infections. Steroids did not have any influence on the course of infection with virulent strains of T.gambiense, T.brucei or T.congolense in rats but the benign strains of T.congolense and T.rhodesiense infections were aggravated by the steroids (Petana, 1964). Thus it

seems, that steroids do not affect the outcome of virulent infections like P.berghei, but they only affect benign strains. This perhaps is the balancing effect of the activation of suppressor T-cells by the virulent organisms (Jayawardena and Waksman 1977) and reduction of antibody production, lymphopenia and prevention of the function of macrophages by the steroids on the one hand and the reticulocytopenia produced by the steroid on the other.

The effect of chloroquine and cortisone on P.berghei infection in mice was similar to that of chloroquine alone both in terms of the course of infection and in mean survival time (exp G2 p 283). However Cantrell and Kendrick (1963) have shown that cortisone in daily doses of 100-200 mg kg<sup>-1</sup> reduced parasitaemia by 25% in P.berghei infected mice and acted synergistically with all the antimalarials. These authors did not think that the effect was due to the depression of reticulocytes.

## H. Malaria and protein-energy malnutrition

1. P.chabaudi infections in mice maintained on protein-energy deficient diets

Two groups of weanling mice were used, one fed on 4% protein diet and the other on a 21% protein diet. PABA (0.05%) was added to the drinking water of all animals.

In mice fed on the 21% protein diet, the initial mean body weight went up from 16.6 g ( $\pm 0.7$ ) to 18 g ( $\pm 1.0$ ) during a period of 4 weeks prior to infection. Mice on the 4% protein diet showed a loss of weight during the same period from 16.5 g ( $\pm 0.6$ ) to 11.4 g ( $\pm 0.9$ ). On day 32 of the malaria infection, mean body weights in the 21% protein and 4% protein groups were 21.3 g ( $\pm 1.7$ ) and 9.3 g ( $\pm 0.7$ ) respectively. At the end of the experiment (day 60) the respective weights were 22.5 g ( $\pm 2.1$ ) and 8.6 g ( $\pm 0.9$ ).

The P.chabaudi inoculum was  $10^7$  infected erythrocytes. Parasitaemia was detectable in both cases on day 1 and maximal on day 6 with 41.8% ( $\pm 1$ ) in mice fed on the 21% protein diet and 38% ( $\pm 4.6$ ) in mice fed on the 4% protein diet. The parasitaemia in the 4% protein diet treated group was more prolonged and resolved on day 28 while the other group resolved on day 17. Also the second peak of the parasitaemia appeared 6 days later in the low protein diet treated group (day 37) than that of the control group (day 33), (fig 48).

There were no deaths in either group.

Figure 10. A model of the primary productivity in a river  
 program (1) in a river (2) and (3) in a  
 pond (4) of the same basin (5) in a  
 lake (6).

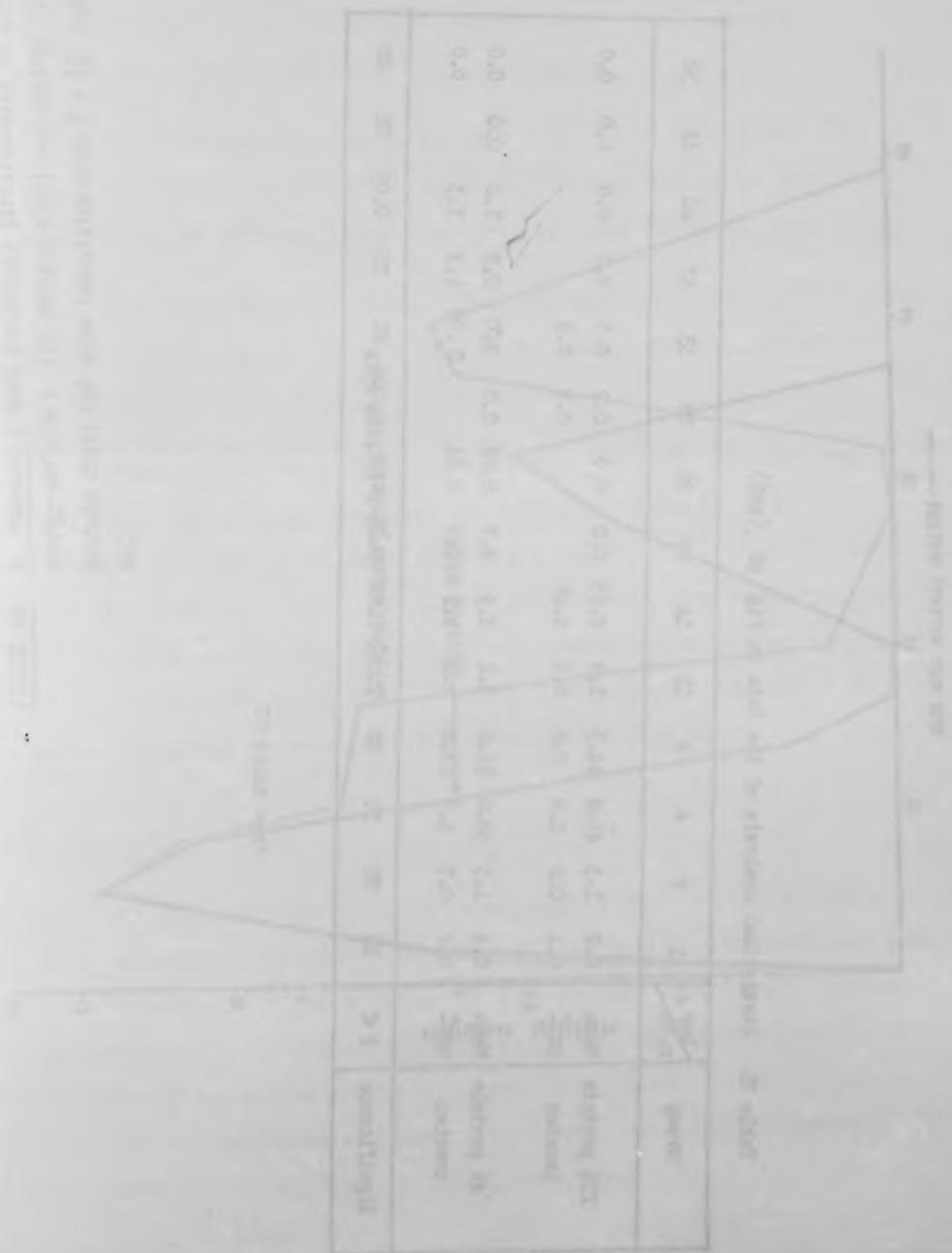
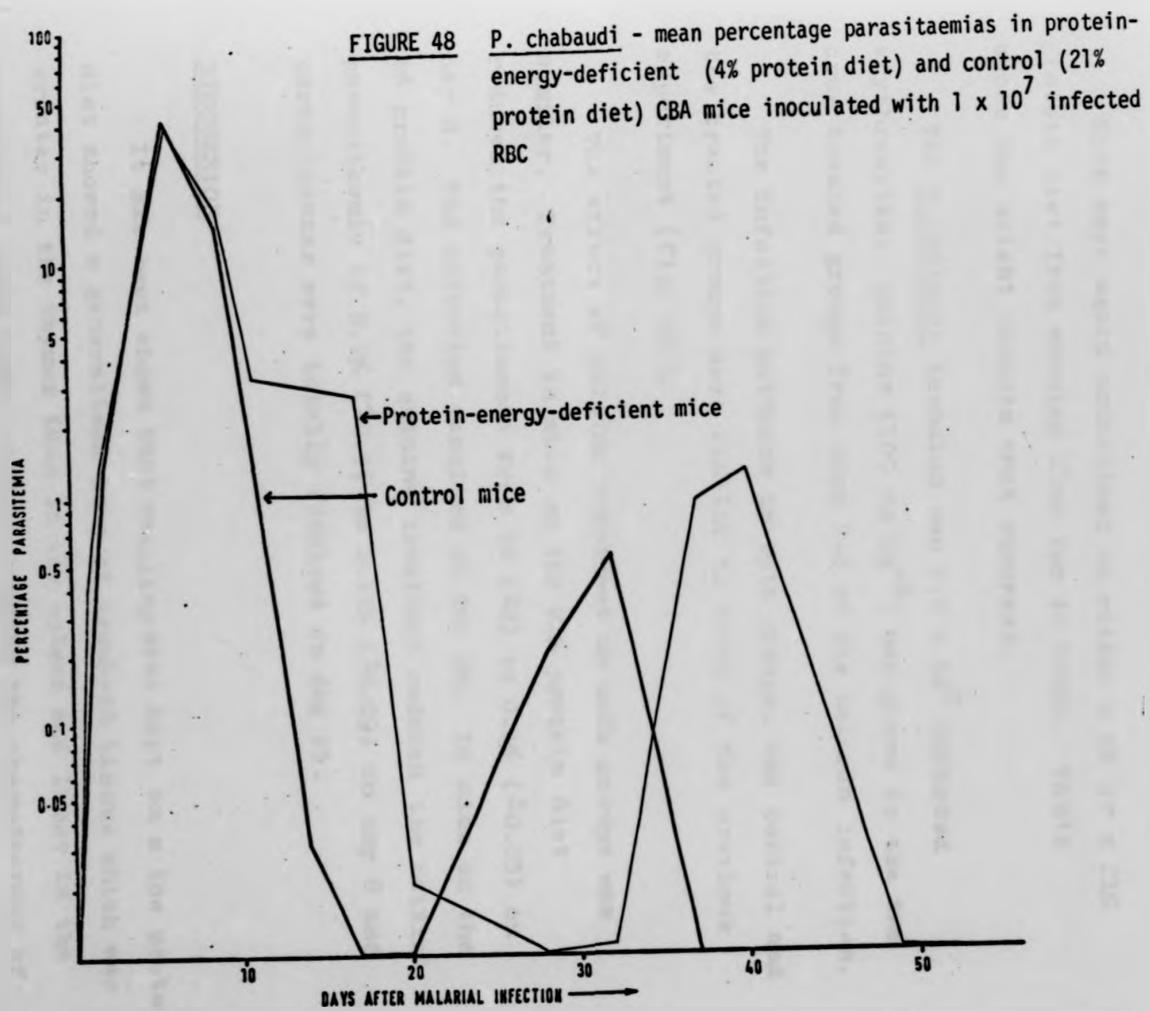


Table XI. Statistical analysis of the data in Fig 48 (n=6)

Group	Day Data	1	2	6	9	11	14	17	20	28	33	37	40	49	52
21% protein treated	Mean	0.2	1.3	41.8	14.3	1.3	0.03	0.0	0.0	0.2	0.5	0.0	0.0	0.0	0.0
	SD ±	0.1	0.2	1.0	3.6	0.6	0.05			0.1	0.9				
4% protein treated	Mean	0.4	1.3	38.0	16.8	3.2	2.9	2.7	0.02	0.0	0.01	0.9	1.2	0.0	0.0
	SD ±	0.2	0.7	4.6	13.0	0.4	1.7	2.7	0.04		0.02	1.4	1.3		0.0
Significance	P <	NS	NS	NS	NS	0.001	0.01	0.05	NS	0.001	NS	NS	0.05	NS	NS

\*see table VI



## 2. Quinine treatment of P.chabaudi infections in protein-energy deficient mice

Mice were again maintained on either a 4% or a 21% protein diet from weaning time for 40 weeks. Table shows the weight changes that occurred.

The P.chabaudi inoculum was  $1.6 \times 10^7$  infected erythrocytes. Quinine ( $100 \text{ mg kg}^{-1}$ ) was given to the two drug treated groups from days 2-8 of the malaria infection.

The infection patterns in both groups, the control and the treated groups were similar to those of the previous experiment (fig 49).

The effect of quinine treatment in both groups was similar. Treatment in mice on the 21% protein diet reduced the parasitaemia from 7% ( $\pm 2$ ) to 0.1% ( $\pm 0.05$ ) on day 8. The infection resolved on day 20. In mice on the 4% protein diet, the quinine treatment reduced the initial parasitaemia of 9.2% ( $\pm 0.5$ ) to 0.12% ( $\pm 0.09$ ) on day 8 and parasitaemias were totally resolved on day 25.

### DISCUSSION

It has been shown that weanling mice kept on a low protein diet showed a generalised loss of lymphoid tissue which was greater in the thymus than in the spleen and least in the mesenteric lymph node. After studying the chemotherapy of malaria infections in T-cell deprived mice, a study was made of the effects of drug treatment of malaria infections in "nutritionally T-cell deprived" mice.

1. The first part of the paper is devoted to a study of the  
 2. properties of the function  $f(x)$  defined by the series  
 3.  $f(x) = \sum_{n=0}^{\infty} a_n x^n$  where  $a_n = \frac{1}{n!}$ . It is shown that  
 4.  $f(x)$  is an entire function of order 1. The second part  
 5. of the paper is devoted to a study of the function  
 6.  $g(x) = \sum_{n=0}^{\infty} b_n x^n$  where  $b_n = \frac{1}{n!}$ . It is shown that  
 7.  $g(x)$  is an entire function of order 1. The third part  
 8. of the paper is devoted to a study of the function  
 9.  $h(x) = \sum_{n=0}^{\infty} c_n x^n$  where  $c_n = \frac{1}{n!}$ . It is shown that  
 10.  $h(x)$  is an entire function of order 1.

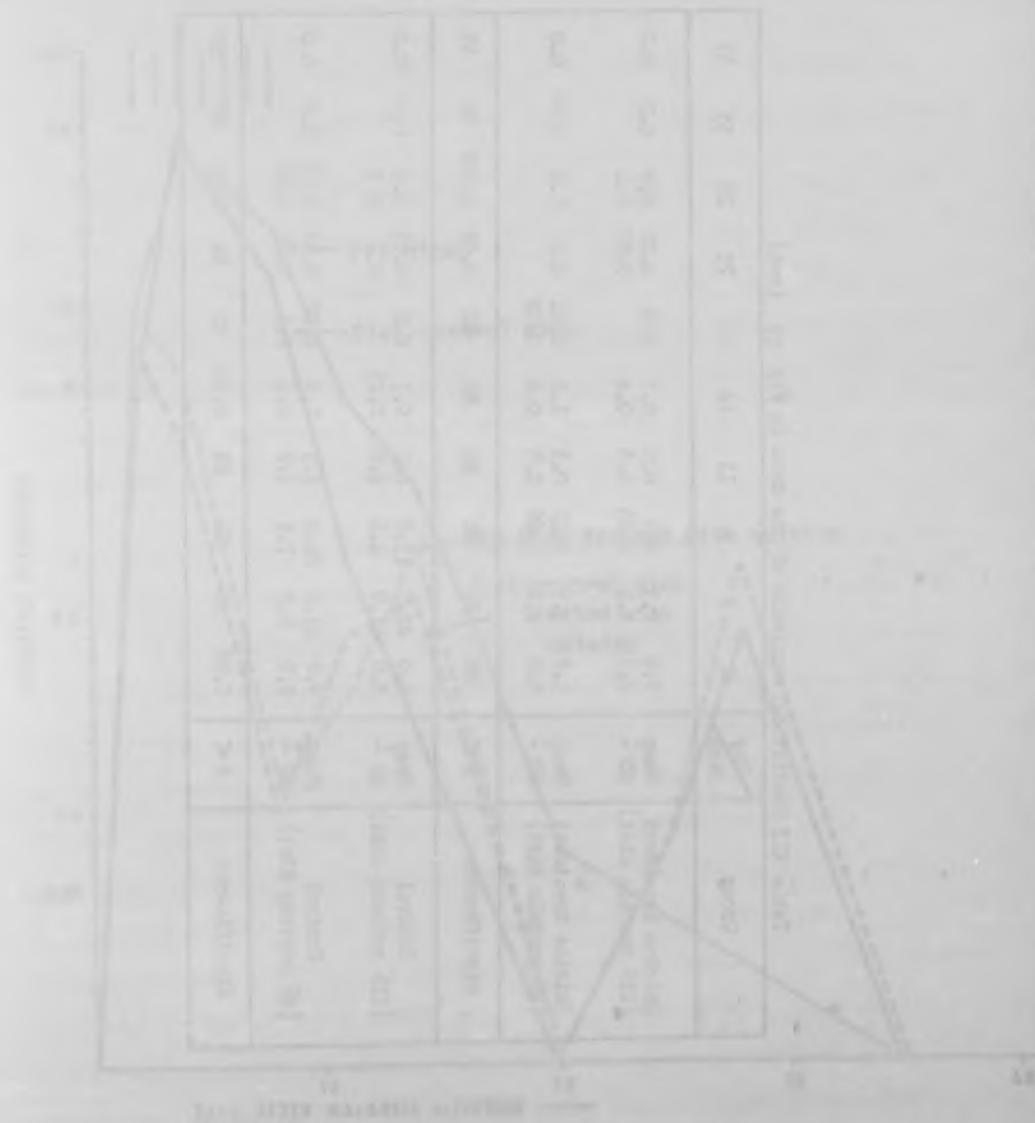
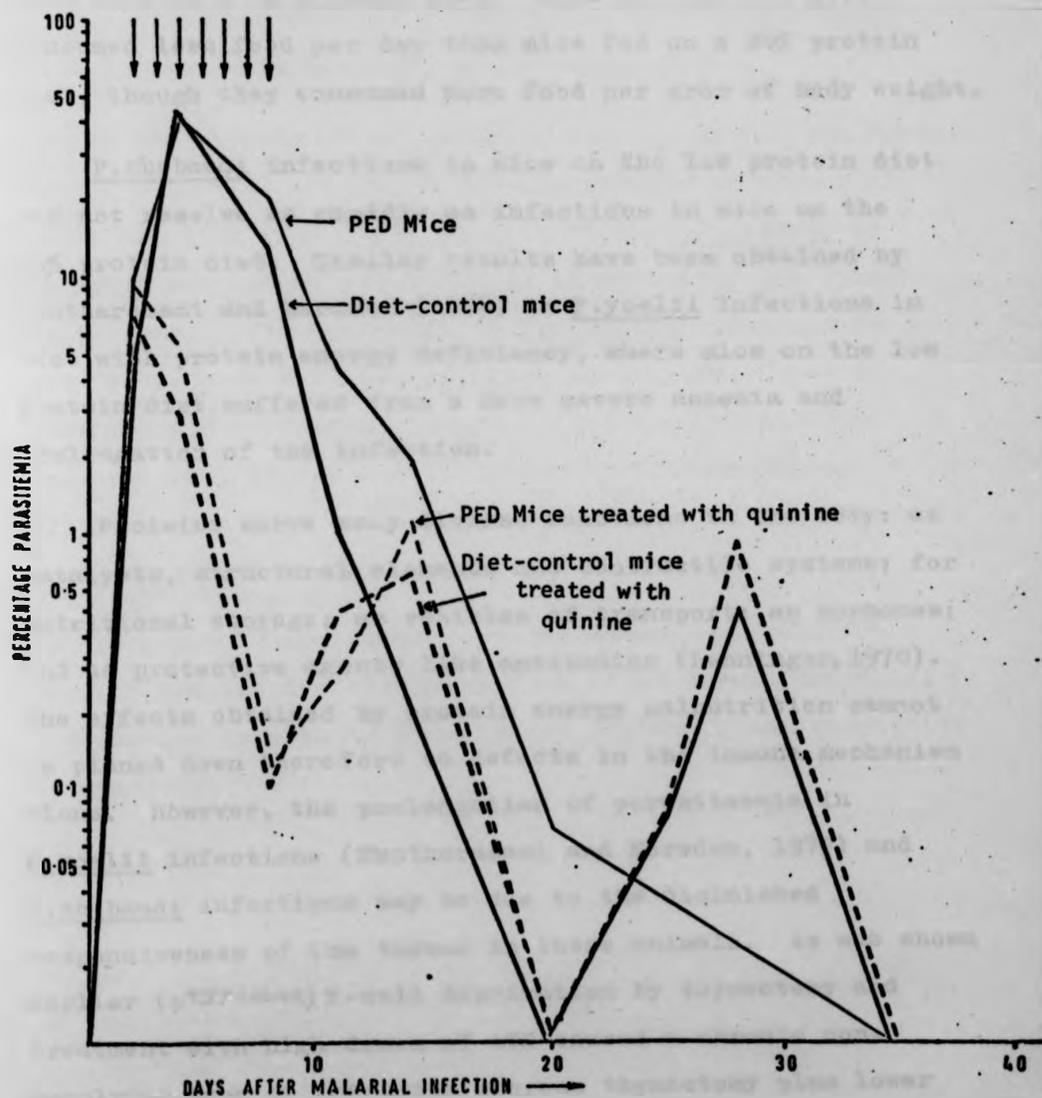


Table XLI Statistical analysis of the data in Fig 49 (n=6)

Group	Day	2	4	8	11	14	20	25	28	35	39
	Data										
Quinine treatment (21% protein diet)	Mean	7.0	2.7	0.1	0.5	0.7	0.0	0.08	0.9	0.0	0.0
	SD	2.0	1.1	0.05	0.3	0.4		0.07	0.1		
Quinine treatment (4% protein diet)	Mean	9.2	5.3	0.12	0.3	1.1	0.01	0.0	0.0	0.0	0.0
	SD	5.0	4.2	0.09	0.5	0.6	0.02				
Significance	P <	NS	NS	NS	NS	NS	NS	0.02	0.001	NS	NS
Control (21% protein diet)	Mean	5.0	44.0	13.0	2.2	0.3	0.0	0.09	0.5	0.0	0.0
	SD	3.0	11.0	4.6	2.0	0.25		0.1	0.3		
Control (4% protein diet)	Mean	9.2	41.0	20.0	4.3	1.9	0.07	0.04	0.03	0.0	0.0
	SD	2.0	6.8	7.6	3.2	0.6	0.1	0.01	0.02		
Significance	P <	0.02	NS	NS	NS	0.001	NS	NS	0.01	NS	NS

\*see table VI

FIGURE 49 - *P. chabaudi* The effect of quinine treatment ( $100\text{mg kg}^{-1}\text{ day}^{-1} \times 7$ ) on protein-energy-deficient mice inoculated with  $1 \times 10^7$  infected R.B.C.



The mean body weight of the mice on the low protein diet went down from 16.5 g ( $\pm 0.6$ ) to 8.6 g ( $\pm 0.9$ ) after 100 days on the diet while the weight of the control mice increased from 16.6 g ( $\pm 0.7$ ) to 22.5 g ( $\pm 2.1$ ). Bell et al. (1976) have also shown the decrease in body weight of the mice kept on a 4% protein diet. Mice fed on this diet consumed less food per day than mice fed on a 20% protein diet, though they consumed more food per gram of body weight.

P.chabaudi infections in mice on the low protein diet did not resolve as rapidly as infections in mice on the 21% protein diet. Similar results have been obtained by Suntharasami and Marsden (1972) in P.yoelii infections in mice with protein energy deficiency, where mice on the low protein diet suffered from a more severe anaemia and prolongation of the infection.

Proteins serve many diverse functions in the body: as catalysts, structural elements and contractile systems; for nutritional storage; as vehicles of transport; as hormones; and as protective agents like antibodies (Lehninger, 1970). The effects obtained by protein energy malnutrition cannot be pinned down therefore to defects in the immune mechanism alone. However, the prolongation of parasitaemia in P.yoelii infections (Suntharasami and Marsden, 1972) and P.chabaudi infections may be due to the diminished responsiveness of the thymus in these animals. As was shown earlier (p<sup>137</sup>/~~244~~) T-cell deprivation by thymectomy and treatment with high doses of ATS caused a chronic non-resolving type of infection whereas thymectomy plus lower doses of ATS caused a chronic infection which apparently resolved after 86 days. Thus, the prolongation of infection

depends on the level of depletion of T-cells. Bell et al. (1976) have shown that mice fed on low protein diet show involution of the thymus. Also, many have shown impairment of cell-mediated immunity in protein-energy deficiency (Smythe et al., 1971; Chandra, 1974; Ferguson et al., 1974) and selective effects on thymus-dependant antibody responses (Malave and Layrisse, 1976).

Quinine treatment of infected mice on 4% and 21% protein diets was equally effective whereas quinine treatment of T-cell deprived mice infected with either P.chabaudi or P.berghei was not as effective as treatment of the intact control mice. The treatment of P.chabaudi infection in mice fed on a 4% diet was expected to be less effective. Skinsnes and Woolridge (1948) showed that antibiotic treatment of pneumococcus infection in protein-energy deficient mice (fed on 1.8% protein diet) took significantly longer than that of normal controls.

In natural conditions, protein-energy malnourished children are usually suffering from other virus, bacterial or parasitic infections which might aggravate and complicate the condition.

I. Concurrent infections with malaria  
parasites and schistosomes.

Both malaria and schistosomiasis infections have been well documented for their immunosuppressive effects on various antigens. WHO Chron (1975) graded malaria as the most important, and schistosomiasis the second most important, parasitic disease of the world. Again, these two diseases co-exist together in some parts of Africa. So it will be of great interest to study the interaction of S.mansoni and P.chabaudi infections in the laboratory.

1. The effect of S.mansoni infection on P.chabaudi infection

The experimental mice were exposed percutaneously to infection with 15 cercariae on two occasions separated by an interval of one week.

The mice were separated into 4 groups. Group A mice were challenged by an inoculum of  $10^7$  P.chabaudi infected erythrocytes, 4 weeks after exposure to S.mansoni. Groups B, C and D were given similar challenge infections 6, 9 and 12 weeks after the schistosome infection. Malaria parasitaemias and mortalities were recorded and at the end of the experiment, the mice were perfused to show the worm loads. Body weight, spleen weight and the liver weights were also recorded.

In group A mice (P.chabaudi infection 4 weeks after exposure to S.mansoni) malaria parasitaemia was comparable in both the experimental and the malaria control groups. Peak parasitaemias were 31.8% ( $\pm 8.7$ ) in group A and 36%



Table XLIIA Statistical analysis of the data in Fig 50 a. (n=6)

Group	<del>Day</del> Data	3	5	7	10	14	17	21	24	28	31	35	46	51
Malaria infected control	Mean $\pm$	2.8	20.2	31.8	3.2	0.9	2.8	0.0	0.1	0.5	0.01	0.1	0.02	0.0
	SD -	1.4	10.9	8.7	4.0	1.0	5.7	0.0	0.1	1.1	0.02	0.2	0.04	0.0
Schistosomiasis + malaria infected (after 4 weeks)	Mean $\pm$	2.5	17.0	36.0	4.0	0.3	0.1	0.3	0.0	0.01	0.2	0.0	0.1	0.0
	SD -	1.9	14.7	10.6	2.9	0.5	0.2	0.5	0.0	0.01	0.4	0.0	0.1	0.0
Significance	P <	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*see table VI

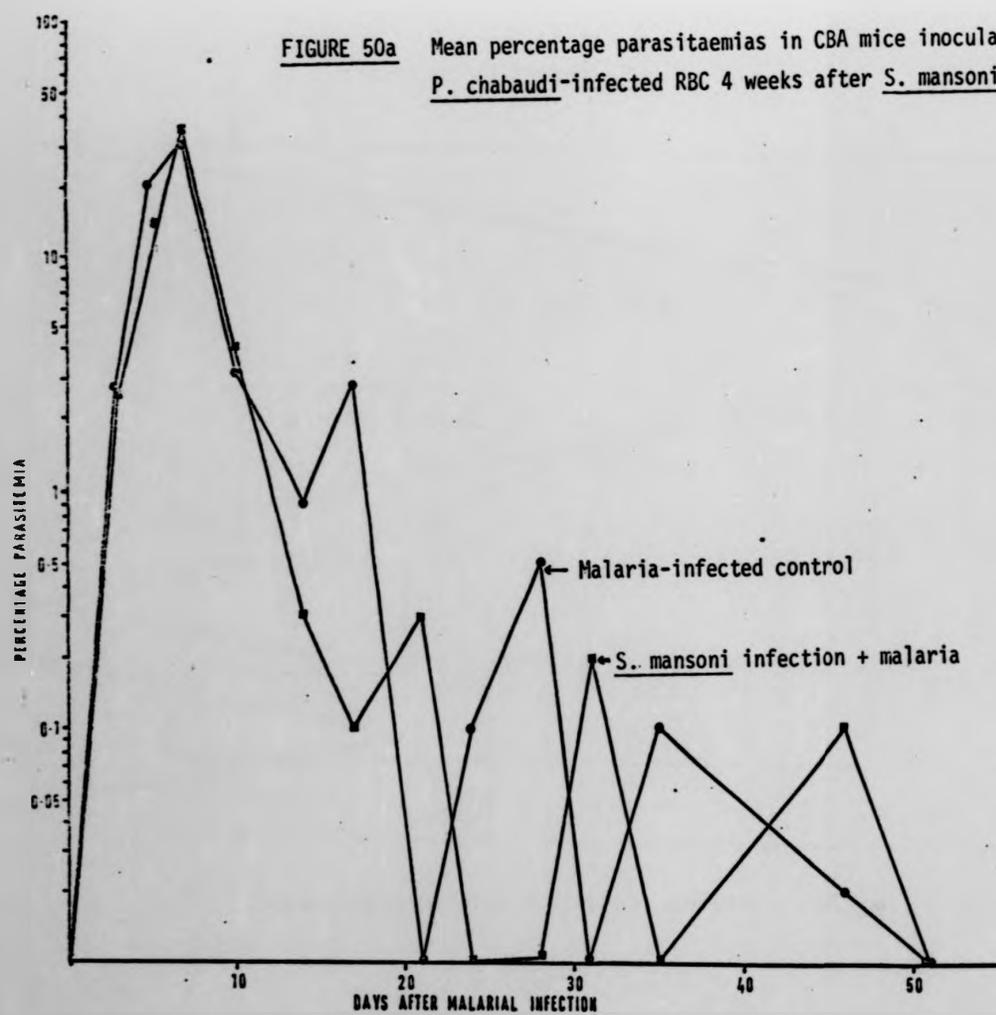




Table XLII B Statistical analysis of the data in Fig 50b. (n=6)

Group	Day	2	5	8	12	16	18	20	23	26
	Data									
Malaria infected control	Mean $\pm$	0.2	9.0	40.0	0.8	0.1	0.01	0.1	0.2	0.0
	SD $\pm$	0.1	6.4	12.5	0.5	0.2	0.0	0.1	0.1	0.0
Schistosomiasis + malaria infected (after 6 weeks)	Mean $\pm$	0.2	5.3	17.7	0.6	0.0	0.01	0.0	0.2	0.0
	SD $\pm$	0.1	2.9	9.8	0.6	0.0	0.0	0.0	0.1	0.0
Significance	P <	NS	NS	0.01	NS	NS	NS	0.05	NS	NS

\*see table VI

FIGURE 50b Mean percentage parasitaemias in CBA mice inoculated with  $1 \times 10^7$  *P. chabaudi*-infected RBC 6 weeks after *S. mansoni* infection

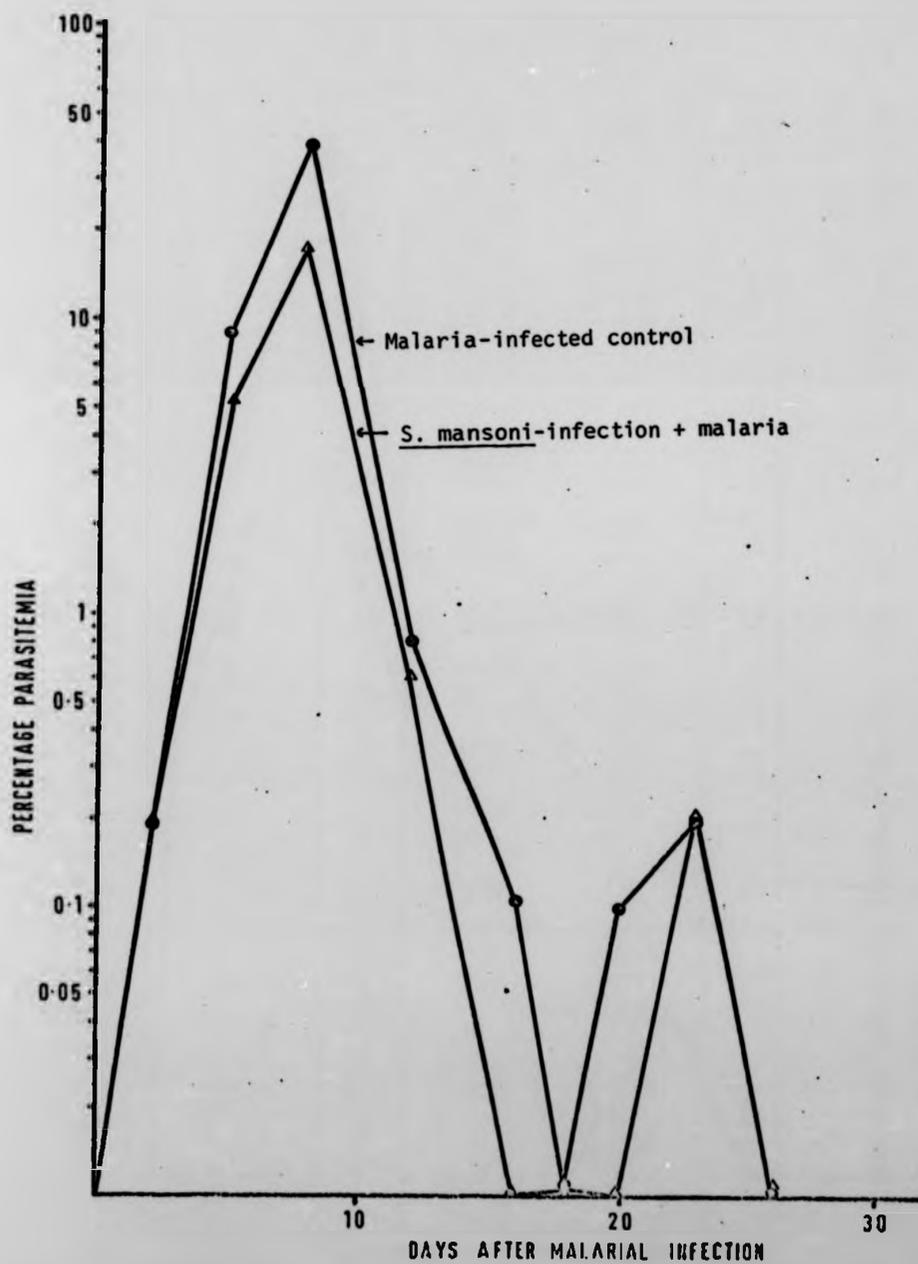




Table 1: Data for the first section of the graph.

Date After Industrial Institution	Percentage of ...	Percentage of ...
1	10	10
2	20	20
3	30	30
4	40	40
5	50	50
6	60	60
7	70	70
8	80	80
9	90	90
10	100	100

Table 2: Data for the second section of the graph.

Date After Industrial Institution	Percentage of ...	Percentage of ...
11	10	10
12	20	20
13	30	30
14	40	40
15	50	50
16	60	60
17	70	70
18	80	80
19	90	90
20	100	100

(See page 317)

(See page 317)

Table XLIIC Statistical analysis of the data in Fig 50C.

(n = 6)

Group	Day Data	1	4	7	10	16	23	30
Malaria infected control	Mean SD ±	0.6 0.3	4.9 2.0	35.6 12.3	1.6 3.0	0.3 0.2	0.0 0.0	0.0 0.0
Schistosomiasis + malaria infected (after 9 weeks)	Mean SD ±	0.3 0.2	1.8 2.0	2.3 1.6	0.4 0.6	0.0 0.0	0.1 0.2	0.1 0.2
Significance	P <	NS	0.05	0.001	NS	0.01	NS	NS

\* see table VI

Table XLIV Statistical analysis of the data in

Fig 50d (n=6)

Group	Day Data	2	6	9	12	16
	Malaria infected control	Mean	3.6	27.3	1.4	0.3
SD		0.9	8.2	0.4	0.1	0.01
Schistosomiasis + malaria infected (after 12 weeks)	Mean	1.8	7.9	1.1	0.0	0.0
	SD	0.9	7.7	1.5	0.0	0.0
Significance	P <	0.01	0.001	NS	0.001	NS

Table XLIIC Statistical analysis of the data in Fig 50C.

(n= 6)

Group	Day Data	1	4	7	10	16	23	30
	Malaria infected control	Mean $\bar{x}$	0.6	4.9	35.6	1.6	0.3	0.0
SD $\bar{s}$		0.3	2.0	12.3	3.0	0.2	0.0	0.0
Schistosomiasis + malaria infected (after 9 weeks)	Mean $\bar{x}$	0.3	1.8	2.3	0.4	0.0	0.1	0.1
	SD $\bar{s}$	0.2	2.0	1.6	0.6	0.0	0.2	0.2
Significance	P <	NS	0.05	0.001	NS	0.01	NS	NS

\* see table VI

Table XLIV Statistical analysis of the data in

Fig 50d (n=6)

Group	Day Data	2	6	9	12	16
Malaria infected control	Mean SD ±	3.6 0.9	27.3 8.2	1.4 0.4	0.3 0.1	0.01 0.01
Schistosomiasis + malaria infected (after 12 weeks)	Mean SD ±	1.8 0.9	7.9 7.7	1.1 1.5	0.0 0.0	0.0 0.0
Significance	P <	0.01	0.001	NS	0.001	NS

FIGURE 50c Mean percentage parasitaemias in CBA mice inoculated with  $1 \times 10^7$  *P. chabaudi*-infected RBC 9 weeks after *S. mansoni* infection

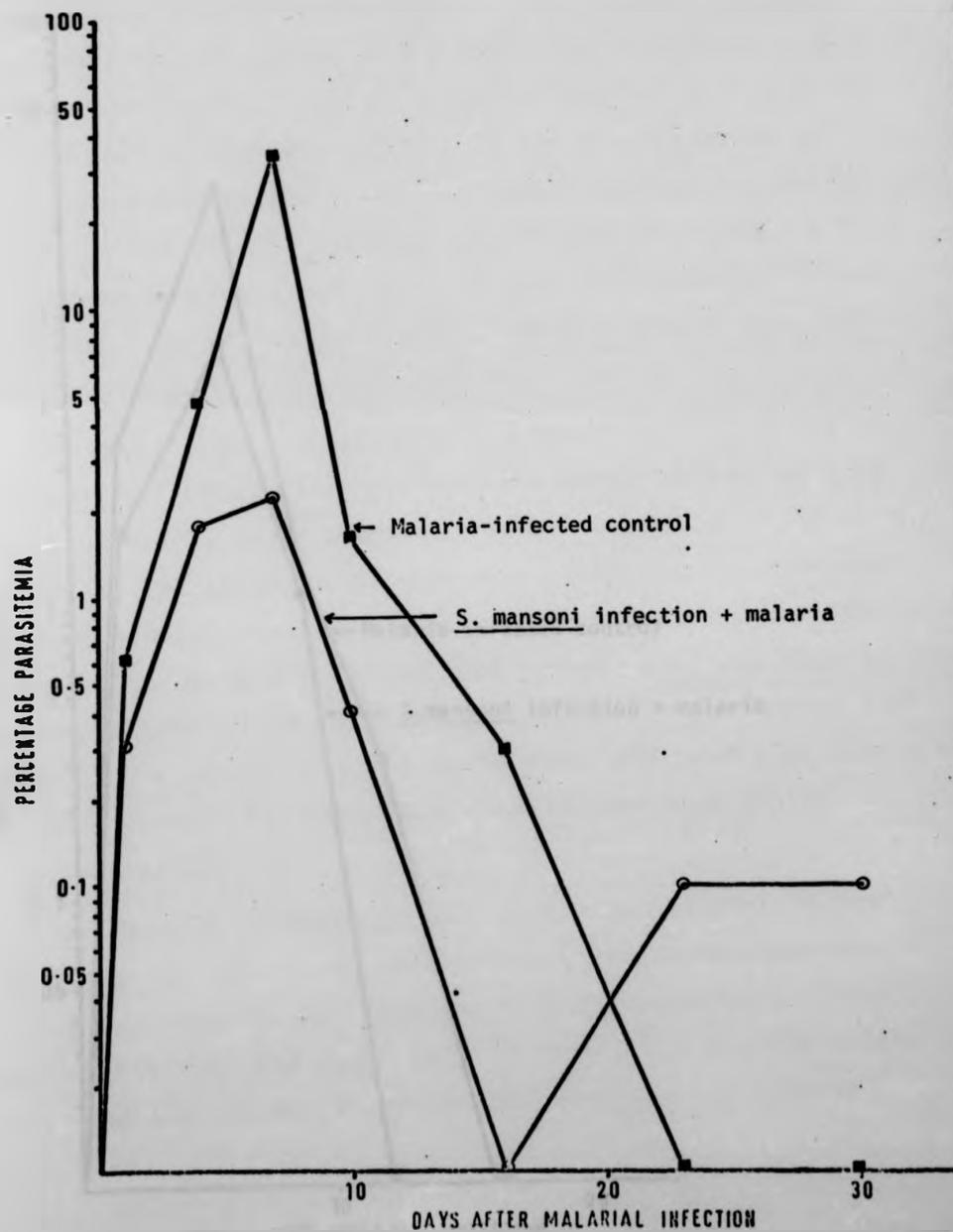
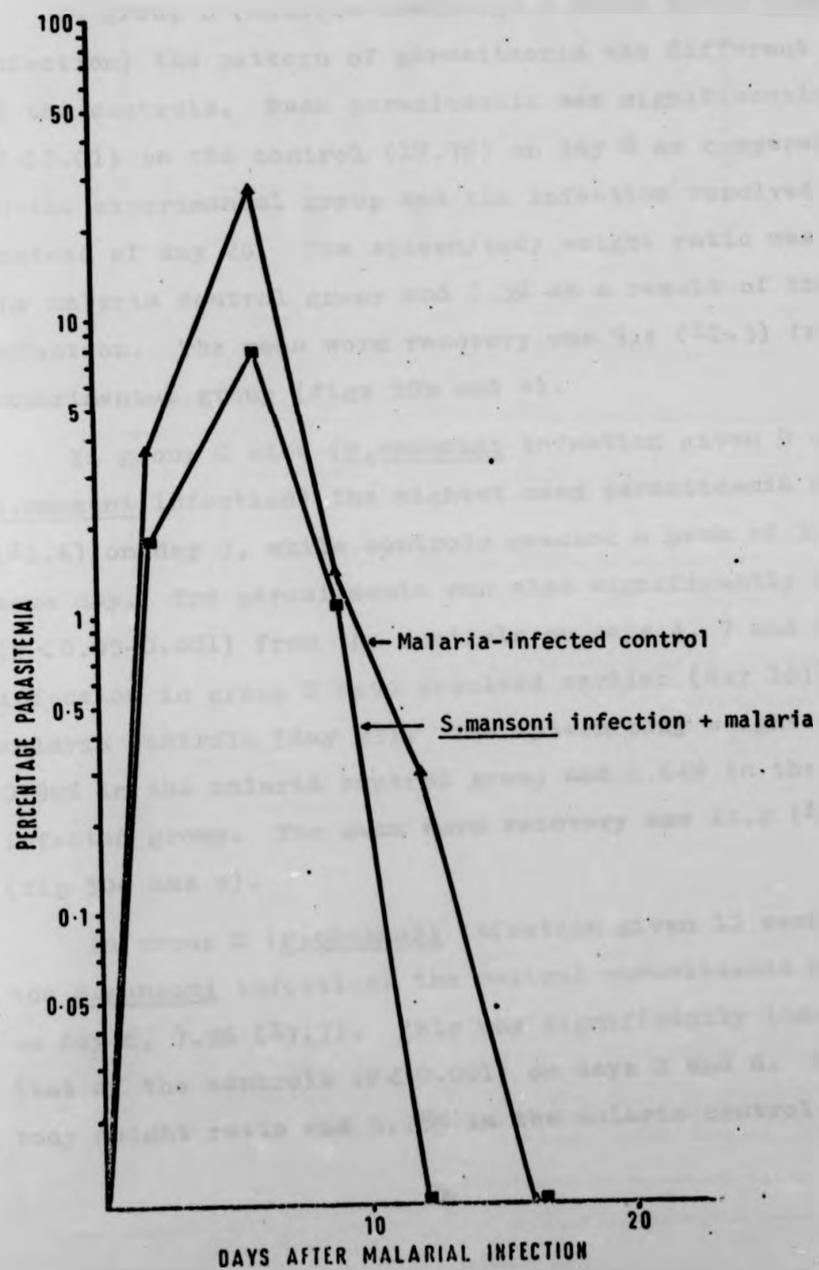


FIGURE 50d Mean percentage parasitaemias in CBA mice inoculated with  $1 \times 10^7$  *P. chabaudi*-infected RBC 12 weeks after *S. mansoni* infection



( $\pm 10.6$ ) in the malaria controls both on day 7. The spleen/body weight ratios were 1.37% with concurrent infections and 0.6% in the malaria controls. The mean worm load in the experimental group was 11.4 ( $\pm 2.07$ ) worms (fig 50a and e).

In group B (malaria challenge 6 weeks after S.mansoni infection) the pattern of parasitaemia was different from that of the controls. Peak parasitaemia was significantly lower ( $P < 0.01$ ) in the control (17.7%) on day 8 as compared to 40% in the experimental group and the infection resolved by day 16 instead of day 26. The spleen/body weight ratio was 0.57% in the malaria control group and 1.3% as a result of the double infection. The mean worm recovery was 9.4 ( $\pm 2.3$ ) from the experimental group (figs 50b and e).

In group C mice (P.chabaudi infection given 9 weeks after S.mansoni infection) the highest mean parasitaemia was 2.3% ( $\pm 1.6$ ) on day 7, while controls reached a peak of 35.6% on the same day. The parasitaemia was also significantly different ( $P < 0.05-0.001$ ) from the controls on days 4, 7 and 16. The infection in group C mice resolved earlier (day 16) than in the malaria controls (day 23). The spleen/body weight ratio was 0.96% in the malaria control group and 1.64% in the doubly infected group. The mean worm recovery was 11.2 ( $\pm 3.2$ ). (fig 50c and e).

In group D (P.chabaudi infection given 12 weeks after the S.mansoni infection) the maximal parasitaemia was seen on day 6, 7.9% ( $\pm 7.7$ ). This was significantly lower than that of the controls ( $P < 0.001$ ) on days 2 and 6. The spleen/body weight ratio was 0.78% in the malaria control group

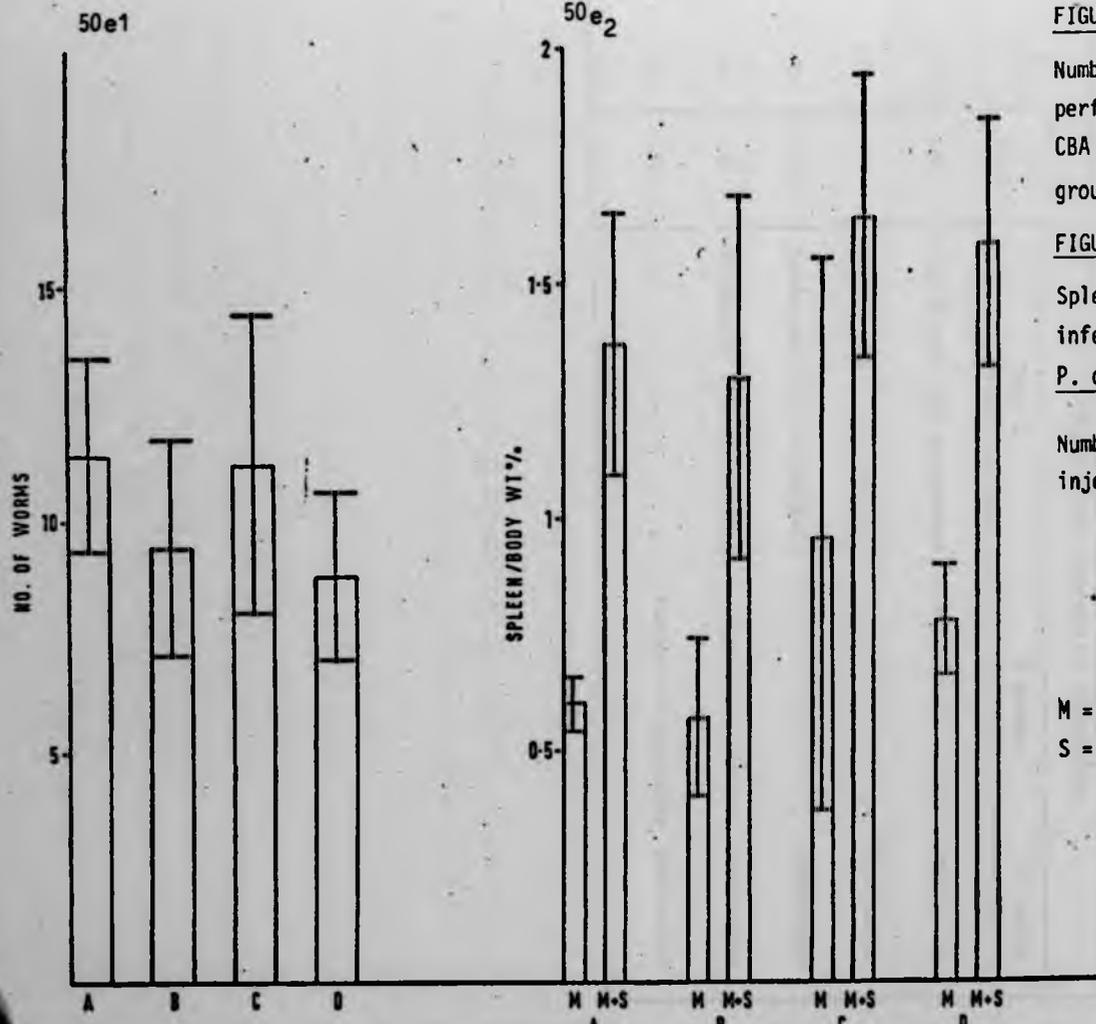


FIGURE 50e<sub>1</sub>

Numbers of worms recovered on perfusion from S. mansoni-infected CBA mice of four experimental groups

FIGURE 50e<sub>2</sub>

Spleen/bodyweight ratios in mice infected with S. mansoni and/or P. chabaudi

Number of weeks P. chabaudi injected after S. mansoni:

A = 4 weeks

B = 6 weeks

C = 9 weeks

D = 12 weeks

M = malaria (P. chabaudi)

S = schistosomiasis (S. mansoni)

Table XLIIIA Protocol for infection of mice with single and bisexual *S.mansoni* and *P.chabaudi* and time of perfusion in experiment I 2.

Group	No. of mice	Days after cercarial infection		
		Day 0	8 weeks	10 weeks
A	6	Single sex (50 cercariae)	-	Perfusion
B	6	Single sex (50 cercariae)	Malaria infection	Perfusion
C	6	Bisexual (35 cercariae)	-	Perfusion
D	6	Bisexual (35 cercariae)	Malaria infection	Perfusion
E	6	-	Malaria infection	Perfusion

and 1.58% in the experimental group. The mean worm recovery was 8.8 ( $\pm 1.8$ ) worms. (fig 50d and e).

2. The effect of single sex *S.mansoni* infection on a subsequent malaria infection

In the previous experiment, there was a suppression of the malaria infection in mice infected with *P.chabaudi* 6, 9 or 12 weeks after exposure to *S.mansoni* cercariae. So this experiment was planned to find out the effect of a single sex infection of *S.mansoni*, which lacks egg production and subsequently is without granuloma formation in the liver. There were five groups of mice. Mice in groups A and B were infected percutaneously with 50 male cercariae and mice in groups C and D were given bisexual infections by exposure to 35 cercariae. *P.chabaudi*  $1 \times 10^7$  infected erythrocytes were given on the 8th week after cercariae infection to groups A, C and E. Groups B and D acted as single and bisexual *S.mansoni* infected control groups and E as the malaria control group. (see table XLIIIA).

The mice were perfused 3 weeks after malaria infection. Body weight, spleen weight and liver weight were recorded at the time of perfusion.

Parasitaemias in malaria control mice were similar to those obtained in previous experiments. The malaria parasitaemias in mice given single sex worm infections were not much different from those of the controls and the infections were resolved by day 16. The spleen/body weight



Table XLIII<sup>R</sup> Statistical analysis of the data  
in Fig 51. (n=6)

Group	Day Data	2	5	9	12	16	19
Malaria infected control	Mean	2.4	37.0	3.2	2.9	0.1	0.0
	SD $\pm$	1.3	3.7	1.5	1.7	0.2	0.0
Single sex Schistosomiasis + malaria infected	Mean	2.0	33.0	6.4	0.5	0.0	0.0
	SD $\pm$	0.7	4.0	2.7	0.8	0.0	0.0
Significance	P <	NS	NS	0.05	0.02	NS	NS

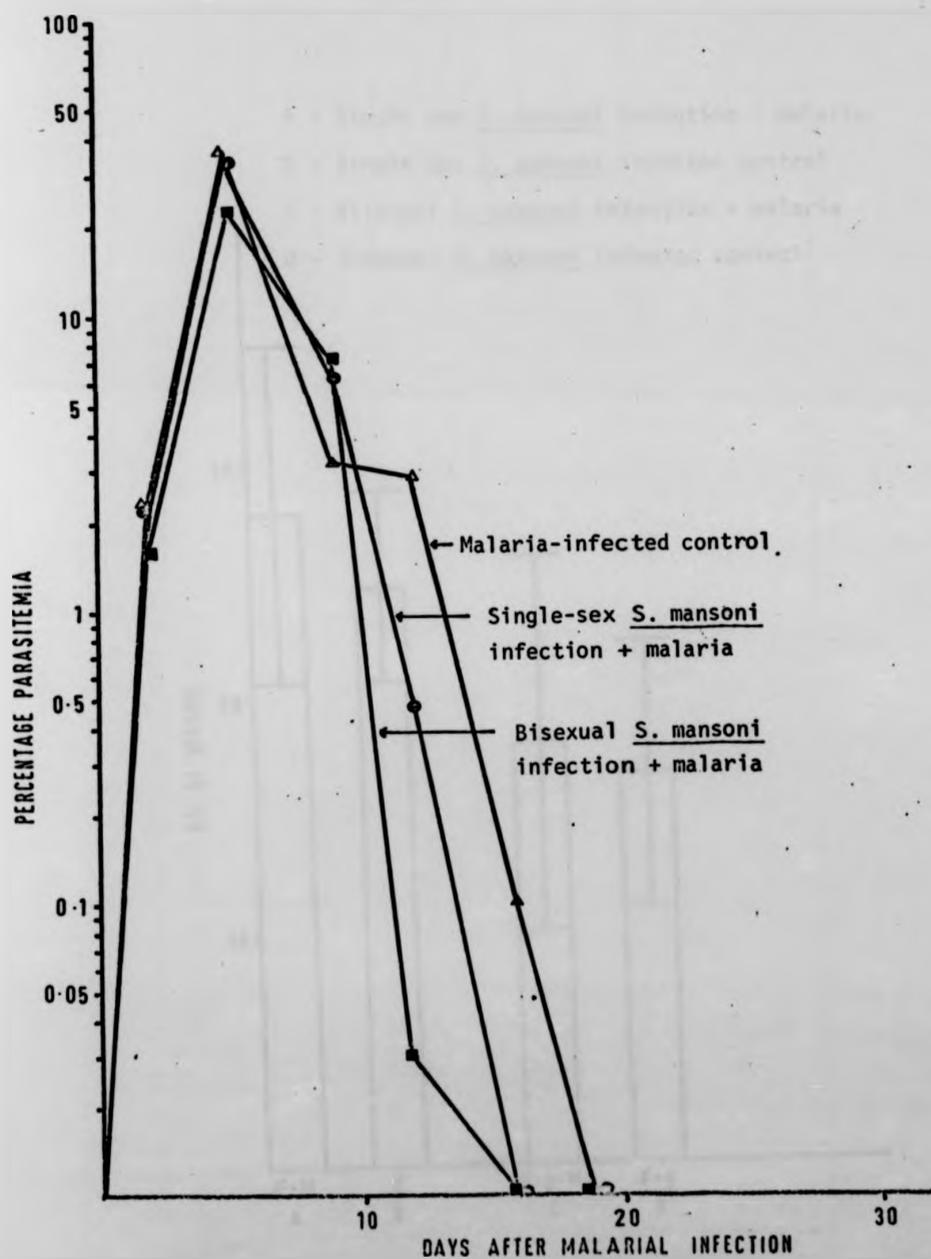
\* see table VI

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Table XLIIIC Statistical analysis of the data  
in Fig 51. (n=6)

Group	Day Data	2	5	9	12	16	19
Malaria infected control	Mean	2.4	37.0	3.2	2.9	0.1	0.0
	SD	1.3	3.7	1.5	1.7	0.2	0.0
Bisexual Schistosomiasis + malaria infected	Mean	1.7	23.0	7.1	0.03	0.0	0.0
	SD	0.6	3.6	2.1	0.03	0.0	0.0
Significance	P <	NS	0001	0.01	0.01	NS	NS

FIGURE 51a Mean percentage parasitaemias in CBA mice inoculated with  $1 \times 10^7$  *P. chabaudi*-infected RBC 3 weeks after bisexual or single-sex (male) *S. mansoni* infection



**FIGURE 51b** The total number of worms S. mansoni recovered from the mice in Fig. 51a

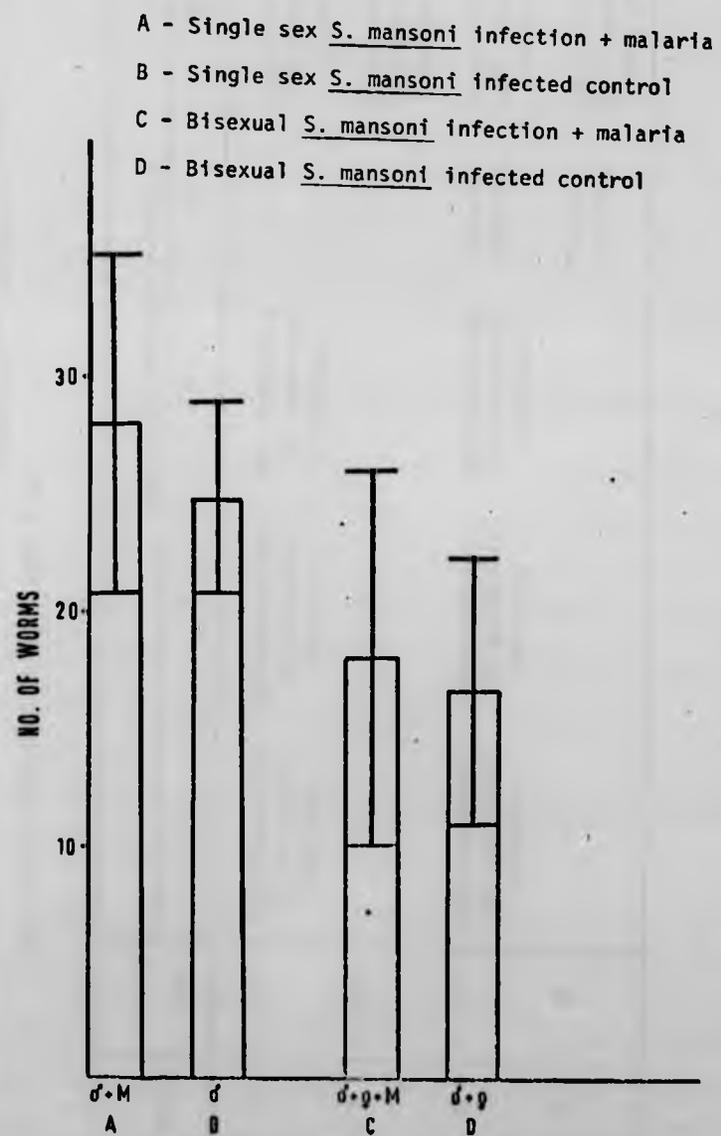


Table XLIVA Protocol for the infection of mice with P.chabaudi and S.mansoni (primary and challenge) and time of perfusion in Experiment I 3a.

Group	No. of mice	Days after cercarial infection					
		0	7	14	49	54	75
A	10	Schistosomiasis (25 cercariae) infection	Schistosomiasis (25 cercariae) infection	Schistosomiasis (25 cercariae) infection	-	Schistosomiasis (200 cercariae) infection	Perfusion
B	10	-	-	-	-	Schistosomiasis (200 cercariae) infection	Perfusion
C	10	Schistosomiasis (25 cercariae) infection	Schistosomiasis (25 cercariae) infection	Schistosomiasis (25 cercariae) infection	Malaria infection	Schistosomiasis (200 cercariae) infection	Perfusion
D	10	-	-	-	Malaria infection	Schistosomiasis (200 cercariae) infection	Perfusion

ratio was higher in the doubly infected mice (0.8%) than in mice infected with male worms alone (0.3%). The malaria infection with mature bisexual S.mansoni cercariae was depressed ( $P < .001$ ) on day 5 with a peak parasitaemia of 23% ( $\pm 3.6$ ) and the infection was resolved on day 16 (Table XLIIIC). The spleen/body weight ratio was 1.27% in the doubly infected group and 0.53% in the S.mansoni alone infected group.

The worm loads in the single sex infections were 28 ( $\pm 7.2$ ) for the experimental group and 24.8 ( $\pm 4$ ) for the control group. The bi-sexually infected groups had 18 ( $\pm 8$ ) worms in the experimental group and 16.7 ( $\pm 5.7$ ) in the control group. (fig 51b).

### 3. The effect of P.chabaudi infection on S. mansoni infection

It was shown in experiment IVB that immunodepression in P.chabaudi infection to SRBC was greatest during the peak period of the parasitaemia, usually days 5 - 7 of the infection. Experiments were planned to determine whether this immunodepression would affect immunizing or challenge infections with S.mansoni.

#### a. The effect of acute P.chabaudi infection on an immunizing S.mansoni infection

There were four groups of mice in this experiment (see table XLIVA)..

P.chabaudi infections of  $1.5 \times 10^7$  infected erythrocytes were given to mice of groups C and D on day 0. On day 5 immunizing doses of 35 S.mansoni cercariae were given

(a)  $\frac{d^2x}{dt^2} + 2\frac{dx}{dt} + x = 0$   
 (b)  $\frac{d^2x}{dt^2} + 2\frac{dx}{dt} + x = 1$

Figure 1.5. The average displacement  $\bar{x}(t)$  and the standard deviation  $\sigma(t)$  of the displacement  $x(t)$  for the system (1.5) and (1.6) with  $\gamma = 2$  and  $\omega = 1$ . The solid line is  $\bar{x}(t)$  and the dashed line is  $\sigma(t)$ . The initial conditions are  $x(0) = 1$  and  $\dot{x}(0) = 0$ .

Figure 1.6. The average displacement  $\bar{x}(t)$  and the standard deviation  $\sigma(t)$  of the displacement  $x(t)$  for the system (1.5) and (1.6) with  $\gamma = 2$  and  $\omega = 1$ . The solid line is  $\bar{x}(t)$  and the dashed line is  $\sigma(t)$ . The initial conditions are  $x(0) = 1$  and  $\dot{x}(0) = 0$ .

TABLE 1.1

Values of  $\bar{x}(t)$  and  $\sigma(t)$  for  $\gamma = 2$  and  $\omega = 1$

Time $t$	$\bar{x}(t)$	$\sigma(t)$	Time $t$	$\bar{x}(t)$	$\sigma(t)$
0.0	1.0000	0.0000	1.0	0.7071	0.7071
0.2	0.9221	0.0778	1.2	0.6323	0.6323
0.4	0.8321	0.1556	1.4	0.5677	0.5677
0.6	0.7309	0.2334	1.6	0.5134	0.5134
0.8	0.6204	0.3112	1.8	0.4693	0.4693
1.0	0.5039	0.3890	2.0	0.4347	0.4347
1.2	0.3851	0.4668	2.2	0.4089	0.4089
1.4	0.2671	0.5446	2.4	0.3905	0.3905
1.6	0.1530	0.6224	2.6	0.3781	0.3781
1.8	0.0367	0.7002	2.8	0.3714	0.3714
2.0	0.0000	0.7778	3.0	0.3701	0.3701

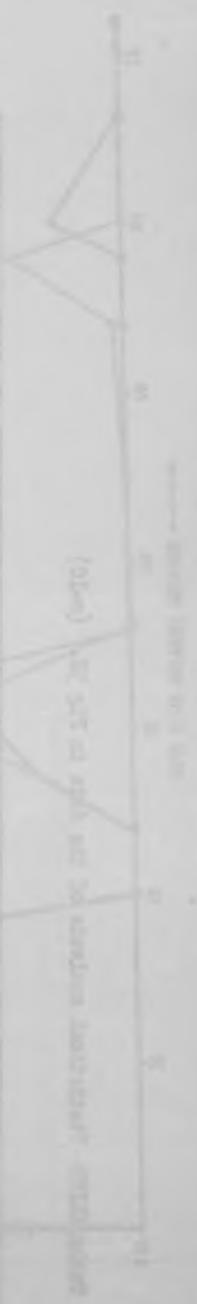


Table XLIVB Statistical analysis of the data in Fig 52. (n=10)

Group	Day Data	1	5	9	12	17	20	24	28	32	36	45	54	58	60	70
D	Mean	2.3	25.0	2.5	0.6	0.2	0.0	0.0	0.03	0.2	0.0	0.0	0.01	0.02	0.0	0.0
	SD $\pm$	1.2	11.0	3.2	0.4	0.2	0.0	0.0	0.02	0.04	0.0	0.0	0.01	0.02	0.0	0.0
C	Mean	1.6	30.8	5.4	0.7	0.5	0.0	0.0	0.03	0.5	0.0	0.0	0.01	0.03	0.0	0.0
	SD $\pm$	1.3	12.3	6.5	0.7	1.0	0.0	0.0	0.02	0.04	0.0	0.0	0.01	0.02	0.0	0.0
Significance	P <	NS	NS	NS	NS	NS	NS	NS	NS	0.001	NS	NS	NS	NS	NS	NS

\* see table VI

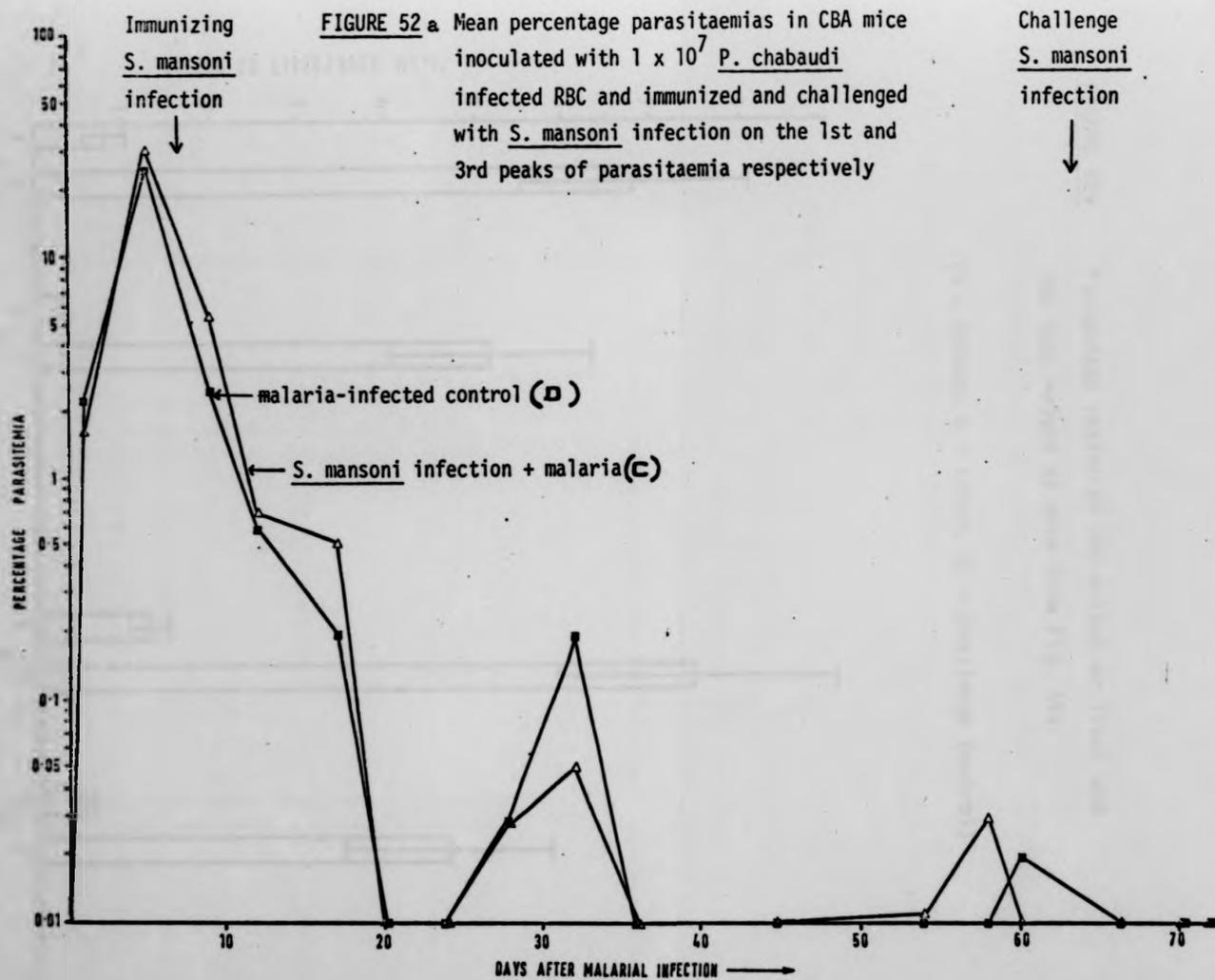
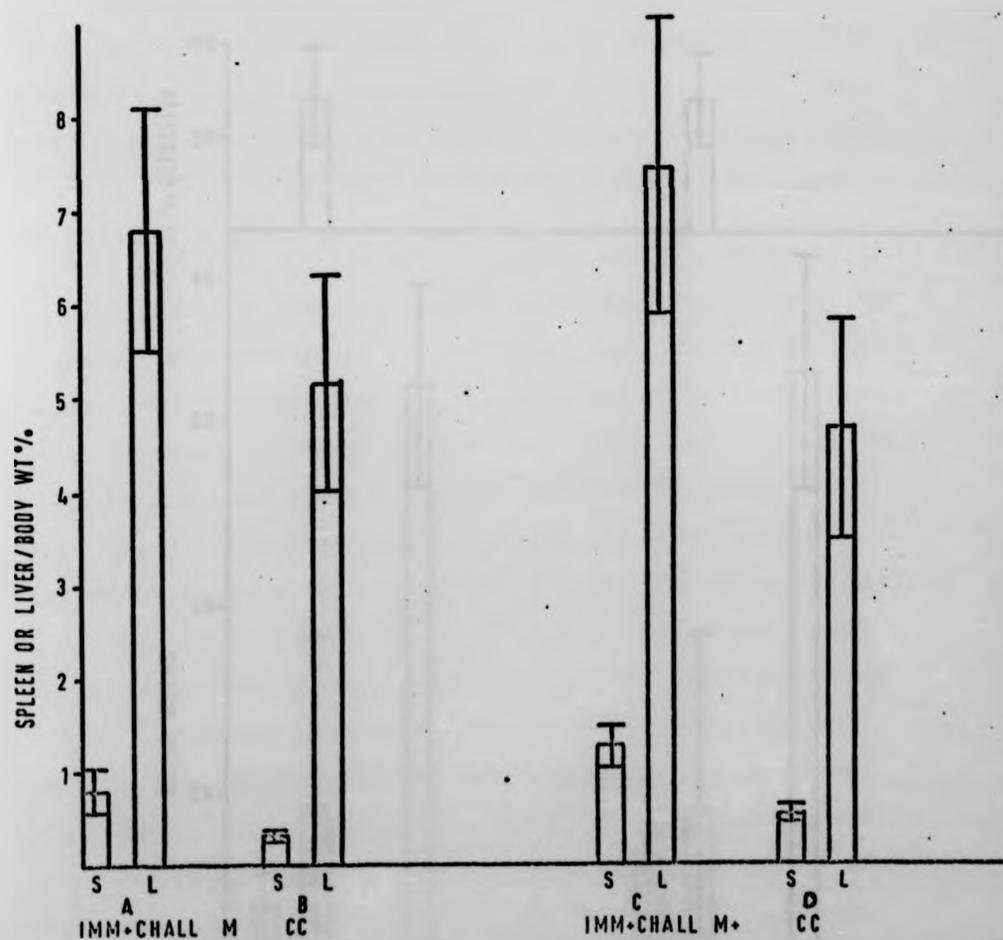


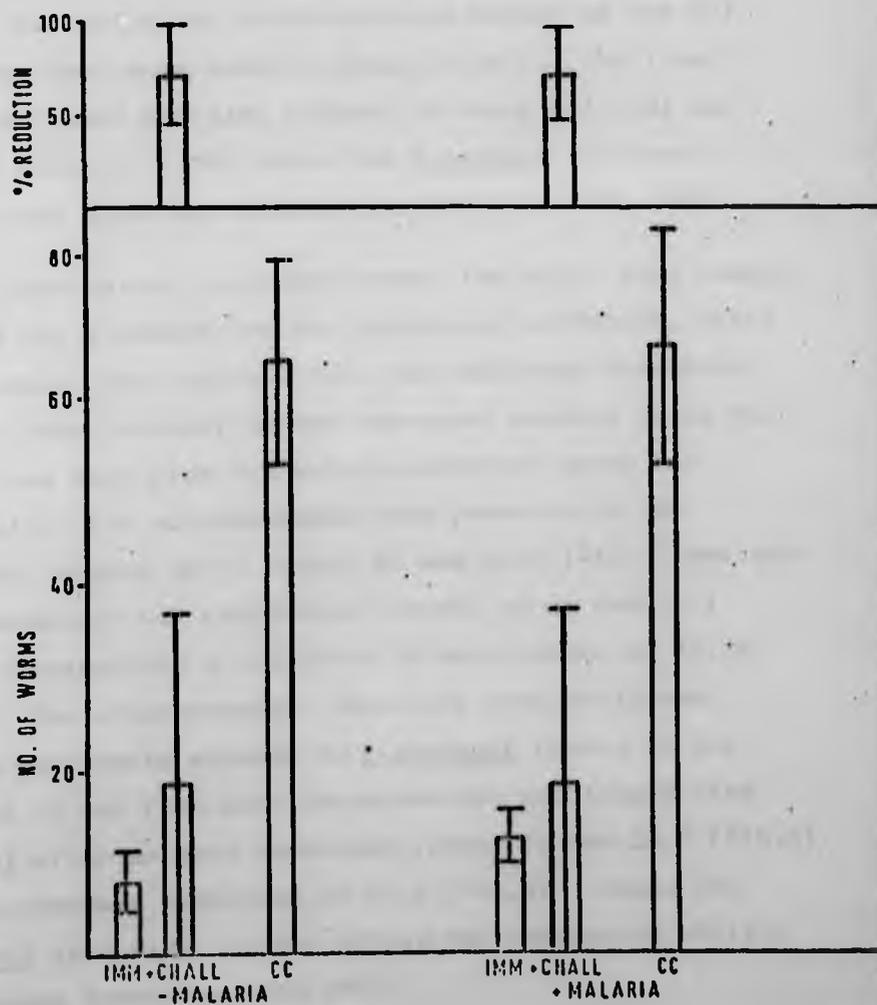
FIGURE 52b Percentage ratios of the spleen or liver and the body weight of mice from Fig. 52a.

(S = spleen, L = Liver, CC = Challenge Control)



**FIGURE 52c** Total number of (*S. mansoni*) worms recovered from the mice immunized (at peak of *P. Chabaudi* infection) and challenged with *S. mansoni* infection

(CC = Challenge control)



percutaneously to animals in groups A and C. Challenging infections (200 cercariae) of S.mansoni were given 6 weeks later. Perfusion for worm recovery was performed 3 weeks after the challenge infection. Malaria parasitaemias in mice of groups C and D were similar to one another (see table XLIVB and fig 52a).

The spleen/body weight ratio was highest in the group C mice (1.27%) infected with malaria, immunized with S.mansoni cercariae and challenged, and lowest in the (B) schistosome challenge control group (0.31%). The liver/body weight ratio was also highest in group C (7.5%) and lowest in group D (4.7%) where the S.mansoni challenge infection was given to malaria-infected mice. (fig 52b).

In counting the recovered worms, the adult worm number reflected the intensity of the immunizing infection, while schistosomula were derived from the challenge infection. The adult worm recovery in the immunized control group was 8 ( $\pm 3.3$ ) and that from the malaria-infected group was 12.1 ( $\pm 5.4$ ). The schistosomula worm recovery in the challenged control group (group B) was 63.8 ( $\pm 11.2$ ) and that of the immunized and challenged control group was 18.4 ( $\pm 18.4$ ) representing a reduction in worm burden of 71.2% ( $\pm 28.9$ ). The schistosomula recovered from challenge controls previously exposed to P.chabaudi (group D) was 65.2 ( $\pm 12.1$ ) and from mice immunized and challenged with S.mansoni after malaria infection (group C) was 18.2 ( $\pm 19.5$ ) with a percentage reduction of 72.2 ( $\pm 29.6$ ). Thus, the P.chabaudi infection did not affect the immunizing ability of S.mansoni cercariae. (fig 52c).

Table XLVA Protocol for the infection of mice with S.mansoni (primary and challenge) and P.chabaudi and time of perfusion in experiment I 3b.

Group	No. of Mice	Days after malaria infection			
		0	5	42	63
A	10	-	Schistosomiasis infection	Schistosomiasis challenge	Perfusion
B	10	-	-	Schistosomiasis challenge	Perfusion
C	10	Malaria infection	Schistosomiasis infection	Schistosomiasis challenge	Perfusion
D	10	Malaria infection	-	Schistosomiasis challenge	Perfusion

Table XLVA Protocol for the infection of mice with S.mansoni (primary and challenge) and P.chabaudi and time of perfusion in experiment I 3b.

Group	No. of Mice	Days after malaria infection			
		0	5	42	63
A	10	-	Schistosomiasis infection	Schistosomiasis challenge	Perfusion
B	10	-	-	Schistosomiasis challenge	Perfusion
C	10	Malaria infection	Schistosomiasis infection	Schistosomiasis challenge	Perfusion
D	10	Malaria infection	-	Schistosomiasis challenge	Perfusion

b. The effect of acute P.chabaudi infection on a challenge infection with S.mansoni.

The plan of the experiment is shown in table. The S.mansoni challenge infection was given when the malaria infection was at its peak. Group A and group C mice were immunized percutaneously with 25 cercariae of S.mansoni on day 0. The immunization was repeated one and two weeks later to ensure that infection occurred and to ensure that mice survived by not giving a single high dose of cercariae. The P.chabaudi infections were given on the 7th week of the S.mansoni infection to animals of groups C and D. All mice were challenged with 200 cercariae of S.mansoni 5 days after the P.chabaudi inoculation. Perfusion for worm recovery was performed on the 3rd week of the challenge infection.

The mean percentage parasitaemia of the P.chabaudi infection in the group previously immunized with S.mansoni (group C) was significantly depressed ( $P < .02 - .001$ ) by comparison with the controls on days 2, 5 and 7 and the infection also resolved earlier on day 13 instead of day 19. (fig 53a).

The spleen/body weight ratio was highest in the group C mice immunized and challenged with S.mansoni and infected with P.chabaudi (3.46%) and was lowest in the malaria-infected control challenged with S.mansoni (group D) (0.98%). (fig 53b)

The numbers of schistosomula recovered on perfusion are shown in fig 53c. There was a highly significant reduction in the numbers recovered from immunized mice but the malaria infection during challenge had no significant.

TABLE 10  
 The percentage of infection in the soil  
 after 10 days of incubation at 20°C. The  
 soil was infected with *B. anthracis* spores  
 and after 10 days of incubation the  
 soil was analyzed for the presence of  
 spores. The results are given in the  
 following table.



TABLE 11  
 The percentage of infection in the soil  
 after 10 days of incubation at 20°C. The  
 soil was infected with *B. anthracis* spores  
 and after 10 days of incubation the  
 soil was analyzed for the presence of  
 spores. The results are given in the  
 following table.

Time (days)	Soil A (%)	Soil B (%)	Soil C (%)
0	0	0	0
1	40	20	10
2	60	30	20
3	70	40	30
4	80	50	40
5	85	60	50
6	90	70	55
7	95	75	60
8	98	80	65
9	100	85	70
10	100	80	60

Table XLVB Statistical analysis of the data in Fig 53A. (n=10)

Group	Day Data	2	5	7	9	13	16	19	25
Malaria +	Mean	1.1	21.0	22.0	2.1	0.1	0.02	0.0	0.0
Schistosomiasis	SD $\pm$	0.4	7.5	9.0	2.6	0.04	0.04	0.0	0.0
Schistosomiasis	Mean	0.5	5.2	8.0	1.7	0.0	0.0	0.0	0.0
+ malaria	SD $\pm$	0.3	4.9	11.9	3.8	0.0	0.0	0.0	0.0
Significance	P <	0.02	0.001	0.001	NS	0.001	NS	NS	NS

\*see table VI.

FIGURE 53a Mean percentage parasitaemias in CBA mice inoculated with  $1 \times 10^7$  *P. chabaudi*-infected RBC 7 weeks after primary *S. mansoni* infection and with a challenge dose of cercariae given on day of the malaria infection

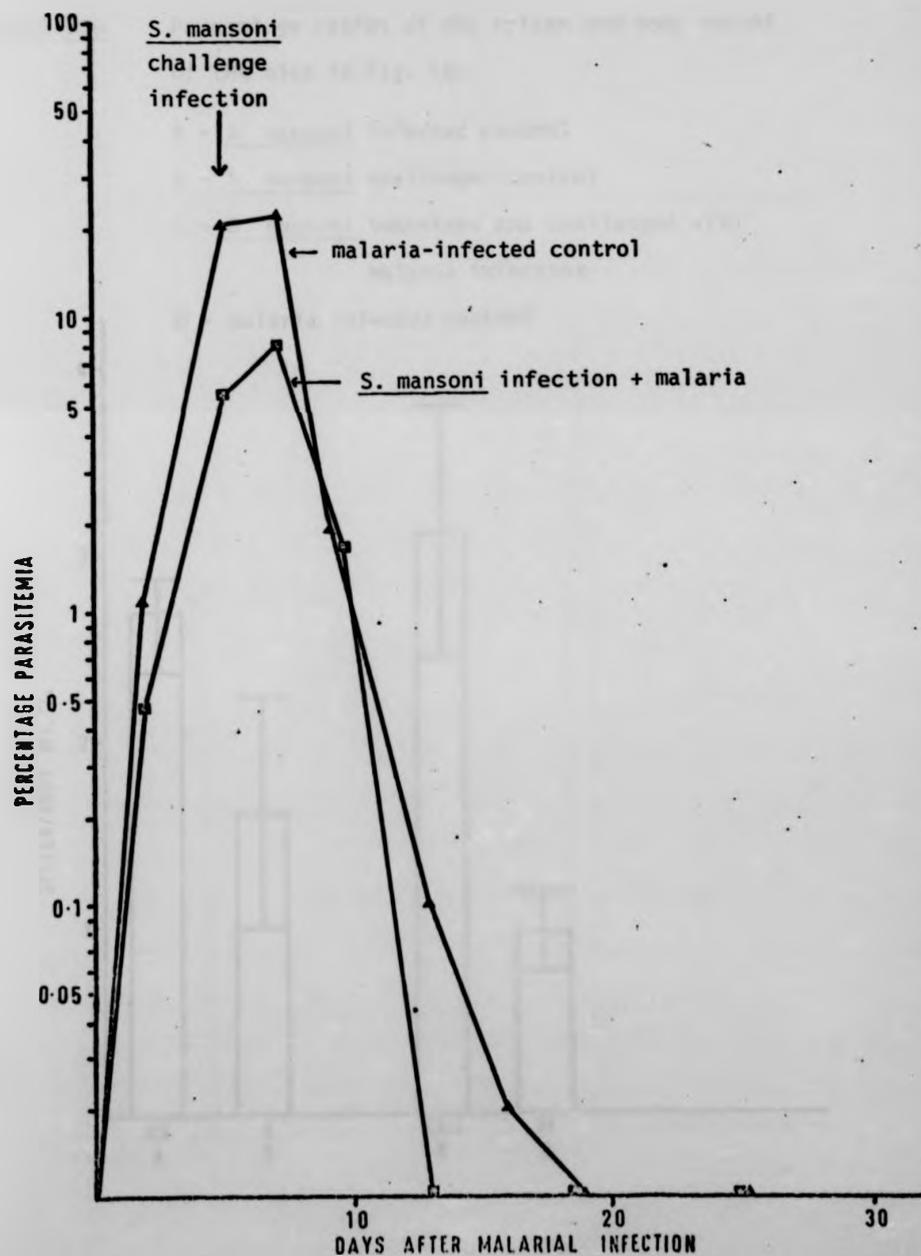


FIGURE 53b Percentage ratios of the spleen and body weight of the mice in Fig. 53a

- A - S. mansoni infected control  
B - S. mansoni challenge control  
C - S. mansoni immunized and challenged with malaria infection  
D - malaria infected control

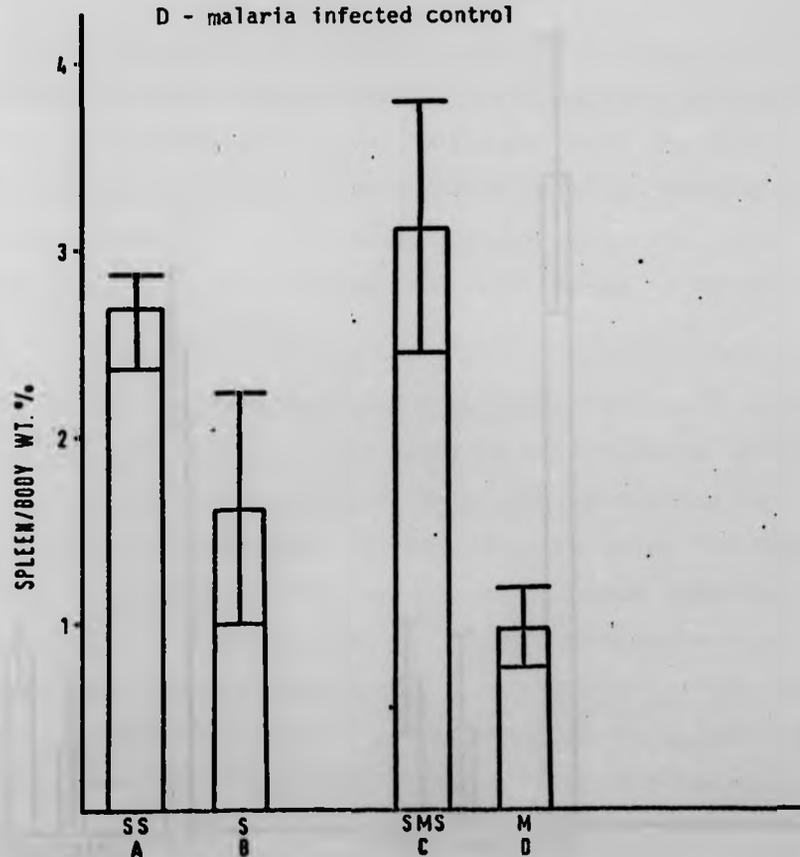
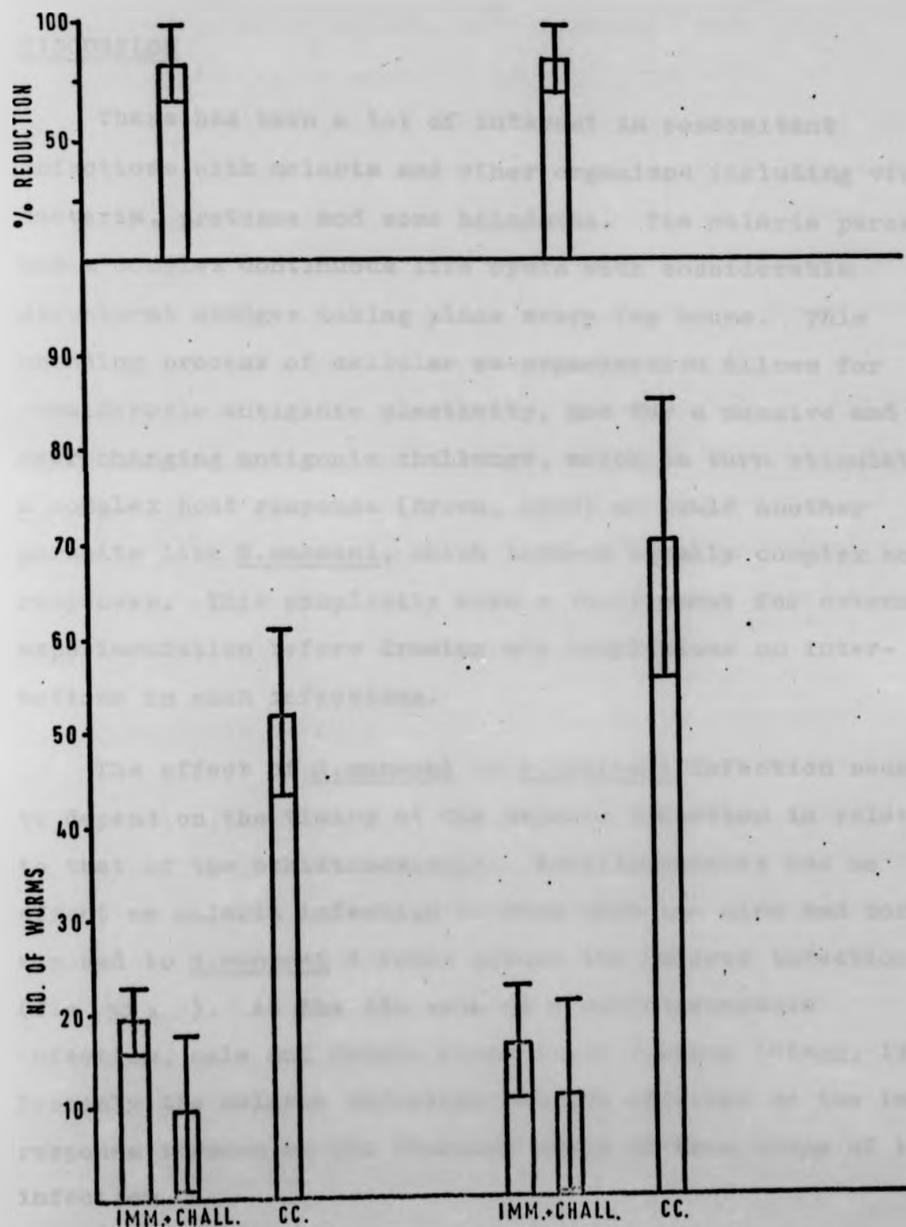


FIGURE 53c Total number of (*S. mansoni*) worms recovered from the mice in Fig. 52a

(Imm = immunized, Chall = challenge, CC = challenge control)



effect. The adult worm recovery in groups A and C were also similar.

#### DISCUSSION

There has been a lot of interest in concomitant infections with malaria and other organisms including viruses, bacteria, protozoa and some helminths. The malaria parasite has a complex continuous life cycle with considerable structural changes taking place every few hours. This unending process of cellular re-organization allows for considerable antigenic plasticity, and for a massive and ever-changing antigenic challenge, which in turn stimulates a complex host response (Brown, 1975) as would another parasite like S.mansoni, which induces equally complex host responses. This complexity sets a requirement for extensive experimentation before drawing any conclusions on interactions in such infections.

The effect of S.mansoni on P.chabaudi infection seemed to depend on the timing of the malaria infection in relation to that of the schistosomiasis. Schistosomiasis had no effect on malaria infection in mice when the mice had been exposed to S.mansoni 4 weeks before the malaria infection (fig 50 a ). At the 4th week of a schistosomiasis infection, male and female worms start pairing (Clegg, 1965). Probably the malaria infection was not affected by the immune response induced by the immature worms at this stage of the infection.

Smithers and Terry (1969) in their review on the

immunology of schistosomiasis suggested that adult schistosome worms are of paramount importance in stimulating immunity. The reviewers drew their conclusions from experiments in which animals were immunized with non-pathogenic strains or species, or with irradiated cercariae, (no mature worms resulting from these immunizing procedures). The immune response on the 4th week of S.mansoni infection has been shown in mice (Sher et al., 1974) and in rats (Perez et al., 1974). In mice, the decrease in recovery of schistosome challenges from the lungs occurs in two separate stages following a primary infection (Sher et al., 1974); a first stage which arises between 1-4 weeks after exposure and a second stage appearing at 7 weeks and onwards. The first stage reflects a delay in the migration of the parasite and this effect can be passively transferred to normal mice with serum fractions which contain mainly IgG<sub>2</sub> antibodies (Sher et al., 1975).

P.chabaudi infection was significantly suppressed in mice exposed to S.mansoni infections 6, 9 or 12 weeks before the malaria infection (fig 50b,c,d). These results are in agreement with that of Yoeli (1956), who had shown that P.berghei infections in the field vole were milder and less virulent when given 4-7 weeks after S.mansoni. P.yoelii parasitaemia was also shown to be markedly suppressed in mice previously infected (5 weeks before) with S.mansoni (Lewinsohn, 1975).

The mechanism of suppression of malaria by the schistosomes after 6 weeks of infection is not known as there are many events happening at this time. Schistosomes start producing

eggs on the 34-35th day (5th week) of the infection (Clegg, 1965). It has also been shown that at the onset of egg production there is an increase in total immunoglobulin levels (Evans and Stirewalt, 1957; Sadun and Walton, 1958; and Smithers and Walker, 1961), or precipitating antibodies (Senterfit, 1958) and of reagin-like antibodies (Ogilvie et al., 1966 and Edwards et al., 1967). In fact, eggs provide a considerable antigenic stimulus to the host leading to the production of circulating antibody and possibly a condition of specific delayed type hypersensitivity (Warren et al., 1967). Immunodepressive effects are also shown after 6 weeks of infection. Brito et al. (1976) have shown the depression of humoral immune responses, and Arujo et al. (1977) and Pelley et al. (1976) have demonstrated the depression of cell mediated immunity at the 8 $\frac{1}{2}$  and 12th week respectively (see page 51 ). Mota-Santos et al. (1977) claimed that the immunosuppression was induced by adult worms and not by eggs. Pronounced pathological lesions due to granuloma formation around eggs in the liver are found from the 6 th week of the infection (Dewitt and Warren, 1959).

The suppression of malaria infection may be due to non-specific antibody induced by both eggs and adult worms. It has been shown that specific IgG to schistosomes was less than 4% of the total IgG in monkeys infected with S.mansoni (Freeman et al., 1970). So there may also be a polyclonal activation of B-cells in S.mansoni infection as suggested for T.brucei infection in mice (Hudson et al., 1976). If polyclonal activation of B-cells exists in S.mansoni infection a certain amount of antibody reacting to malaria parasites which could be produced could suppress malaria infection.

Other possible mechanisms include the non-specific activation of macrophages, either via T-cells (mediators) or directly causing them to liberate mediators which produce degeneration of parasites within circulating erythrocytes (Allison and Clark, 1977).

Portal hypertension in mice infected with S.mansoni starts to increase after 5 weeks of infection (Warren, 1961). This can cause a decreased flow of blood in the liver and a slowing down of circulation in the enlarged spleen which might help to clear the malaria infection quicker. Bowdler (1975) has reviewed the functions of the spleen and stated that the spleen acts as a filter to remove particulate materials from the circulation (both microscopic and sub-microscopic particles), which is principally mediated by vascular structures. The spleen contains the densest accumulation of macrophages in the body and it serves as a focus of phagocytosis. However, Cheever and Warren (1964) have shown that hepatic blood flow per 100g body weight remained within normal limits in mice infected with S.mansoni up to 11 weeks after infection, despite the development of portal hypertension.

Single sex (male) infections did not confer immunity to malaria infection given 8 weeks after cercarial infection. Though the result is not conclusive, it seems as if egg production is necessary for the suppressive effect on the malaria infection. The eggs produced by bisexual infection can act either through the stimulation of immune mechanisms (Warren et al., 1967) or by producing hepatic granuloma formation causing the increase of portal hypertension as

discussed earlier. But before drawing any conclusion some more experiments should be performed with malaria infection and irradiated cercariae, which do not give rise to mature egg-producing worms. This would give information on whether suppression of malaria depends on adult worms (bisexual) or not. Malaria infection should be given at intervals after intravenous injection of viable eggs to find out the effect of schistosome egg antigens in immunity to malaria infection.

The effect of P.chabaudi infection on both primary and secondary (challenge) infections with S.mansoni was also studied. It had no demonstrable effect on either numbers of adult worms (primary infection) or schistosomula (challenge infection) recovery, even though the primary cercarial infection was given at the peak of the malaria infection (fig 52a p331 ). This may have been because the immunosuppressive effect of the P.chabaudi infection was not potent enough or did not last long enough. However, Abdul-Wahab (1974) showed a decrease in the size of granulomas around S.mansoni eggs injected intravenously into mice 16 days after P.berghei infection.

The immunodepressive effect of P.chabaudi has been shown to correlate with the parasitaemia level (Exp . B la p158 ). P.chabaudi infection was significantly suppressed in mice infected 6, 9 or 12 weeks after S.mansoni infection (Exp. F 1 p313 ). So at this low level of parasitaemia with relatively less immunodepressive effect there may not be any significant effect on S.mansoni infection, either on the number of adult worms or on schistosomula recovered.

## V. SUMMARY AND CONCLUSIONS

1. P.chabaudi in CBA mice produces a benign synchronous and reproducible pattern of infection with 3 waves of parasitaemia. The infection in T-cell deprived mice, also, is not fatal but parasitaemia is maintained for longer than 120 days.

P.berghei infection in CBA mice is invariably fatal. The parasitaemias in both intact and in T-cell deprived mice are similar but the mean survival time is significantly longer in T-cell deprived mice. Thymectomy alone does not alter the infection in any way.

2. The magnitude of the primary antibody response to SRBC in P.chabaudi infections was related to the degree of parasitaemia. When the parasitaemia was low, either early in the infection or during the chronic phase, the antibody response to SRBC was not depressed as it was during the primary peak of high parasitaemia.

The effect of P.chabaudi on the secondary and tertiary responses to SRBC also varied according to the parasitaemia at the time the priming dose of SRBC was given. If the primary immune response to SRBC was significantly depressed due to SRBC being given at the time of the peak of parasitaemia, the subsequent responses to SRBC were also very much suppressed. In all cases there was a more rapid fall off in anti-SRBC antibody titres in malaria infected mice than in uninfected controls. Though this effect seemed to be

due to increased catabolism of immunoglobulins in malaria infected mice, it could not be confirmed by experiments on the passive transfer of antibody to sheep cells.

3. The antimalarial drugs quinine and pyrimethamine did not have any immunodepressive effect on the antibody response to SRBC. On the other hand chloroquine at a high dose level ( $50 \text{ mg kg}^{-1}$ ) showed some immunodepression of anti-sheep cell antibody titres but low doses ( $5 \text{ mg kg}^{-1}$ ) did not do so.
4. P.chabaudi infection in CBA mice produces a sterile, solid type of immunity. The immunity conferred by suppressive chemotherapy depended on the duration and the intensity of the recrudescence parasitaemia, both of which depend on the drug dosage schedule. Prolonged maintenance of a low level of parasitaemia by means of subcurative chemotherapy produced a solid immunity. On the other hand, the immunity bestowed by curative chemotherapy was related to the timing of the treatment. Though immunity could be induced by treatment on the first day of the infection, the maximal immunity was obtained by treating late in the infection.
5. When comparing the effectiveness of chemotherapy on the intact and T-cell deprived mice, the two parasites, P.chabaudi and P.berghei, behaved differently. In P.chabaudi infections an intact immune system is beneficial to the host and helps to resolve the

infection. Chemotherapy was more effective in intact than in T-cell deprived mice and the possible role of the immune mechanism is considered.

6. P.berghei infections kill both intact and deprived mice but the deprived mice have significantly longer mean survival times. In this case, antimalarial chemotherapy is more effective in T-cell deprived animals than in the intact controls. Though the exact mechanism is not known, the possible involvement of the immune mechanism is again considered.
7. When the recrudescence populations from both intact and T-cell deprived mice after chemotherapy of both P.chabaudi and P.berghei infections were tested for drug sensitivity they were always similar to that of the stocks used to initiate the infections.
8. Hydrocortisone acetate treatment of P.chabaudi infections in mice invariably produced a fatal outcome. Antimalarial chemotherapy combined with hydrocortisone acetate was less effective than the antimalarial given alone (quinine or chloroquine).

The hydrocortisone did not modify P.berghei infections when given alone or in combination with antimalarials. The importance of reticulocyte depression induced by the cortisone as well as its immunodepressive effect is discussed.

9. Protein energy deficiency in CBA mice infected with malaria induced a severe weight loss, anaemia and prolonged parasitaemia. However, chemotherapeutic effects in such mice were similar to those on malaria-infected controls.
  
10. The effect of S.mansoni infection on P.chabaudi infection was studied in mice. The malaria parasitaemia was significantly suppressed in mice infected with S.mansoni 6, 9 or 12 weeks previously but not 4 weeks previously. Single sex (male) infections given 8 weeks before did not modify the malaria parasitaemia. The possible nature of non-specific immunity conferred by the eggs and/or adult worms was discussed.

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