IMMUNOSUPPRESSION AND MALARIA

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Ethical Considerations

The research protocol for the Malumfashi Malaria Project was submitted to the Ethical Committee of the Ahmadu Bello University, Zaria, Nigeria by Dr Alan Williamson before the start of the study and the ethical considerations were approved by this Committee.

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SUMMARY

The aims of the Malumfashi Malaria Project were to study the effect of subclinical malaria on the antibody response to vaccines used in routine immunisation schedules in infants and children, to study the development of malaria antibodies in children protected from malaria by chemoprophylaxis and in unprotected children and to assess the nutritional status of these children.

Two groups of children matched for age, sex and ethnic origin were given either weekly chloroquine or weekly vitamin C tablets from the first week of life onwards. All children were seen every week for this purpose and if they were ill they were treated by standard methods according to their diagnosis. All babies were given a full course of immunisations while blood samples for antibody levels and haematological measurements were taken at regular intervals. Anthropometric measurements were made every three months.

There were no significant differences in the antibody responses to vaccination between the two groups of children except for the responses to group A and group C meningococcal polysaccharide vaccines which were better in protected than in unprotected children. Malaria antibodies developed in both groups of children although their development in the protected group was slower than in the control group of children.

As subclinical malaria infection does not depress the immune response to vaccinations, except to the meningococcal vaccines, it is not necessary to eradicate subclinical malaria before giving routine immunisations to babies and children in areas where malaria is found.

PREFACE AND ACKNOWLEDGEMENTS

This thesis is written as a tribute to Dr Alan Williamson who died tragically during the study in 1978 just before his thirtieth birthday. Alan had spent two years working on the study before his death during which time he had trained his team of local staff with patience and skill. He had inspired such loyalty among his staff that after his sudden death the team carried on their exacting routine so effectively that the project continued without a break despite the fact that the field workers had had no supervision for several weeks. As no permanent replacement could be found to take his place it was decided that I should try and continue the supervision of the study from Zaria as I had been involved in the earlier investigations from which the project had developed.

This plan could not have been considered without the knowledge that Dr Andy Bradley and his family at Malumfashi were ready and willing to help in every possible way to keep the study going. This they achieved by many hours of detailed work in the field and in the office and by the understanding care and kindness shown to our daughters, my husband and myself on our frequent visits to their home at Malumfashi. When their contract came to an end a year later this role was taken over very ably by Dr Andrew Tomkins and his family who always gave us a generous welcome.

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CHAPTER 1 - INTRODUCTION

1. INTRODUCTION

In 1962 McGregor and Barr showed that the antibody response to tetanus toxoid was lower in Gambian children with malaria than in protected controls. At first little attention was paid to this important observation. Seven years later Salaman et al. (1969) reported that the antibody response to sheep red blood cells was suppressed in mice infected with Plasmodium berghei, thus providing a laboratory model for the situation described in man. Since 1969 many studies have been undertaken in experimental animals which have confirmed the immunosuppressive properties of P. berghei and which have given some indication of how this immune defect is brought about. It is now realised that impairment of the immune response is an accompaniment of many bacterial, viral and protozoal diseases, although the characteristics and the severity of the immune defect vary from infection to infection (Greenwood and Whittle, 1981). However, the immune defect produced by malaria is of special importance because of the frequent occurrence of this infection.

2. IMMUNOSUPPRESSION IN EXPERIMENTAL ANIMALS WITH MALARIA

2.I Characteristics

In 1969 Salaman et al. reported that the immune response of mice to immunisation with sheep red blood cells, as assessed by the Jerne plaque technique, was depressed by infection with P. berghei. This observation has been confirmed by several groups of workers (Greenwood et al., 1971a; Whitmore, 1972; Lourie, 1972). The number of both IgG and IgM plaque forming cells is reduced and serum antibody levels are depressed. The antibody response of P. berghei infected mice to a number of other antigens has been investigated (Table 1a). The immune response to most, but not to all antigens, is reduced. A normal antibody response to bacteriophage ØX 174 (Barker, 1971) and to DNP- Ficoll (Brown et al., 1977) has been reported. DNP- Ficoll is thought to be a macrophage

independent antigen. Even when antibody is produced, its affinity may be lower than normal (Steward and Voller, 1973). The defect in humoral immunity associated with P. berghei malaria can be partially overcome if an antigen is given with an adjuvant (Greenwood et al., 1971a; Tarzaali et al., 1977).

Initial investigations into the integrity of the cellular immune response of mice infected with malaria gave conflicting results. Greenwood et al. (1971a) found that skin graft rejection, oxazolone sensitisation and the response of splenic lymphocytes to stimulation with phytohaemagglutinin (PHA) were normal in mice infected with P. berghei yoellii. Similar results were obtained by Lourie (1972). However Golenser et al. (1975) and Spira et al. (1976) showed that the cellular immune response of splenic lymphocytes to stimulation with non-specific mitogens was reduced in rats infected with P. berghei. The probable reason for these apparently conflicting results was provided by experiments carried out by Jayawardena et al. (1975). These workers showed that cellular immunity, as assessed by PHA responsiveness and oxazolone sensitisation, was reduced in mice with severe P. berghei infections but that it was little affected by infection with the less virulent parasite P. berghei yoellii. Thus it appears that cellular immunity is only suppressed during the course of murine malaria infections when the infection is very severe.

The duration of the defect in humoral immunity associated with rodent malaria is influenced by the nature of the test antigen. Thus, the immune response to sheep red blood cells returns rapidly to normal following treatment or following spontaneous resolution of the infection (Salaman et al., 1969; Greenwood et al., 1971a) whereas the antibody response to pneumococcal polysaccharide vaccine is suppressed for several weeks after an acute infection (McBride et al., 1977). The latter finding is of interest in relation to the differential

response to vaccination with protein and polysaccharide vaccines observed in children with P. falciparum malaria (see below). Mice with chronic malaria produced by infection with P. berghei yoellii followed by infection with P. berghei, show a persistent defect in the humoral immune response to pneumococcal polysaccharide (Wedderburn and Dracott, 1977).

Most experimental studies of immunosuppression induced by malaria have used the rodent parasite P. berghei. However, an immunosuppression has been demonstrated in chickens infected with P. gallinaceum (Weidanz and Rank, 1975).

2.2 Mechanism

Some of the possible ways in which malaria parasites might cause immunosuppression are indicated in Fig. 1a and these are considered below.

a. Impaired function of B lymphocytes

Malaria infected rodents are able to synthesise immunoglobulin effectively. Serum immunoglobulin levels are increased in P. berghei infected rodents (Poels and van Niekerk, 1977) and increasing titres of malaria antibodies are produced as the infection progresses. The number of B lymphocytes and of plasma cells in the spleen is increased by P. berghei infection (Moran et al., 1973; Graveley et al., 1976). The cause of this non-specific proliferation of B lymphocytes is uncertain but it is likely that a parasite derived mitogen, acting either directly on B lymphocytes or through the help of T lymphocytes, plays some part in its production (Greenwood, 1974; Greenwood and Vick, 1975; Freeman and Parish, 1978). It is possible that over-stimulation of B lymphocytes by a non-specific mitogen renders them refractory to stimulation with specific antigens in a manner analogous to that described for endotoxin.

b. Impaired function of T lymphocytes

The number of T lymphocytes in the spleen and in the thymus of rodents

infected with P. berghei is reduced (Krettli and Nussenzweig, 1974; Graveley et al., 1976). This depression is most marked in animals with the most severe infections (Jayawardena et al., 1975). Although the overall number of T lymphocytes is reduced in infected animals it is possible that an imbalance between suppressor and helper T lymphocytes might occur, thus resulting in immunodepression. The generation of T suppressor cells has been demonstrated in experimental trypanosome infections (Jayawardena and Waksman, 1977) but not in animals infected with P. berghei.

c. Impaired reticuloendothelial cell function

The spleen and lymph nodes of malaria infected rodents show extensive anatomical changes. The spleen is frequently grossly enlarged, and on microscopy shows a heavy infiltration with plasma cells and pale mononuclear cells (Moran et al., 1973). The size of the germinal centres is increased (Greenwood et al., 1971b). It is possible that this gross anatomical disorganisation of the reticuloendothelial system interferes with the normal traffic of lymphocytes necessary for an ordered immune response.

Studies on the phagocytic capabilities of the reticuloendothelial system in malaria infected rodents have given variable results dependent upon the stage of the infection at which measurements are made. However, most studies have demonstrated enhanced clearance of particles from the circulation during acute infection (Lucia and Nussenzweig, 1969; Greenwood et al., 1971b; Cantrell and Elko, 1976). These observations do not necessarily imply that other functions of the reticuloendothelial system are also stimulated by the infection. Thus, Loose et al. (1971) found that macrophages obtained from mice infected with P. berghei were unable to detoxify endotoxin normally. This finding led these workers to study the effect of malaria on antigen processing by macrophages. The results of transfer experiments with adherent and non-adherent cells showed that the defective ability to produce anti-

bodies of splenic mononuclear cells obtained from mice with malaria could be restored by the addition of normal macrophages to the in vitro culture system (Loose et al., 1972). These results have been confirmed by Warren and Weidanz (1976) and by Brown et al. (1977). Warren and Weidanz (1976) noted that the uptake of horse red blood cells by macrophages from animals infected with malaria was reduced. Therefore, it appears that defective processing of antigens by macrophages is an important cause of the immune defect associated with P. berghei malaria, a view in keeping with the fact that the antibody response to DNP - Ficoll, a macrophage independent antigen is normal in infected mice (Brown et al., 1977) and that the humoral defect to some antigens can be overcome by administering them with an adjuvant (Tarzaali et al., 1977).

d. Conclusion

The results of the experiments reviewed above suggest that the immune defect associated with rodent malaria has a complex aetiology. Anatomical disturbances of the immune system, defective macrophage processing of antigens and over-stimulation of B lymphocytes probably all play a part in its pathogenesis (Table 1a).

2.3 Consequences

Three biological consequences of rodent malaria may be related to the immunosuppression associated with the infection.

a. Rodents infected with malaria show an increased susceptibility to other infections. In 1965 Kaye et al. reported that Salmonella typhimurium infections were more severe in mice infected with P. berghei than in normal mice. Subsequently it has been shown that rodent malaria potentiates infection with Toxoplasma gondii (Strickland et al., 1972), Trypanosoma muscula (Cox, 1975) and Leishmania enrietti (Belehu et al., 1976). Depression of the immune response may not be the only mechanism by which malaria

potentiates these infections. Thus, changes in iron metabolism associated with malaria induced haemolytic anaemia may play a part in increasing the susceptibility of malaria infected mice to salmonella.

b. The incidence of lymphomas is increased in malaria infected animals. In 1968 Jerusalem reported that the incidence of spontaneous lymphoma was increased in P. berghei infected mice. It is likely that his colony of mice was infected by a naturally occurring tumour virus. These observations were carried a stage further by Wedderburn (1970) who showed that the incidence of tumours in mice deliberately infected with Moloney lymphomagenic virus (MLV) was increased by concomittant infection with P. berghei. Tumour development was enhanced when P. berghei was injected one week before to one week after injection of the oncogenic virus. Mice infected with malaria showed lower virus neutralising antibody titres than controls.

c. The development of spontaneous autoimmune disease is reduced in mice infected with malaria in early life. Greenwood and his colleagues studied the effect of malaria infection on NZB and NZB/NZW hybrid mice which spontaneously develop an autoimmune disease similar to disseminated lupus erythematosus. Infection of mice with P. berghei at the age of 1 month prevented the development of immune complex nephritis in NZB/NZW mice and prolonged their life span. This effect was observed despite the fact that malaria parasitaemia could not be detected at the age of 5-6 months when mice of this strain first show clinical signs of renal disease (Greenwood et al., 1970a and b).

3. IMMUNOSUPPRESSION IN HUMAN MALARIA

3.1 Characteristics

In 1962 McGregor and Barr reported that the antibody response to tetanus

toxoid of Gambian children protected against malaria was significantly higher than that of control children who were not protected against this infection. The response to other immunological stimuli was not investigated in this study. In 1972 Greenwood et al. reported the results of a more detailed study of the immune response of a group of Nigerian children acutely infected with P. falciparum. The results obtained in this group of children were compared with those obtained in a group of febrile children without malaria and with those obtained in healthy control children. Both humoral and cellular immune response was investigated. Children with acute malaria showed a significantly lower antibody response to the O antigen of S. typhi and to tetanus toxoid than did the healthy or sick controls. No significant difference in the antibody response to the H antigen of S. typhi was found and the mean titre of heterophile antibody was similar in the three groups. No defect in cell mediated immunity was found in the children with acute malaria because the proportion of children with malaria who responded to skin tests with candida and streptococcal antigens and to skin sensitisation with di-nitro chlorobenzene (DNCB) was similar to that observed in sick and healthy controls. Lymphocytes from children with acute malaria gave a normal proliferative response to stimulation with PHA at an optimum concentration of mitogen. A more detailed study of the PHA response of lymphocytes of children with acute malaria undertaken in The Gambia by Moore et al. (1974) confirmed that the PHA response of lymphocytes from infected children was normal under optimal conditions but showed that at sub-optimal PHA concentrations a defective response was obtained.

Williamson and Greenwood (1978) investigated the duration of the diminished response to immunisation produced by an acute episode of malaria. They found that the immune response to immunisation with S. typhi O antigen returned to normal one week after treatment of an attack of acute P. falciparum malaria but that the immune response to

group C meningococcal polysaccharide vaccine was still depressed 4 weeks after the acute illness, a finding comparable to that observed in malaria infected mice immunised with pneumococcal polysaccharide (McBride et al., 1977).

The studies of Greenwood et al. (1972) and Williamson and Greenwood (1978) were concerned with the immune response of children who were acutely ill with P. falciparum malaria. Such episodes are uncommon, even in areas where malaria is endemic. Little is known as to whether asymptomatic malaria, which may be present in up to half the population of highly endemic areas at any one time, has any effect on the immune response. Some indirect evidence which suggests that it may have an effect was obtained during the course of an investigation into the factors controlling the immune response to meningococcal polysaccharide vaccines. During the course of a village survey undertaken in a malaria endemic area of northern Nigeria Greenwood et al. (1980) found that the antibody response to group C meningococcal polysaccharide vaccine was higher in subjects with the haemoglobin genotype AS than in subjects with the genotype AA. The effect was most marked in young subjects. This association was not found in a second study carried out in healthy Nigerian secondary schoolboys at a time of the year when little malaria transmission was taking place (Blakebrough et al., 1981). These findings suggest that the haemoglobin genotype AS exerts its beneficial effect on the immune response to meningococcal vaccine through its influence on malaria rather than by any direct effect on immune responsiveness.

3.2 The mechanism

Little information is available about the mechanism of the immune defect found in patients with acute malaria. Immunoglobulin synthesis is not impaired by the infection because patients with acute malaria have elevated IgG and IgM levels (Abele et al., 1965) and show increased immunoglobulin synthesis (Cohen et al., 1961). Autoantibodies

and heterophile agglutinins may be formed (Greenwood and Whittle, 1981).

A number of observations on the changes in lymphocyte sub-populations that accompany acute malaria have been made (Wyler, 1976; Greenwood et al., 1977; Wells et al., 1979). These studies have shown depressed T lymphocyte counts, normal or increased B lymphocyte counts and increased counts of null cells in patients with acute malaria. Whether helper T lymphocytes or suppressor T lymphocytes are selectively effected is not known. Two possible mechanisms for the T lymphocytopenia found in patients with acute malaria have been put forward. Wyler et al. (1976) suggested that T lymphocytes are selectively trapped in the spleen whilst Wells et al. (1980) suggest that cold acting lymphocytotoxins, which can be demonstrated in the sera of many patients with acute malaria, contribute to the lymphopenia observed in these patients. Despite the T cell lymphopenia found in patients with acute malaria, functional tests of cell mediated immunity are well preserved (see above).

Little is known about function of the reticuloendothelial system in patients with acute malaria. The phagocytic ability of the liver and spleen is probably enhanced. Sheagren et al. (1970) demonstrated accelerated splenic uptake of large doses of aggregated albumin in patients with P. vivax malaria and Greenwood et al. (unpublished) obtained similar results in children with acute P. falciparum malaria. Enhanced splenic uptake of platelets may contribute to the thrombocytopenia found in many patients with acute malaria (Skudowitz et al., 1973). Demonstration of enhanced reticuloendothelial phagocytic activity in patients with acute malaria does not exclude the possibility that a macrophage defect is responsible for the immunodepression found in patients with this condition because more complicated functions of macrophages, such as antigen processing, may be impaired at a time when uptake of particles by macrophages is enhanced. Although no studies

of macrophage handling of antigens have been made in patients with acute malaria, partly because of the difficulties of obtaining a suitable cell population, it seems likely that a macrophage defect plays an important part in the aetiology of the immunodepression found in patients with this infection as it does in rodent malaria (Table 1b).

3.3 Clinical signficance

There are at least 4 areas in which the immunosuppression induced by malaria might have important clinical consequences.

a. Immunosuppression induced by malaria might impair the ability of the host to mount the immune attack necessary to eliminate the causative parasite thus causing a chronic infection. Some evidence for such a process has been obtained in experimental trypanosome infections during which immunosuppression induced by the parasite may have impaired the antibody response to a new antigenic variant appearing later in the course of the infection (Hudson et al., 1979) but there is no evidence that this phenomenon occurs in human malaria. Plasmodium falciparum has not been shown to underto antigenic variation and the formation of protective antibody against the homologous parasite can be demonstrated in many patients on recovery from the infection (Wilson and Phillips, 1976).

b. Immunosuppression accompanying malaria might increase susceptibility to a secondary infection. Chest infections and gastroenteritis are common complications of acute malaria and it is possible that some of these infections are caused by opportunist organisms. However, there is little direct evidence to support this supposition although it was noted many years ago (Hayasaka, 1933) that salmonella infections were sometimes precipitated by the use of malaria for the treatment of advanced syphilis. Recently it has been shown in The Gambia that many young children

with non S. typhi salmonellosis are severely anaemic and that many of them have malaria parasitaemia. This association with malaria is not found in children of the same age group who present with Escherichia coli septicaemia (Mabey, personal communication). The possible relationship between acute malaria and acute bacterial infections is worth further study.

c. It is possible that alterations of the immune status of a patient by acute or recurrent malaria alters the way in which the patient reacts to another agent causing disease. This alteration in immune response could be either harmful or beneficial. It is now widely believed that Burkitt's lymphoma is caused by infection with Epstein Barr virus (EBV). However, this tumour occurs frequently only in areas where malaria is highly endemic (Burkitt and Wright, 1970). The mechanism of this association is still uncertain. It has been suggested that the oncogenic potential of EBV is enhanced if infection occurs at a time when a patient is immunosuppressed as a result of an attack of acute malaria. Alternatively an increased rate of B lymphocyte turnover induced by recurrent malaria might favour oncogenic change in this group of cells. In contrast it is possible that recurrent infections with malaria and other parasites increase resistance to autoimmune disease for these conditions are encountered infrequently in many areas of the tropics where malaria is hyperendemic (Greenwood, 1968).

d. Immunosuppression induced by malaria might interfere with the response to routine immunisation. As has been described above the immune response to tetanus, S. typhi and meningococcal vaccines is impaired in children with acute malaria. Whether asymptomatic malaria has any effect on the immune response to immunisation has not been established.

4. AIMS OF THE PRESENT STUDY

Many vaccination schemes undertaken in tropical, developing countries have had only limited success. Some studies have failed because of poor planning or because of failure of the cold chain. However, it has been suggested that poor results are inevitable in communites in which malaria and malnutrition are common. The main aim of the present study was to determine whether the prevention of malaria throughout the first 2 years of life by regular administration of chemoprophylaxis could improve the immune response to routine immunisation. The study has provided additional information on the effects of malaria prophylaxis on mortality, morbidity and nutrition in children of this age group.

TABLE 1a

Characteristics of the immune defect observed in rodents infected with P. berghei

	Test system	Reference
Humoral immunity		
↓	Sheep red blood cells	1,2,3,4,5
↓	Tetanus toxoid	6
↓	Diphtheria toxoid	7
↓	Bordetella pertussis vaccine	8
↓	Human γ - globulin	2
↓	Pneumococcal polysaccharide	9,10
→ or ↓	Human serum albumin	2,11
→	Bacteriophage	3
→	DNP- Ficoll	12
Cellular immunity		
	Recall skin tests	2
	DNCB sensitisation	2
→ or ↓	Oxazolone sensitisation[b]	2,13
→ or ↓	PHA stimulation[b]	2,13,14,15

Notes: a. → or ↓ indicate normal or diminished immune response.
b. response determined by the severity of the infection.

References: [1] Salaman et al., 1969; [2] Greenwood et al., 1971a; [3] Barker, 1971; [4] Whitmore, 1972; [5] Lourie, 1972; [6] Voller et al., 1972; [7] Tarzaali et al., 1977; [8] Viens et al., 1974; [9] McBride et al., 1977; [10] Wedderburn and Dracott, 1977; [11] McBride and Micklem, 1977; [12] Brown et al., 1977; [13] Jayawardena et al., 1975; [14] Golenser et al., 1975; [15] Spira et al., 1976.

TABLE 1b

Characteristics of the immune defect observed in children infected with acute P. falciparum malaria

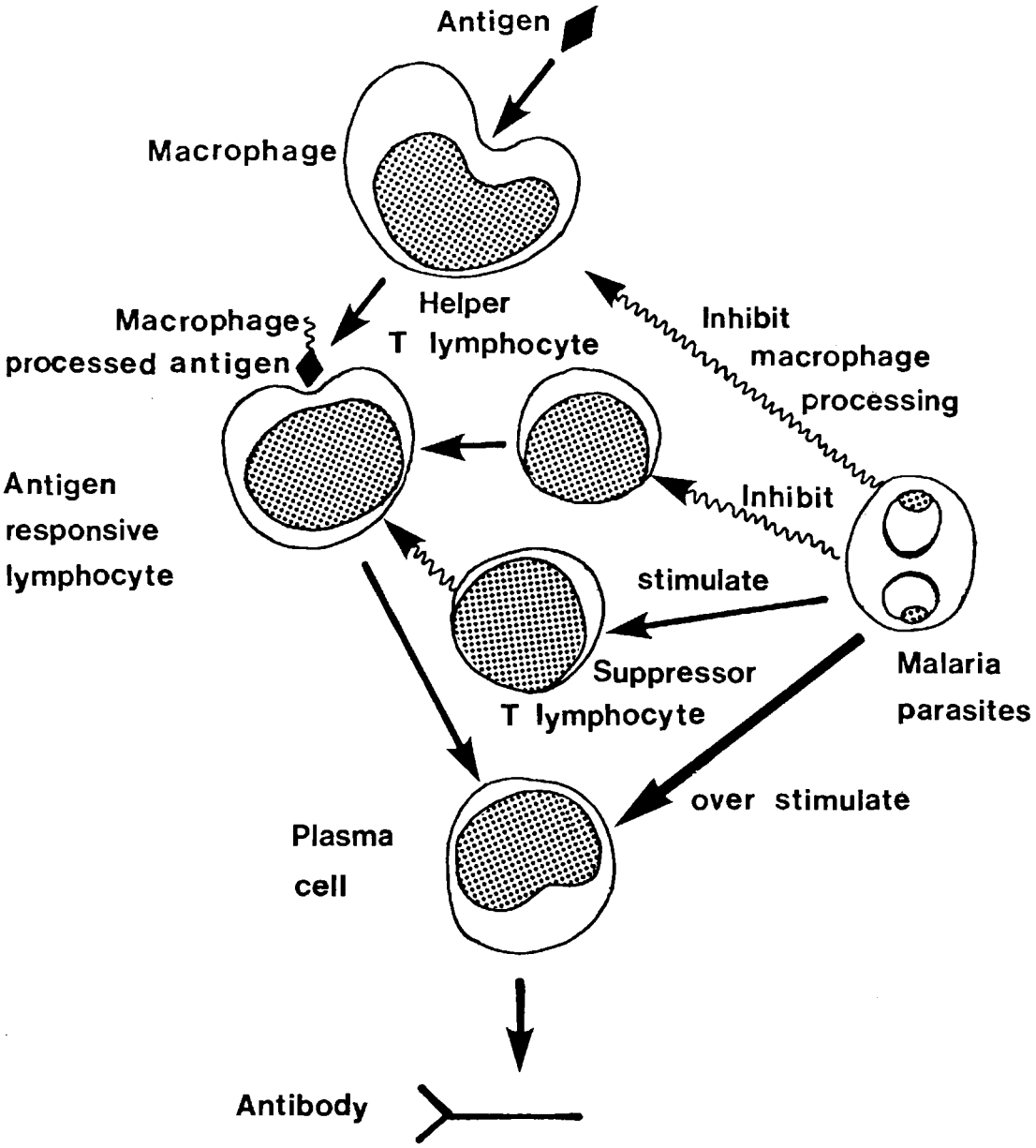
	Test system	Reference
Humoral immunity		
↓	Tetanus toxoid	1
↓	*S. typhi* O antigen	1,2
↓	Meningococcal vaccine	2
→	*S. typhi* H antigen	1
Cellular immunity		
→	Recall skin tests	2
→	DNCB sensitisation	2
→ or ↓	PHA stimulation [b]	2,3

Notes: a. ↓ or → indicates diminished or normal response.

b. response determined by the dose of PHA used.

References: [1] Greenwood *et al.*, 1972; [2] Williamson and Greenwood, 1978; [3] Moore *et al.*, 1974.

Fig. 1a. Schematic representation of the way in which malaria parasites might impair the immune response of the host to immunisation.



CHAPTER 2 - SUBJECTS, MATERIALS AND METHODS

I. INTRODUCTION

In this chapter the study area, the population studied and the laboratory methods employed in the investigation are described. This study was undertaken as part of the Malumfashi Endemic Diseases Research Project. Further details of the geography and demography of the area are given by Bell and Howells (1973) and Bradley et al. (1977).

2. THE STUDY AREA AND ITS PEOPLE

2.1 Gamzago village area

The study was carried out in and around the village of Gamzago near Malumfashi in northern Nigeria (Fig. 2a). Gamzago village area comprises thirteen villages and hamlets (Fig. 2b). Gamzago gari, the central village, is 25 km east of the town of Malumfashi where there is an 80 bed general hospital run by Ahmadu Bello University. The laboratory and office facilities used by the staff of the Endemic Diseases Research Unit are situated at Malumfashi Hospital.

The countryside of the Malumfashi area is flat with some deposits of laterite and alluvial areas near water courses. There are occasional rocky outcrops to enliven the landscape and some interesting deep river beds. The road from Malumfashi to Gamzago gari is rough laterite and it is passable during both the dry season and the wet season. However, the small tracks from Gamzago to surrounding villages and hamlets are narrow and tortuous and they are only suitable for Land Rovers and similar vehicles. Two big rivers in the area can be forded in the dry season but in the wet season the water level rises too high to allow vehicles to pass. This means that wide detours are often necessary to reach villages that are close to each other. Under such circumstances it was not possible to work in more than one village on any one day, and so the schedules for immunisation and sample collection had to be carefully planned.

Malumfashi district lies within the Sudan savanna. It is an area

of intense cultivation, the main crops being guinea-corn, cowpeas and groundnuts. Millet, maize, cassava and sweet potatoes are grown as well. Fine herds of Zebu cattle are kept by the Fulani, while the Hausa and Maguzawa keep small numbers of goats, sheep and poultry. Generally, the community is a prosperous one.

2.2 Population

The population of the study area is approximately 10,000 and it comprises a mixture of Hausa, Fulani and Maguzawa peoples with the Hausa predominating. The characteristics of these three cultural groups are summarised in Table 2a. The Hausa and the town-dwelling Fulani form relatively stable populations while the nomadic Fulani move frequently from area to area with their cattle. Maguzawa women work freely in the fields while Hausa women tend to remain within their family compounds. Members of the three different tribes live within their own groups but they mingle freely in the markets.

All three tribal groups carry out a traditional Naming Ceremony. A newborn baby is not recognised until it is seven days old. On the seventh day after birth the child is given a name by a malam and then he or she becomes a member of the family. Even though locally elected men from each village acted as project helpers in their own villages it was often impossible to find out about the birth of a baby until it was seven days old and so babies were never admitted to the study before they reached this age.

2.3 Climate

The climate in Gamzago follows the pattern characteristic of the dry savanna with a long dry season which lasts from late September until early May and with a wet season which lasts from May until September. The annual rainfall is in the region of 1,000 mm per year but this figures varies widely from year to year. The range was 536-1225 mm per year between the years of 1962-1976 (Bradley et al., 1977).

3. STUDY OUTLINE

3.1 Project babies

Newborn babies were identified and admitted to the project as early as possible, usually between the ages of one and two weeks. They were randomly divided into two groups; one group received weekly chloroquine, the other control group received a placebo. The two groups were well matched for sex and tribe (Table 2b).

Each baby was given a project number when he or she entered the trial and all relevant information about the baby was recorded on a mastercard held in the office at Malumfashi. The mother was issued with a small card which recorded the baby's name, number and date of birth and she was asked to bring this card with her whenever she came for prophylaxis or whenever she attended hospital. The small card was slipped into a thick plastic envelope and stapled into place and most of the mothers looked after the cards very carefully. If a card was reported lost, or chewed up by the baby, a new card was issued but only after the identity of the baby had been carefully checked.

Babies who entered the study between August 1976 and June 1977 were given chloroquine or placebo for two years, whilst those who were admitted between June 1977 and April 1978 were treated for one year only. Babies were followed for another six months after stopping the administration of chloroquine or placebo tablets to ensure that they did not come to any harm following the withdrawal of prophylaxis. During this final follow-up period the field worker visited the study villages every week as usual and saw those mothers and babies who wanted to come to her, but she did not give them any chloroquine or placebo tablets. Other mothers and babies were visited in their homes every 1 or 2 weeks. Any child who was ill was treated in the appropriate way.

Table 2c shows the number of children who entered the study and summarises their subsequent fate. The original protocol for the study

envisaged three groups of babies, one group followed for three years, one group followed for two years and one group followed for one year only. However, following the death of Dr Williamson, it was not possible to continue the study for a full three years as originally planned and the group of children due to be followed for three years was combined with the group due to be followed for two years, and both were followed for the latter period. This change in study design explains the fact that the group of children followed for 2 years is considerably larger than the group followed for 1 year. Only 45% of children completed the full course of injections and blood sampling. Twenty-five per cent moved away from the study area during the course of investigations, some at the time of weaning as is customary in the Malumfashi area. Nineteen per cent withdrew from the study because of their resistance to blood sampling and 11% died. The latter group is considered in detail in Chapter 3.

3.2 Prophylaxis

Local girls were trained as field workers. They were allocated to an area in which they worked consistently so that they came to know the project mothers and their babies well and they were able to establish good relations with their families.

Chloroquine was used for prophylaxis. It was given to study children as soon as the child joined the project and weekly thereafter. The dose of chloroquine given was 100 mg base for the first year and 200 mg base in the second year.

On the appropriate day for drug prophylaxis in a village the field worker waited at the customary meeting place until the mothers and their babies had collected. Then she checked which group each baby belonged to, administered the relevant medicine and marked it off on her register. These attendances were marked on the master card at the end of each day. Occasionally a baby's mother would be unable to come to

the field worker and she would send a message instead. In that case the field worker would either go to the baby's compound or ask one of the other mothers to bring the baby to her. Whenever possible the medicine was given to the baby by the field worker and it was not given to a third party to pass on to the mother. This was particularly important as often the third party was a small sister, very willing to help, but quite unaware of the implications of forgetting her errand half way home. Any defaulters were noted and on Thursdays the field workers made a special trip to try and find them.

Chloroquine tablets were crushed between two teaspoons and the powder was mixed in a little water. The resulting liquid was given to the baby by the field worker and the child was returned to his or her mother for a breast feed. The mother was asked to wait nearby and the baby was watched for vomiting for half an hour. If vomiting occurred the drug was given again. If vomiting occurred after the second tablet the baby was given an equivalent dose of chloroquine syrup (5 ml of syrup containing 100 mg of chloroquine base). If a baby consistently proved intolerant of chloroquine tablets he or she was given regular chloroquine syrup instead. Generally the tendency to vomit decreased as the babies grew older. Placebo tablets (sugar coated vitamin C) were given to the babies in the control group in exactly the same way as the chloroquine was given to babies in the study group.

3.3 Routine clinical care

As few medical facilities were available in the project area a general clinic was run in each village in turn, advance notice being sent through the village helper or the village head. Attendance rates at these clinics were high. (The clinics were run independently of the regular visits of the field workers to give out malaria prophylaxis and placebo). All patients seen at these clinics who had clinical features of malaria were treated with chloroquine regardless of whether or not

they were study babies. Medicines were carried in project vehicles to deal with emergencies and anyone who was in need of hospital treatment was brought back to Malumfashi Hospital by project transport.

When project babies attended general clinics their name and project number, the date of attendance, their symptoms, a working diagnosis, whether or not a blood test was done, and the treatment given were carefully recorded on a small printed card. These cards were taken back to Malumfashi where the information was transcribed onto the master cards together with the results of any blood tests that had been done. In this way a record was obtained of the number of babies in the two study groups who attended general clinics, the number of babies with a presumptive diagnosis of malaria in each group and whether or not this diagnosis was subsequently proven or discounted by examination of a blood smear.

3.4 Immunisations

A schedule of immunisations was given which follows closely that in current use in northern Nigeria except that BCG was given later than normal and except that all children were immunised with S. typhi and meningococcal vaccines (Table 2d). Table 2e lists the sources of the vaccines used and their antigen content.

3.5 Blood samples for antibody assays

Fingerprick blood samples were collected into heparinised capillary tubes (Gelman Hawksley). Usually it was possible to obtain 5 capillary tubes of blood, approximately 0.3 ml from each child. Tubes were sealed with critaseal (Gelman Hawksley). All capillaries from the same child were placed in a plastic tissue culture tube with a cap and labelled with the baby's name and number and the date. That evening sera and cells were separated. Sera were stored in a deepfreeze at $-20^{o}C$ until they were tested. Some red cell deposits were used for genotype determinations.

Antibody levels were measured on fingerprick samples taken immediately before and at different times after immunisations, as shown in Table 2f.

3.6 Skin tests

Skin tests were performed at one year or two years of age. Mantoux testing was carried out at the start of the final session. An injection of 0.1 ml of standard tuberculin solution, (i.e. 5 T.U.) (Evans Medical Ltd, London) was given intradermally into the left forearm. The resulting induration was observed 48 hours later. The response was measured as a mean of two diameters, taken at right angles to each other, across the raised and indurated area. Candida skin testing was carried out in a similar way. An injection of 0.1 ml of 1% *Candida albicans* antigen (Bencard) was injected into the right forearm and the response was measured in the way described for the mantoux test.

3.7 Anthropometry

Two field workers were allocated to anthropometry and worked together throughout the project. Measurements were made as soon as a baby joined the project and they were repeated at three monthly intervals for as long as the child remained in the study. A continuous cycle of anthropometry was required to obtain these regular measurements.

Measurements were made of height, weight, head circumference, chest circumference, upper arm circumference and skinfold thickness.

Height: This was measured with the child lying flat on his or her back on a specially constructed board with a vertical foot piece. Special care was taken to see that the baby's head was firmly against the top of the board, that his or her knees were straight and that the soles of his or her feet were flat on the vertical foot piece. Measurements were made in cm. (Fig. 2c).

Weight: Babies were weighed on a Salter spring scale (Fig. 2c). A

baby was suspended in a pair of plastic pants and, whilst one field worker held him or her up gently, the other read and recorded the weight in Kg.

Head circumference: This was measured around the head using a non-elastic cotton tape measure calibrated in cm.

Chest circumference: This was measured around the nipple line with the same tape as that used for measuring the head circumference.

Upper arm circumference: This was measured around the thickest part of the arm above the elbow with the arm extended.

Skinfold thickness: The thickest fold of skin over the triceps was measured using 'Harpenden' skin calipers, but results obtained in this way proved to be inaccurate and inconsistent and the data obtained was not used in the final analysis.

At least two observations were made for every measurement and the mean values were transcribed from the work book used in the field onto the child's master card held at Malumfashi.

4. LABORATORY METHODS

4.1 Parasitological and haematological determinations

a. Malaria parasitaemia

Thick and thin blood films collected in the field were marked with the baby's name and study number in wax crayon and placed in a specially designed box to protect them from dust and flies. Thin blood films were fixed in methanol for one second and then stained with 8% Giemsa stain at pH 7.2. These films were used for species identification of parasites. Thick blood films were stained with 3% Giemsa (pH 7.2) for 30 minutes and they were examined for parasites using a x 100 oil immersion lens. The number of high power fields that were positive for malaria parasites out of the 200 fields examined was recorded and the number of parasites in each high power field was also determined. (An average of ten parasites or more in each high power field was taken to

mean a heavy infection.) Examination of 200 high power fields took approximately ten minutes. The procedure used for examining positive blood films was the same as that employed during the WHO survey undertaken at Garki in northern Nigeria (Molineaux and Gramiccia, 1980).

b. Haemoglobin, packed cell volume (PCV) and white blood cell (WBC) counts

Haemoglobin, PCV, WBC and other haematological indices were measured with a Coulter Counter ZN (Coulter Electronics Ltd). This machine was kept at the project laboratory at Malumfashi. Plastic bottles, each containing 10 ml of Isoton (Coulter Electronics Ltd) were taken to the field and an initial dilution of blood was made directly a sample was obtained. Twenty µl of blood, obtained with a calibrated Drummond microcap, were added to 10 ml of Isoton to give a 1:500 dilution of blood which was used for WBC determination. At the base laboratory 0.1 ml of this diluted sample was added to 10 ml of Isoton to give a final dilution of 1 in 50,000 which was used for red blood cell counts and haemoglobin determinations.

Preliminary studies showed that the results obtained when an initial dilution was made in the field were comparable to those obtained on dilution of whole blood samples in the base laboratory, provided that the diluted sample was not held for more than 6 hours before being tested. Reference blood samples were used to calibrate the Coulter Counter.

c. Genotype determination

Haemoglobin genotype determination was carried out on red cell eluates by cellulose acetate electrophoresis by Mr A. Akintunde, Department of Haematology, Ahmadu Bello University Hospital, Zaria.

d. Folate determinations

Serum and whole blood folate levels were determined by Mr E.D.E. Attai, Department of Haematology, Ahmadu Bello University Hospital, Zaria. The

method used was a modification of the Lactobacillus casei assay described by Fleming et al. (1971). This technique is a microbiological method using L. casei which requires folates for normal growth.

Assays may be done on serum or red blood cells. Red cell folate (RCF) was calculated by the equation

$$RCF = \frac{WBF - SFA\ (1 - PCV/100)}{PCV/100}$$

where WBF = whole blood folate, SFA = serum folate and PCV = packed cell volume.

e. Serum ferritin determinations

These determinations were carried out by Professor F.V. Flynn and his colleagues in the Department of Haematology, University College Hospital, London. The assay used was a radioimmunoassay which measures the inhibition by a test sample of the binding of purified I^{125} labelled human ferritin to rabbit anti-ferritin antiserum.

4.2 Antibody measurements

a. Tetanus antibodies

Tetanus antibodies were measured both by a haemagglutination test, based on the method of Levine et al., 1960, and by an enzyme linked immunosorbent (ELISA) assay (Voller et al., 1979).

Haemagglutination assay: Sheep red blood cells, collected into Alsevers solution, were spun down and washed four times in phosphate buffered saline (PBS) pH 7.2. Two hundred ml of a 25% solution of these cells were placed in a conical flask to which was added a dialysis sac containing 50 ml of 40% formaldehyde. The flask was gently rotated on a mixing wheel. The concentration of formalin to which the cells were exposed built up slowly during the next few hours until it was equal inside and outside the membrane. Then the bag was opened and the cells were left overnight with gentle mixing. Next day they were washed five times in PBS. This procedure rendered the red cells very stable and a batch large enough to test all the study sera was made up

at the same time.

Formalinised cells were tanned as follows. A 5% solution of formalinised cells was added to an equal volume of 1/100,000 tannic acid, mixed and left at room temperature for 20 minutes. Then the cells were spun down and washed twice in PBS pH 7.2.

Tanned formalinised cells were coated with tetanus toxoid by the following procedure. The cells were washed once in PBS pH 6.4 and a 2.5% solution of cells was made up in this slightly acid buffer. A mixture of 2.5% cells was added to an equal volume of purified tetanus toxoid (Lf 2,900/ml, kindly provided by the Institut Mérieux) at a dilution of 1/100 and mixed in a shaking waterbath for 30 minutes. In preceding experiments a dilution of tetanus toxoid of 1/100 had been found to be the optimum concentration for this assay. Coated cells were washed three times in PBS at pH 7.2.

A PBS pH 7.2 buffer containing 2% normal rabbit serum (NRS) previously absorbed with sheep red blood cells was prepared. The cells were washed for the last time in this protein-enriched buffer and then they were suspended in it at a concentration of 0.5%.

Test sera were heat inactivated (56^{o}C for 20 minutes) and absorbed with sheep red blood cells overnight to remove heterophile antibodies. Serial dilutions of test sera were made in 'V' microtitre plates (Flow Laboratories) using PBS 2% NRS buffer. Twenty-five µl of coated cells were added to each well, the plates were gently shaken and read on a viewing box after standing overnight at 4^{o}C.

A positive and a negative control serum and a horse antiserum containing one unit of antibody per ml were included in each assay to ensure consistency between results obtained on different days.

ELISA assay: Polystyrene, flat bottomed microtitre plates (Dynatech M129A) were coated with tetanus toxoid (Wellcome Tetanus Vaccine, B.P. Tet/vac/FT) as follows. Two hundred µl of antigen diluted 1:200 in

carbonate/bicarbonate buffer pH 9.6 were added to each well and the plates were then incubated overnight at 4°C. They were then washed twice in PBS/Tween buffer (PBS pH 7.4 + 0.05% Tween 20) and once in distilled water. Plates were then dried at 37°C for 30 minutes, packed in air-tight plastic bags containing silica gel and stored at 4°C until used.

To perform an assay 200 µl of test sera, diluted 1:200 in PBS/Tween buffer were added in duplicate to antigen-containing wells. Plates were then incubated at room temperature for 2 hours. Sera was removed by 3 washes in PBS/Tween buffer. Two hundred µl of a 1:5,000 dilution of sheep anti-human immunoglobulin antiserum conjugated with peroxidase by a 2-step glutaraldehyde procedure (Voller et al., 1979) were than added to each well. Following incubation for 3 hours at room temperature the conjugate was removed and the plates washed 3 times in PBS/Tween buffer. Two hundred µl of the peroxidase substrate orthophenylenediamine, (40 mg of orthophenylenediamine in 100 ml of phosphate citrate buffer pH 5.0 + 40 µl of 30% H_2O_2) were added to each well to develop a colour reaction. This was stopped after 20 minutes by the addition of 50 µl of 2.5N H_2SO_4. Plates were read on a Flow multiskan machine using a 492 nm filter.

Control positive and negative sera were included in each batch of assays and results were corrected to give a reference reading with the control positive serum of 1.0. The optimum concentrations of antigen, antibody and conjugate for the assay of test sera were determined in a series of preliminary experiments.

b. Diphtheria antibodies

Diphtheria antibodies were measured by an ELISA technique very similar to that described above for tetanus antibodies. The coating antigen used for this assay was purified diphtheria toxoid obtained from Burroughs Wellcome. The antigen preparation, which contained 6250

Lf/ml and 3.75 mg protein/ml, was diluted 1:30,000 for coating.

Control positive and control negative sera were included in each batch of tests as described above.

c. Poliomyelitis antibodies

Poliomyelitis antibodies were measured by Dr J. Craske (Public Health Laboratories Service, Manchester) using a standard virus neutralisation assay.

Tests were carried out in sterile flat-bottomed tissue culture grade microtitre plates with lids (Flow Laboratories Ltd). Plates were sealed with non-toxic plate cellophane sealers (Flow Laboratories Ltd). Sera were diluted in 25 µl volumes using disposable micropipettes (Drummond Scientific Ltd).

The prototype Sabin strains of poliovirus types 1, 2 and 3 were used. These were grown in Hep_2 cells, and virus stocks were prepared by infecting monolayers grown in bottles. Viruses were harvested when the cells showed 75-100% cytopathic effect. These were then frozen and thawed once and then stored at $-80^{o}C$ in volumes of about 0.5 ml. One volume of each prototype strain was thawed for each batch of tests and then discarded. Hep_2 cells were grown in Eagle's basal medium supplemented by 10% foetal calf serum with 2 ml HEPES buffer per 100 ml of medium. Cells were maintained in 2% foetal calf serum with 2 ml HEPES buffer per 100 ml.

Because of the very small volume of serum available for many specimens - less than 25 µl - an accurate dilution of some serum samples was not possible. A volume of 25 µl of serum was sufficient for an accurate titration of serum against all three serotypes of poliovirus with a starting dilution of serum of 1/12. In some instances, the 25 µl micropipette was half filled with serum, and this was assumed to be a 1/2 dilution when made up to 25 µl with maintenance medium. Dilutions were made in a WHO plate, previously sterilised by ultra-

violet light irradiation. Dilutions were made from 1/12 to 1/12288 in fourfold steps. Twenty-five µl of each dilution of serum were transferred to each of 2 wells of a microtitre plate for each virus serotype. A separate plate was used for each serotype. Twenty-five µl of a dilution of each serotype of polio virus containing 100 $TCID_{50}$ were added to each of 2 wells of the microtitre plate per dilution of serum. Controls included a back titration of each virus using dilutions of 100, 10, 1.0 and 0.1 $TCID_{50}$ as well as a serum control for each specimen.

Serum/virus mixtures were incubated at 37°C for 3 hours. Twenty-five µl of a suspension of Hep_2 cells containing approximately 3-4 x 10^5 cells per ml in growth medium were added to each well. Then plates were sealed and incubated at 37°C, and they were examined for the presence of a cytopathic effect (cpe) after 48 and 72 hours incubation. The titre of a serum was taken as the highest dilution where virus growth in 50% of the wells was inhibited for each virus. The end titre of a serum was estimated for each serotype when the virus controls showed the presence of between 32 and 320 $TCID_{50}$ in the virus/growth mixture. This was usually after 72 hours incubation.

d. Measles antibody

Measles antibody levels were measured by a haemagglutination inhibition technique. Test sera were heat inactivated and absorbed overnight with green monkey red blood cells to remove non-specific agglutinins. Doubling dilutions of 25 µl of absorbed sera were made in 'V' microtitre plates using PBS, pH 7.2 containing 0.5% bovine serum albumin as the diluent. To each well 25 µl of measles haemagglutinating antigen (Behringwerke, Ltd) diluted 1:2 56 in PBS albumin buffer, were added. This concentration of antigen represented 4 minimal haemagglutinating units as determined by a preliminary titration of the haemagglutinin against a 0.5% solution of green monkey red cells. After mixing, the trays were incubated at room temperature for 1 hour.

Twenty-five µl of 0.5% green monkey red cells suspension were added to each well, the trays were shaken and incubated overnight at 4°C. The highest dilution of serum showing inhibition of agglutination was then recorded. Positive and negative controls with and without haemagglutinin were included. A standard serum was included in each assay to ensure reproducibility of results undertaken on different days.

e. Salmonella typhi antibodies

Patients' sera were inactivated by heating to 56°C for 20 minutes and doubling dilutions of sera were made in 'U' shaped microtitre plates using PBS pH 7.2 as diluent. One drop of S. typhi 'O' suspension (Burroughs Wellcome), diluted 1 in 5 was added to each well. Plates were read for the pattern of agglutination after overnight incubation at room temperature. Standard positive and negative sera were included in each assay.

f. Meningococcal antibodies

Meningococcal antibody levels were measured by a haemagglutination test. Human group O rhesus negative red blood cells were collected in heparin, washed and allowed to stand in Alsevers solution for 3-5 days. Cells from the same donor were used in all experiments. Prior to coating, cells were washed three times in PBS pH 6.9 and a 5% solution of cells was made up in PBS pH 6.9. An equal volume of this cell suspension was added to an equal volume of purified meningococcal polysaccharide at an optimal concentration made up in the same buffer (5 mg/L for group A polysaccharide and 1 mg/L for group C polysaccharide). The mixture was left for 45 minutes at 37°C in a waterbath with gentle shaking and then the cells were washed three times in PBS pH 6.9 to remove free antigen. The last wash was carried out with PBS enriched with 0.5% rabbit serum previously absorbed with human red blood cells. Finally the cells were made up

at a concentration of 0.5% PBS pH 6.9 and 0.5% rabbit serum.

Patients' sera were inactivated by heating to 56°C in a water-bath and then doubling dilutions of sera were made in PBS pH 7.2 supplemented with 0.5% rabbits serum in 'V' microtitre plates (Flow Laboratories Ltd). Twenty-five µl of cells were added to each well. The plates were read after standing overnight at 4°C. Standard positive and negative sera were included in each assay.

g. Malaria antibodies

Malaria antibody levels were determined by the ELISA assay, and by the indirect immunofluorescent assay and by gel diffusion tests.

ELISA assay: An ELISA assay was carried out as described in section 4.1a except that malaria antigen was used as the test substrate. The malaria antigen employed was prepared from Aotus monkeys infected with P. falciparum. A soluble antigen, prepared from infected blood by sonication was used at a dilution of 1:5,000.

Immunofluorescence: Antigen prepared as above was spotted onto "multi-spot" slides which were stored at -70°C until used. Doubling dilutions of test sera were made in PBS pH 7.2 from a dilution of 1:20 to a dilution of 1:640. Ten µl of each dilution were placed on an antigen spot and the slides were incubated for 30 minutes at room temperature in a moist chamber. After washing 3 times in PBS pH 7.2 for 15 minutes, 10 µl of a fluorescin-labelled anti-human IgG conjugate diluted 1:20 were added to each spot and the slides were incubated for a further 30 minutes at room temperature. Following 3 more washes the slides were mounted and examined for fluorescence on a Carl Zeiss UV microscope. Positive and negative control sera were included in each assay.

Gel-diffusion test: Selected sera were tested for the presence of precipitating malaria antibodies against an antigen obtained from the placenta of a Gambian woman infected with P. falciparum. This placenta had been extracted as described previously (McGregor et al,

1966) and subsequently stored at -70°C. Assays were carried out in 1.5% agar made up in 0.3M PO_4 buffer, pH 8.0. After incubation for 48 hours at 4°C slides were washed in saline and stained with amido black. The number of precipitin lines present was recorded.

4.3 Other laboratory assays

a. Immunoglobulin determinations

Serum IgG and IgM levels were measured by radial immunodiffusion. Glass photographic plates 10 cm x 10 cm were coated with 1.5% agar (Difco Noble agar) dissolved in barbitone buffer pH 8.6 containing anti IgG or anti IgM antisera (Hyland Laboratories) at the optimum concentration (1 in 30 for IgG and 1 in 100 for IgM). Twenty-five 3 mm wells were punched in the agar and filled with 5 µl of test serum or standard using an accurately calibrated Drummond microcap (Shandon). Plates were incubated at room temperature for 48 or 72 hours and then the size of the precipitin ring was measured using a magnifying glass and a graticule. Immunoglobulin values were determined by reference to a standard curve constructed from the results obtained with 5 dilutions of a local serum standard. This standard has been calibrated against a WHO standard serum thus allowing results to be expressed in International Units.

b. C3 determinations

C3 levels were determined by radial immunodiffusion as described above using an anti C3 serum (Hyland Laboratories) at a final dilution of 1:60. Results are expressed as a percentage of a local serum standard prepared from samples obtained from health Nigerian adults.

c. Serum albumin

Serum albumin levels were determined by the bromcresol green method (Doumas et al., 1972). A stock solution of bromcresol green was prepared in 0.1M succinate buffer pH 4.0 and stored at 4°C until used. Before each assay a working solution of bromcresol green was prepared

by adding 1 volume of stock solution to 3 volumes of succinate buffer. Four ml of Brij 35 were added to each litre of working solution and the pH adjusted to 4.2. Twenty-five µl of serum were added to 5 ml of dye and, after incubation at room temperature for 10 minutes, the colour reaction was read on a Pye Unicam SP 600 UV spectrophotometer at a wavelength of 628 nm. A curve was plotted using dilutions of a Behringwerke serum standard and albumin levels in test sera were related to this serum standard.

d. Pre-albumin

Pre-albumin levels were determined by radial immunodiffusion (see section a) using an anti-pre-albumin serum (Behringwerke Ltd) at a final dilution of 1:30. Results were expressed as a percentage of a locally prepared adult serum standard.

e. Urinary chloroquine

Urine samples were tested for chloroquine by the method of Haskins (1958).

5. DATA HANDLING

5.1 Computer

All data was analysed on the CDC 6600 computer at the University of London Computer Centre by Ms Betty Kirkwood.

5.2 Data preparation

Data was copied from the record books onto specifically designed coding sheets. Then it was punched and verified onto 80 column computer cards and transferred onto magnetic tape.

5.3 Data checking

Range checks were performed on most of the variables using a London School of Hygiene programme called CONKER. Some consistency checks were also performed. Some examples are (1) "Was the season given correct for the date?" (2) "Was the same group and treatment coded on all cards belonging to the same child?"

5.4 Data analysis

The data were analysed using SPSS, the Statistical Package for the Social Sciences and specially written Fortran programmes. The statistical analyses used were the X^2 test and student's 't' test unless it is specifically stated in the text that another test was needed to elucidate a particular point.

6. DISCUSSION

The field area covered by the Endemic Diseases Research Unit was extensive, covering 470 square miles and a population of 43,216. The Gamzago village area was chosen from within this bigger area for three main reasons. Firstly the area did not have a dispensary and it was several miles distance from the nearest pharmacist. Thus, it was difficult for mothers to obtain chloroquine for their children from anyone except project staff. Secondly the population of Gamzago village area, approximately 10,000, was large enough to yield a significant number of babies within the time available to the study. Finally, the study area was near enough to Malumfashi Hospital for the facilities of the hospital to be used for laboratory work and for the diagnosis and treatment of the seriously ill patients encountered during the course of the survey. Despite the availability of a hospital it was difficult to obtain a definitive cause of death in study babies. There is a strong tradition in northern Nigeria that death should occur at home and mothers often took their critically ill babies away from hospital for this reason. Even when deaths took place in the ward no post mortems could be performed because of the strong cultural resistance to this practice in the Malumfashi area.

Each baby in the project had his or her own set of records. The small identity card, held by the baby's mother, proved to be most valuable; she was asked to produce it whenever identification of the baby was needed. These cards were particularly useful towards the

end of the study when it was necessary to identify babies who had moved to other villages in the area and to identify babies who had not been seen by the project workers for some weeks. The baby's project number was married to the number that he or she had been given in the full demographic survey (Bradley, 1979). This provided much useful information about his or her compound and family background. Master cards which formed a repository for all the information collected about each baby were held at Malumfashi. The results of all measurements or tests were recorded on these master cards as they became available. These cards are still held at Malumfashi Hospital as part of the records of the Endemic Diseases Research Unit and provide information on a group of well documented children that could form the basis for further studies in the area.

On the whole prophylaxis proceeded smoothly. Field workers became adept at persuading infants to take chloroquine and they quickly identified those who were likely to vomit their tablets. This was a small but significant problem in some young babies. Fortunately chloroquine syrup proved to be an acceptable alternative in all cases and, despite some earlier fears, no babies had to be removed from the protected group because of failure to take their medication. The incidence of vomiting decreased steadily as the children grew older, although occasionally older babies spat out their tablets deliberately. A separate small group of babies who were receiving pyrimethamine (Daraprim) for malaria prophylaxis did not vomit, an important advantage of Daraprim over chloroquine (Greenwood, A.M., unpublished).

The two field workers who had been trained to carry out anthropometric measurements worked together throughout the study with only an occasional locum who was necessary to cover illnesses or holidays. These two workers were taught the principles of the measurements that were used and the importance of taking two readings for each measurement and recording the mean at once. Most of the data collected by

them were within acceptable limits of accuracy. Readings made by the field workers were checked by Dr Williamson initially and any doubtful readings were repeated the following week. However, skinfold thickness proved to be very difficult to measure accurately. This was partly because babies disliked the pinch from the calipers and so struggled when they saw the instrument, and partly because the calipers are difficult to use, even in a cooperative subject. The results of skinfold thickness measurements were so inconsistent that they were omitted from the final analysis.

When the original protocol for the study was devised it was planned to take routine fingerprick blood samples every three months for estimation of malaria parasitaemia and haematological measurements, in addition to the samples collected at the times of immunisation. Unfortunately, mothers became increasingly unhappy about this apparently unnecessary blood sampling, although the reasons for it had been explained to them by the village leaders, and routine blood tests had to be discontinued. The original protocol also envisaged the collection of a final venous blood sample for detailed immunological and haematological assays. However, venepuncture proved to be totally unacceptable to mothers, despite many efforts at reassurance by project staff and local leaders. Thus, only a few 10 ml venous blood samples were obtained. Several mothers withdrew their babies from the study at the time that venepuncture was first attempted and there is little doubt that persistence in attempts to collect venous blood would have led to collapse of the survey.

Because of the resistance of mothers to blood testing and because of the impossibility of obtaining venous blood multiple laboratory assays had to be carried out on very small blood samples obtained by fingerprick. The use of a Coulter Counter enabled a full haematological profile to be obtained on only a very small amount of blood

(20 µl). Preliminary studies showed that the initial dilution of blood for Coulter counting could be made safely in the field, using an accurately calibrated capillary tube to collect the blood sample. The Coulter Counter was operated in the field laboratory by laboratory assistants with little formal training who received only occasional supervision from staff of the main teaching hospital at Zaria, 70 miles away. The technically complex Coulter Counter, operated in a small rural hospital, provides a means of obtaining haematological data on field populations that would be difficult to obtain in any other way.

The ELISA technique is ideally suited for antibody measurements on small blood samples, 5 µl - 10 µl of serum being sufficient for each assay. This technique was successfully used to measure antibodies to tetanus toxoid, diphtheria toxoid and malaria. A good correlation was found between the malaria antibody levels of individual samples as measured by ELISA and FAT (see chapter 3). Attempts to develop a polio ELISA assay were unsuccessful and finally polio antibodies had to be measured by a standard viral neutralisation assay which requires a moderate amount of serum. Thus, this antibody assay could not be carried out in all patients. No problems were encountered in measurement of the antibody response to other vaccines by standard micro-haemagglutination or haemagglutination inhibition assays.

TABLE 2a

Characteristics of the 3 main cultural groups in the Malumfashi area. (From Bradley, 1980).

Cultural Group	Language	Religion	Settlement Type	Farming Pattern
HAUSA	Hausa	Muslim	Compact	Cultivation of cereals etc.
MAGUZAWA	Hausa	Traditional + Christian	Dispersed, large units	Cultivation of cereals etc.
FULANI				
Nomadic Fulani	Fulani	Nominally Muslim	Dispersed, small units	Herding cattle
Semi-settled	Hausa + Fulani	Muslim	Compact	Cultivation + Herding
Settled Fulani	Hausa	Muslim	Compact	Cultivation

TABLE 2b

A comparison of the sex and tribe of the two groups of children admitted to the study

		Protected	Controls
NUMBERS	Total	199	186
	1 year follow-up	90	82
	2 year follow-up	109	104
SEX	Male	101	87
	Female	98	99
TRIBE	Hausa	155	135
	Maguzawa	19	22
	Settled Fulani	10	15
	Nomadic Fulani	15	14

TABLE 2c

The outcome of children admitted to the study

GROUP	OUTCOME				
One year follow-up:	Completed	Died	Refused	Moved	Total
Chloroquine group	41	12	14	23	90
Unprotected group	32	16	14	20	82
TOTAL	73	28	28	43	172
Two year follow-up:					
Chloroquine group	53	9	24	23	109
Unprotected group	49	7	20	28	104
TOTAL	102	16	44	51	213
Combined follow-up:					
Chloroquine group	94	21	38	46	199
Unprotected group	81	23	34	48	186
TOTAL	175(45%)	44(11%)	72(19%)	94(25%)	385(100%)

TABLE 2d

Immunisation schedule employed in the study

Age	Immunisation
4 months	1st injection of triple vaccine, (diphtheria, tetanus and pertussis vaccines)
	1st dose of oral poliomyelitis
5 months	2nd injection of triple vaccine
	2nd dose of oral poliomyelitis vaccine
6 months	3rd injection of triple vaccine
	3rd dose of oral poliomyelitis vaccine
7 months	Measles immunisation
1 year or 2 years	BCG vaccine if mantoux test negative
	Meningococcal vaccine Group A + Group C
	Salmonella typhi vaccine

TABLE 2e

Composition and sources of the vaccines employed in the study

	Vaccine	Dose	Route of administration	Composition per dose	Suppliers
1.	Trivax	0.5 ml x 3	IM	Diph. toxoid 25 lf Tet. toxoid 5 lf B. pertussis $2x10^{10}$	Burroughs Wellcome Laboratories, UK
2.	Polio	0.2 ml x 3	Oral	Type 1 virus $1x10^{6}$ Type 2 virus $1x10^{5}$ Type 3 virus $3x10^{5}$	Connaught Laboratories, Canada
3.	Measles	0.5 ml	IM	Live attenuated* Schwarz strain	Duncan Flockhart, UK or Institut Mérieux, France
4.	Meningococcal	0.5 ml	SC	50 µg group A polysaccharide + 50 µg group C polysaccharide	Institut Mérieux, France
5.	Typhoid	0.5 ml	IM	Heat killed S. typhi $5x10^{8}$ organisms	Burroughs Wellcome Laboratories, UK
6.	BCG	0.1 ml	ID	Dried Tub/vac/BCG Standardised potency	Glaxo Laboratories, UK

(IM = intramuscular; SC = subcutaneous; ID = intradermal)

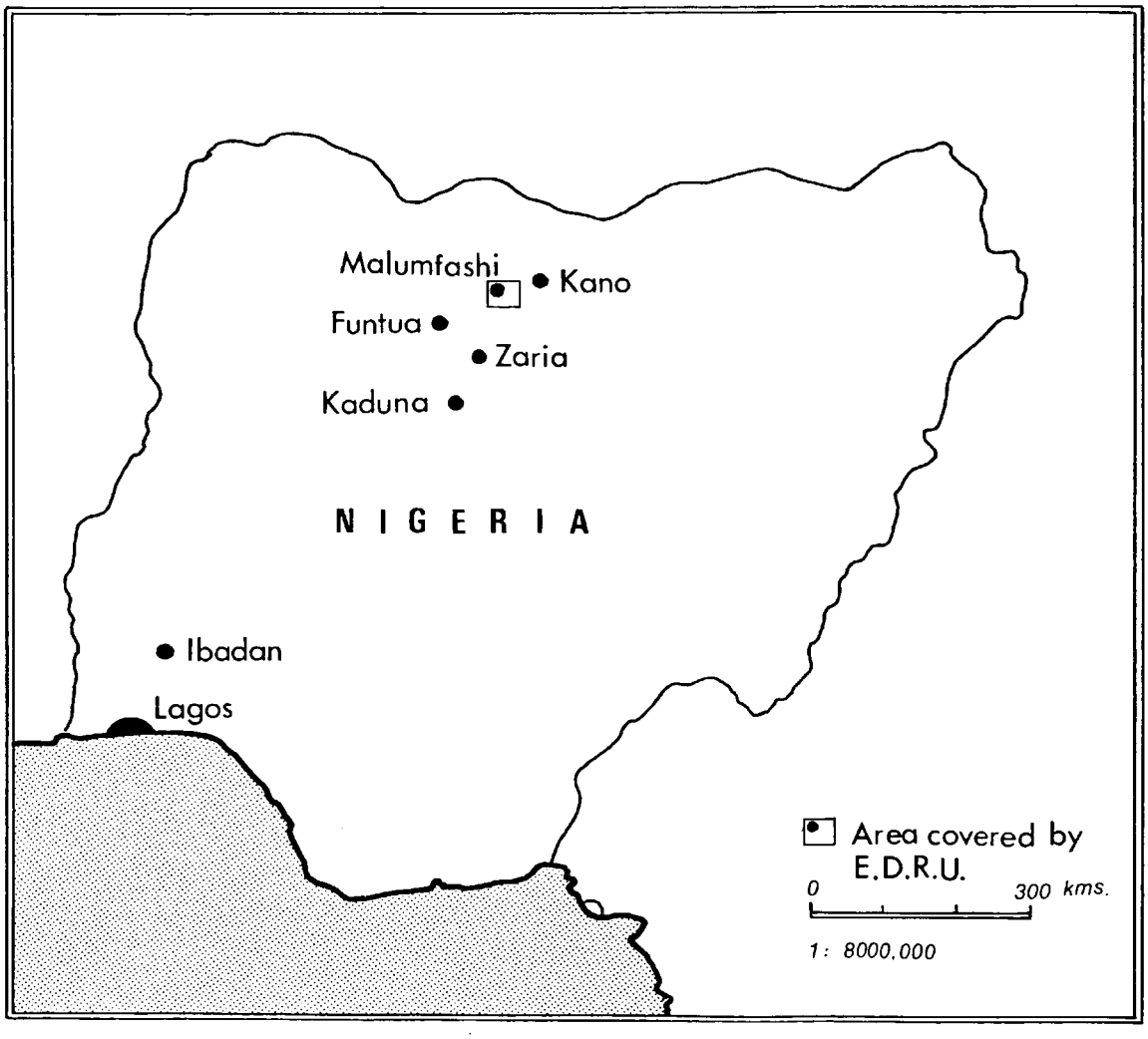
*Potency greater than 1,000 $TCID_{50}$ on assessment by Dr H.C. Whittle

TABLE 2f

The schedule of blood sampling for antibody measurements

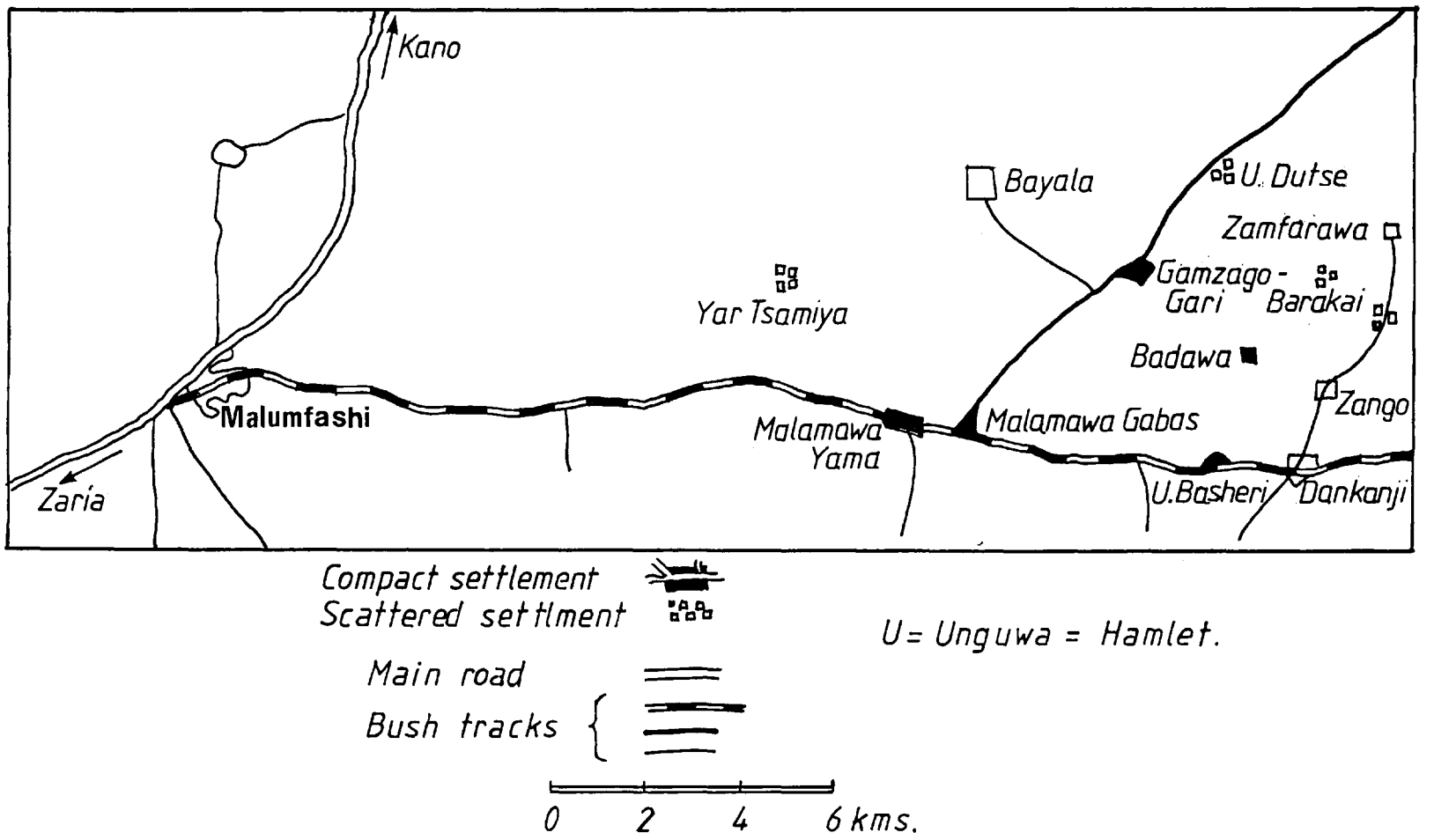
Age in months	Blood sample	Antibody measurements
4	1	Pre-vaccination diphtheria, tetanus and polio
		Malaria
7	2	Post-vaccination diphtheria, tetanus and polio
		Pre-vaccination measles
8	3	Post-vaccination measles
		Malaria
12 or 24	4	Pre-vaccination typhoid and meningococcal
13 or 25	5	Post-vaccination typhoid and meningococcal
		Final diphtheria, tetanus measles and polio
		Malaria

Note: Coulter counter samples for routine haematological investigations and thick blood films for malaria parasite determination were obtained at each sampling.



Map of Nigeria showing area of project.

THE STUDY AREA





Field team distributing chloroquine and placebo on their weekly visit to the village



Immunisation session with capillary blood sampling in the village



One of the study babies with her Malaria Project cards



The malaria study team. 1978



Weighing the Project babies in the field



Measuring the Project babies in the field

CHAPTER 3 - ASSESSMENT OF MALARIA PROPHYLAXIS

I. INTRODUCTION

The aim of this study was to determine whether the regular administration of malaria chemoprophylaxis to young children improved their immune response to vaccination. An essential component of the study was, therefore, to determine whether the scheme of prophylaxis used, weekly administration of chloroquine, was effective in preventing malaria in the study children. Both direct measurements of the effect of chemoprophylaxis on malaria parasitaemia and indirect measurements of its effect on mortality and morbidity were made. The results of these investigations are presented in this chapter.

2. COMPLIANCE

The number of occasions on which the administration of chloroquine or placebo was noted on a child's record card was determined. A high level of compliance was recorded. For children followed for 1 year, administration of chloroquine or placebo was noted on an average of 95% of occasions on which one or other drug should have been given (range 87%-100%). For children followed for 2 years the equivalent figure was 94% (range 80%-100%).

In order to check the validity of field workers' records of chloroquine administration a number of random urine samples from babies in both groups were tested for chloroquine by a colorimetric method (Haskins, 1958). Positive tests were obtained with urine from children receiving chloroquine but also with urine obtained from control children and with urine from healthy adult Nigerians who had not been taking prophylaxis. Therefore, it was considered that this assay was an unreliable means of determining chloroquine excretion in Nigerians and this approach to the measurement of drug compliance was not pursued any further.

3. MORTALITY

All deaths among study children during the period of chemoprophylaxis

and during a further six month period after chemoprophylaxis was stopped were investigated. Twenty-one of the 199 children receiving chemoprophylaxis died during the study period (10.6%), a figure comparable to the 23 deaths (12.4%) recorded among 186 children in the control group ($P > 0.5$, log rank test). Figure 3a shows survival by age in the two groups of children. Only one further death occurred among 93 children followed for a further six months after they had stopped chemoprophylaxis at the age of 1 year or 2 years.

It was difficult to determine the cause of the death of many babies, even when they had been seen during their final illness. Table 3a shows the likely causes of death among babies in the two groups. Malaria was accepted as the probable cause of death when malaria parasites were found in a blood film obtained within 24 hours of the death of a child who had an illness clinically suggestive of malaria. Five such deaths occurred among control children but only one among children in the protected group ($P = 0.1$; Fisher's exact test). Malnutrition played a major part in the death of 6 unprotected children but only 1 child taking chemoprophylaxis was severely malnourished at the time of his death ($P = 0.06$; Fisher's exact test). One patient died from measles just before he was due to be vaccinated and another baby probably died from poliomyelitis. Other likely causes of death are shown in Table 3a. Terminal diagnoses in individual patients are listed in Table 3b.

4. MORBIDITY

A record was made of all attendances of project babies at the routine clinics held in study villages by dispensers or by doctors associated with the project (Table 3c). Significantly fewer protected children than control children attended the clinic with proven malaria or with an illness clinically suggestive of malaria that was not investigated further ($P < 0.001$ in each case). There was no difference in clinic

attendance with diarrhoea between babies in the two groups (143 attendances for protected babies and 131 attendances for control babies).

Protected babies were slightly heavier than control babies and they had higher PCV and Hb levels (see Chapters 5 and 6 respectively).

5. PARASITAEMIA

In addition to the blood films made when a child was seen with an illness clinically suggestive of malaria thick and thin blood films were prepared whenever a fingerprick blood sample was collected from a child for antibody determinations. On average each child had 4 routine blood films examined. Routine blood films obtained from unprotected children were positive for sexual and asexual forms of P. falciparum and for asexual forms of P. malariae significantly more frequently than films obtained from children taking chloroquine (Table 3d) ($P < 0.001$ in each case). However, when P. falciparum infection occurred in protected children heavy infections, as judged by the proportion of films containing 10 parasites or more per high power field, were recorded as frequently as in control children (52% compared with 54%). Similarly the number of positive films with parasites in each group of 200 high power fields was almost identical in the two groups (68% and 71% respectively). The prevalence of P. falciparum asexual parasitaemia increased progressively with age among children in both groups (Fig. 3b). Similarly the prevalence of P. falciparum gametocytes and P. malariae asexual forms increased with age. No sex difference in the prevalence of malaria parasitaemia was observed.

The influence of season on the prevalence of malaria in children in both groups was determined. During the wet season, as defined in Chapter 2, blood films from unprotected children were positive for P. falciparum asexual forms significantly more frequently than during the dry season (54% compared with 39%; $P < 0.001$). The intensity of infection, as shown by the number of positive films with greater than 10

parasites per high power field and the number of films with parasites in each of 200 high power fields, was also significantly higher among control children during the wet season than during the dry season (65% compared with 41%, and 78% compared with 63% respectively; $P < 0.01$ for each comparison). Among protected children parasitaemia was also more frequent during the wet season than during the dry season (13% compared with 7%; $P < 0.01$) but no significant seasonal variation in the intensity of infection was found. No seasonal variation in the proportion of blood films positive for P. falciparum gametocytes was observed among protected or among control children. By chance blood films were obtained during the wet season from 1-2 year old children significantly more frequently than from children under the age of 1 year. As parasitaemia increased progressively with age (Fig. 3b) this age inbalance may have been responsible for the seasonal changes in the prevalence of malaria that were observed.

6. SEROLOGICAL RESPONSE TO MALARIA

Both non-specific and specific immune responses to malaria were investigated in protected and in control children.

a. Immunoglobulins

The mean IgG level of children who had received malaria chemoprophylaxis for 1 or 2 years was significantly lower than the mean IgG level of control children (Table 3e). However, the mean IgM level (154 ± 81 IU/ml) of 75 protected children was very similar to the mean IgM level (158 ± 70 IU/ml) observed in 72 control children.

b. Malaria antibody levels

The antibody response of protected and of control children to malaria, as assessed by the ELISA technique, is shown in Figures 3c and 3d. Positive tests for malaria antibody (absorbance 0.2 or greater) were found significantly more frequently among control children than among protected children at all ages (Fig. 3c) but by the age of 2 years the

gap between the 2 groups had narrowed. Mean antibody titres are shown in Figure 3d. At the age of 2 years there was no longer any significant difference between the mean antibody level of protected children and the mean antibody level of control children.

In order to confirm the results obtained by ELISA randomly selected sera collected at the age of 1 or 2 years were tested for malaria antibodies by the immunoprecipitin technique and by immunofluorescence. The results of precipitin tests against an antigen prepared from a placenta infected with P. falciparum are shown in Table 3f. These are similar to the results obtained by ELISA. At the age of 1 year sera from most control children gave a positive precipitin reaction (91%) whilst only one half of sera (48%) obtained from protected children were positive ($P = 0.02$). However, by the age of 2 years nearly all sera contained precipitins and there was no longer a significant difference between the two groups. The mean number of precipitin lines given by positive sera did not differ between protected and control children.

The results obtained with immunofluorescent tests for malaria antibody are shown in Table 3g and in Figure 3e. In contrast to the results obtained with the ELISA and precipitin tests differences between protected and control children were more marked at the age of 2 years than they were at the age of 1 year. Nevertheless, a significant correlation was found between the results obtained by ELISA and by FAT ($r = 0.61$; $P < 0.001$). A wide range of ELISA results was obtained for sera with an FAT titre in the middle range.

7. DISCUSSION

Three main points for discussion arise from the results presented in this chapter.

a. Control of malaria by chemoprophylaxis

Children who received weekly chemoprophylaxis with chloroquine

experienced significantly fewer episodes of clinical malaria than control children and they had significantly fewer episodes of asymptomatic malaria parasitaemia. However, children who received chemoprophylaxis were not protected completely from malaria for 9% of random blood films were positive for P. falciparum. This had been the experience of others who have used chemoprophylaxis alone to control malaria. Thus, in The Gambia, McGregor et al. (1956) recorded 3 clinical attacks of malaria in 20 infants who received weekly chloroquine and in Uganda Harland et al. (1975) noted episodes of parasitaemia in 31 of 36 children aged 0-3 years who received monthly chemoprophylaxis with pyrimethamine. Lucas et al. (1969) found parasites in 10%-20% of a group of Nigerian schoolchildren who received weekly prophylaxis with pyrimethamine; better results were obtained with a pyrimethamine and sulphonamide combination. When chemoprophylaxis has been used as a means of mass malaria control results have generally been even less satisfactory than those observed in these carefully controlled investigations (Clyde and Shute, 1957; Ricossé et al., 1960; Escudié et al., 1961; Escudié et al., 1962; Cavalie and Mouchet., 1962). However, when on a study at Garki in northern Nigeria, chemoprophylaxis with sulfalene-pyrimethamine was combined with propoxur spraying the prevalence of P. falciparum parasitaemia was kept to less than 2% during the dry season and to less than 5% during the rainy season (Molineaux and Grammicia, 1980).

Three possible reasons for the occurrence of parasitaemia among protected children have been considered. Occasionally, children did not receive their chloroquine because they were away from home or because they could not be found by a field worker. However, chloroquine was apparently given on over 90% of the occasions on which it should have been administered. Although the field workers involved in administration of drugs were diligent and conscientious it would have been surprising if there had not been occasional errors in their record

keeping. Unfortunately, attempts to check on drug compliance by urine testing were unsuccessful because technical difficulties were experienced with the colorimetric assay that was used. Development of an ELISA test that could detect antimalarial drugs in urine simply and reliably, along the lines of the test for urinary dapsone developed by Huikeshoven et al. (1980), would provide a valuable means for assessing compliance in mass malaria chemoprophylaxis programmes.

Vomiting is another possible explanation for the failure of weekly chloroquine to prevent malaria completely in the study children. Vomiting often occurred after chloroquine administration in a proportion of study infants, but the tendency to vomit decreased as these children became older. Although children were watched for 30 minutes after chloroquine administration it is possible that vomiting occasionally occurred shortly afterwards before adequate amounts of chloroquine had been absorbed.

A third unlikely explanation for the occurrence of parasitaemia in children who received chloroquine is that chloroquine resistant strains of P. falciparum were present in the Malumfashi area. This is very unlikely for no treatment failures with chloroquine were observed among project babies or among any of the many patients admitted to Malumfashi Hospital with malaria during the study period (Tomkins, personal communication). In a study undertaken at Malumfashi Hospital in 1978 eradication of parasites was documented in all of 79 children with acute P. falciparum malaria treated with conventional doses of chloroquine (Williamson and Greenwood, 1978).

Thus it is likely that the failure of weekly chemoprophylaxis with chloroquine to control P. falciparum infection completely resulted from occasional failures of drug administration and from the tendency of chloroquine to make young infants vomit. The importance of the latter is suggested by the fact that better malaria control was achieved in a small group of children who received weekly pyrimethamine,

rather than weekly chloroquine, from the same group of field workers (unpublished data).

b. Effect of malaria control on mortality and morbidity

Despite the fact that chloroquine chemoprophylaxis achieved a considerable degree of malaria control mortality among protected and among control children was similar. The number of deaths during the first year of life was 80 per 1,000 among protected children and 106 per 1,000 among control children (combined value 93 per 1,000). These values are not true infant mortality rates as deaths during the first week of life were not recorded. On the basis of the data obtained by Bradley in the Malumfashi area during 1977-1978 (Bradley, 1980) it is likely that the infant mortality rate among the study children was of the order of 130 per 1,000. The figure falls between the values of 80 per 1,000 obtained by Bradley for the Malumfashi area as a whole using data collected by registration and the value of 200 per 1,000 obtained using the retrospective enumeration technique. Determination of the cause of death in individual children was often impossible. Deaths which were probably due to malaria were less frequent in protected children than in control children and so were deaths from malnutrition. In neither instance was the difference between the two groups statistically significant although in each case the trend suggested that chemoprophylaxis might have had a positive effect.

Several studies in Africa have shown that deaths from malaria can be reduced by control of malaria with insecticides (Kouznetsov, 1977). In the Pare region of Tanzania deaths among children were reduced by household spraying with dieldrin (Draper and Smith, 1960) and similar results were obtained at Kisumu in Kenya (Fontaine et al., 1978). Whether malaria control by chemoprophylaxis alone reduces childhood mortality is much less certain. At Garki some reduction in the infant mortality rate was observed in treated villages compared with control villages (102 per 1,000 compared with 192 per 1,000) but this analysis

was based on only a small number of deaths (20) and insecticide was used in addition to chemoprophylaxis in this programme. Most studies of chemoprophylaxis have concentrated upon the parasitological and entomological consequences of intervention and little attention has been paid to the effects of chemoprophylaxis on the health of the recipients. I have been unable to find any study which shows convincingly that malaria chemoprophylaxis given alone reduces mortality in young children (Table 3h).

The effect of chemoprophylaxis on morbidity from malaria has been discussed already. Diarrhoea was not reduced in protected children suggesting that malaria is an infrequent cause of this condition in the Malumfashi region. The effects of chemoprophylaxis on nutrition and on haematological measurements are discussed in Chapters 5 and 6 respectively.

c. Rebound malaria

It has been suggested that malaria control schemes which employ chemoprophylaxis could be more harmful than beneficial because severe, and possibly fatal, malaria might occur in protected subjects once chemoprophylaxis was stopped. These fears stem from the observation that attacks of malaria with higher than normal parasitaemia may occur after only a short period of prophylaxis (Pringle and Avery-Jones, 1966) and from the observation that malaria antibody formation is diminished in protected infants (Voller and Wilson, 1964; Harland et al., 1975; Molineaux and Gramiccia, 1980). However, one year after the termination of a successful control scheme in Tanzania an increase in the number of reported cases of malaria was observed but there was no increase in malaria mortality (Pringle 1967). Similarly at Garki no obvious increase in mortality was observed on the completion of intervention, although this problem was not specifically investigated in this study. Nevertheless, a careful watch was kept on the children in the Malumfashi study for six months after the cessation of prophylaxis

at the age of 1 year or 2 years. (For most of these children this six month period included part of a rainy season.) Only one death, for which no diagnosis could be made, occurred during this period among 93 children and there was no obvious increase in the number of severe febrile episodes that they experienced.

The reason for the lack of severe malaria among the study children after they had stopped taking chloroquine probably lies in the fact that prophylaxis was not totally effective and that this allowed some natural immunisation to occur. This view is borne out by the results of ELISA and immunoprecipitin malaria antibody assays which showed narrowing of the difference in antibody levels between protected and control children by the age of 2 years. Very low antibody levels were found in children receiving prophylaxis in The Gambia (Voller and Wilson, 1964) and at Garki (Molineaux and Grammiccia, 1980) but these children were only followed up to the age of 8 months and 15 months respectively. It is essential that more detailed information is obtained about the effects of chemoprophylaxis on the development of natural malaria immunity before malaria chemoprophylaxis is used on a mass scale in Africa.

TABLE 3a

The suspected cause of death among 199 protected children and among 186 control children.

Cause of death[a]	Protected children	Control children
Malaria	1	5
Fever (probably not malaria)[b]	6	7
Malnutrition	1	6
Anaemia	1	1
Haemoglobinopathy	1	2
Chest infection	2	1
Measles	1	0
Poliomyelitis	0	1
Other, or unknown	12	5

Notes: a. More than one serious condition was present at the time of death in some children and in those cases both diagnoses have been recorded.

b. Blood films negative for malaria or not taken.

TABLE 3b

Likely causes of death in individual protected and control children. In some cases no retrospective diagnosis could be made.

PROTECTED GROUP				UNPROTECTED GROUP			
Number	Age Months	Sex	Cause	Number	Age Months	Sex	Cause
541	1	M	Fever	281	2	F	?
131	1	M	?	132	2	F	?
204	2	M	?	206	3	M	?
205	2	M	?	611	4	M	Sickle cell disease, malaria
226	2	M	?	528	5	M	Fever
242	3	F	Chest infection	182	7	M	Fever
21	4	M	?	22	7	F	Malaria
292	4	M	Multiple abscesses	238	7	M	Malnutrition
613	7	F	Sickle cell disease, fever	166	9	M	?
179	7	F	Measles	130	9	M	Malaria, malnutrition
181	8	M	?	413	9	M	Malaria (twin)
147	9	F	Fever, fits	142	9	F	Fever

TABLE 3b cont.

PROTECTED GROUP				UNPROTECTED GROUP			
Number	Age Months	Sex	Cause	Number	Age Months	Sex	Cause
222	13	F	Malaria	144	10	M	? SS disease
305	13	F	?	180	10	F	Fever
80	15	M	Fever, vomiting	535	10	M	Downs syndrome, chest infection
139	17	M	Pneumonia, anaemia, jaundice	10	11	M	? poliomyelitis
70	20	F	Fever, vomiting	91	12	F	Fever
13	20	M	Marasmus, fever	422	12	M	Anaemia
149	22	M	?	304	18	F	Malaria, malnutrition
92	22	M	?	302	19	M	?
167	24	M	?	20	21	M	Fever, malnutrition
				16	22	M	Malnutrition
				124	24	M	Fever, malnutrition

TABLE 3c

Attendances made at routine clinics by 199 protected children and by 186 control children

	Protected children		Control children	
	Number making a visit	Total number of visits	Number making a visit	Total number of visits
Any illness	*129	398	140	442
Suspected malaria[a]	**15	35	44	104
Proven malaria	**12	15	43	78

Notes: a. Blood films were not made in these patients.

* $P < 0.05$.

** $P < 0.001$.

TABLE 3d

The prevalence of malaria parasitaemia in routine blood films obtained from 199 children receiving chloroquine chemoprophylaxis and in routine blood films obtained from 186 control children.

	Protected children n=711	Control children n=630	Significance
P. falciparum asexual	63 (8.9%)	260 (41.3%)	$P < 0.001$
P. falciparum gametocytes	28 (3.9%)	108 (17.1%)	$P < 0.001$
P. malariae asexual	3 (0.4%)	26 (4.1%)	$P < 0.001$
P. malariae gametocytes	0	1	NS

TABLE 3e

Immunoglobulin G levels (mean ± 1 standard deviation in IU/ml) in children receiving malaria chemoprophylaxis for 1 year or 2 years and in control children of the same age. Figures in brackets indicate the number of observations made.

	Protected children	Control children	Significance
1 year	202 ± 62 (36)	230 ± 60 (27)	$0.05 < P < 0.1$
2 years	223 ± 67 (39)	288 ± 84 (43)	$P < 0.001$

TABLE 3f

Precipitin lines obtained with sera from protected and control children when tested against an antigen prepared from a P. falciparum infected placenta.

Group	Protected children		Control children	
	Number positive	Mean number of lines ± 1 standard deviation for positive sera	Number positive	Mean number of lines ± 1 standard deviation for positive sera
1 year	*10/21 (48%)	1.50 ± 0.53	10/11 (91%)	1.50 ± 0.58
2 years	18/23 (78%)	1.67 ± 0.59	20/22 (91%)	1.40 ± 0.50

* P = 0.02

TABLE 3g

Fluorescent malaria antibody levels in protected and control children at the age of 1 year or 2 years. Results are expressed as the reciprocal of the mean titre ± 1 standard deviation. Figures in brackets indicate the number of observations made.

	Protected children	Control children
1 year	166 ± 205 (10)	263 ± 93 (8)
2 years	*256 ± 382 (10)	672 ± 511 (15)

* $P < 0.05$

TABLE 3h

Some studies of malaria chemoprophylaxis

Country	Drug	Age Group	Parasitaemia	Mortality Morbidity	Nutrition Haematology
Ghana[1] 1955	Pyrimethamine weekly in term time	7 yrs	Cleared (a few episodes after Easter break)	Spleen rate ↓ Absentees at school ↓ No deaths	No weight change
The Gambia[2] 1956	Chloroquine weekly	0-3 yrs	Occasionally in protected	Spleen rate ↓ Hepatomegaly ↓ Y-globulin ↓ 1 death in protected 5 deaths in controls	Small weight rise Hb ≏ 2g% ↑
Nigeria[3] 1956	Pyrimethamine weekly in term time	5-9 yrs	Occasionally in protected	Spleen rate ↓ School absentees ↓	Weight ↑ Hb, WBC →
Nigeria[4] 1969	Pyrimethamine + Sulphormethoxine ± Dapsone weekly	7-8 yrs	< 10% Pyrimethamine < 5% mixture < 5% mixture	Spleen rate ↓	Weight → Fewer anaemic children
Uganda[5] 1975	Pyrimethamine monthly	0-3 yrs	Frequent parasitaemia	Malaria antibody ↓ Clinical malaria in protected	

TABLE 3h cont.

Country	Drug	Age Group	Parasitaemia	Mortality Morbidity	Nutrition Haematology
Senegal[6] 1975	Chloroquine weekly	0-5 yrs	< 10% in protected	Spleen rate ↓	PCV ↑ 11% over controls
Ghana[7] 1979	Pyrimethamine monthly	0-10 yrs	↓ from 40% to 30%	Spleen rate ↓ Mortality possibly reduced	
Liberia[8] 1980	Pyrimethamine or Chlorproguanil monthly	2-9 yrs	Initially ↓ Subsequently ↑ for pyrimethamine	Spleen rate ↓	PCV ↑

Response normal → ; Response depressed ↓ ; Response increased ↑ .

1. Colbourne (1955)
2. McGregor et al. (1956)
3. Archibald and Bruce-Chwatt (1956)
4. Lucas et al. (1969)
5. Harland et al. (1975)
6. Diallo et al. (1975)
7. Danfa Project (1979)
8. Björkman et al. (1980)

Fig. 3a. Figure showing percentage of children surviving at each month of age in the protected and in the control groups

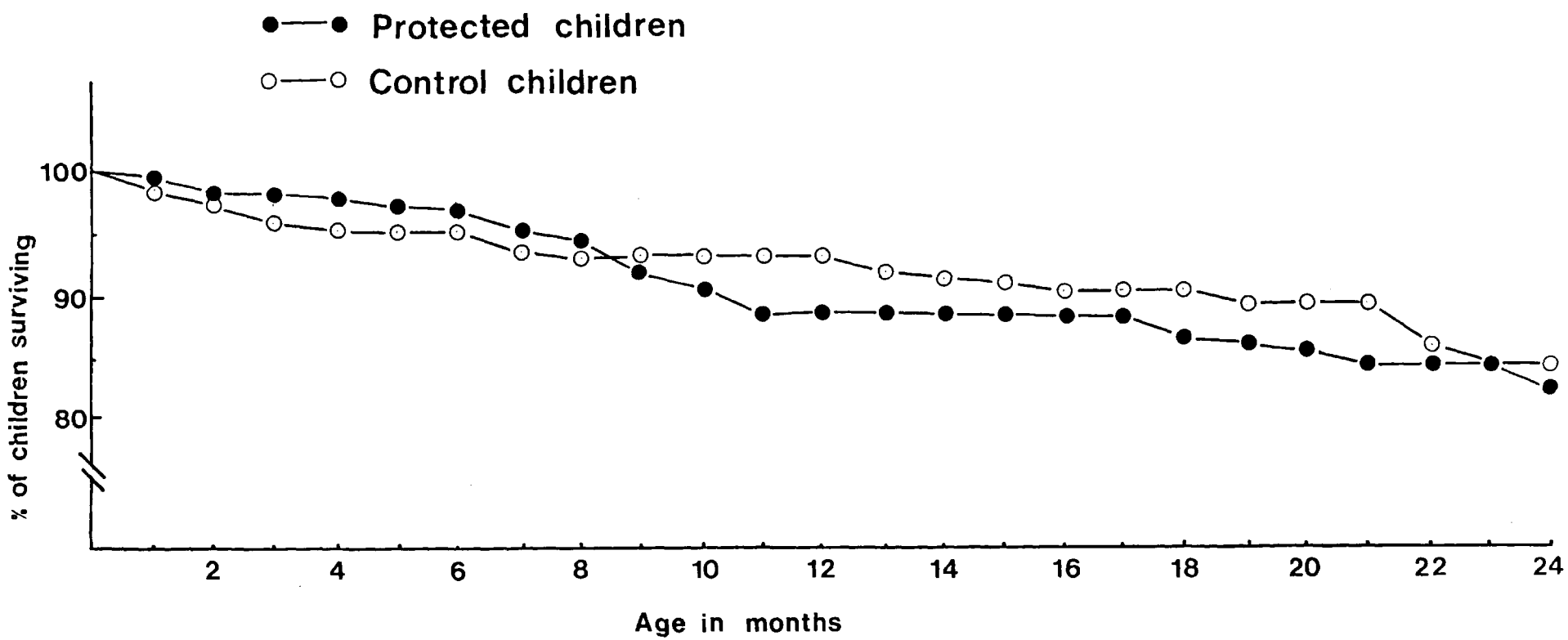


Fig.3b. The percentage of children with parasitaemia at different ages in the protected group and the control group.

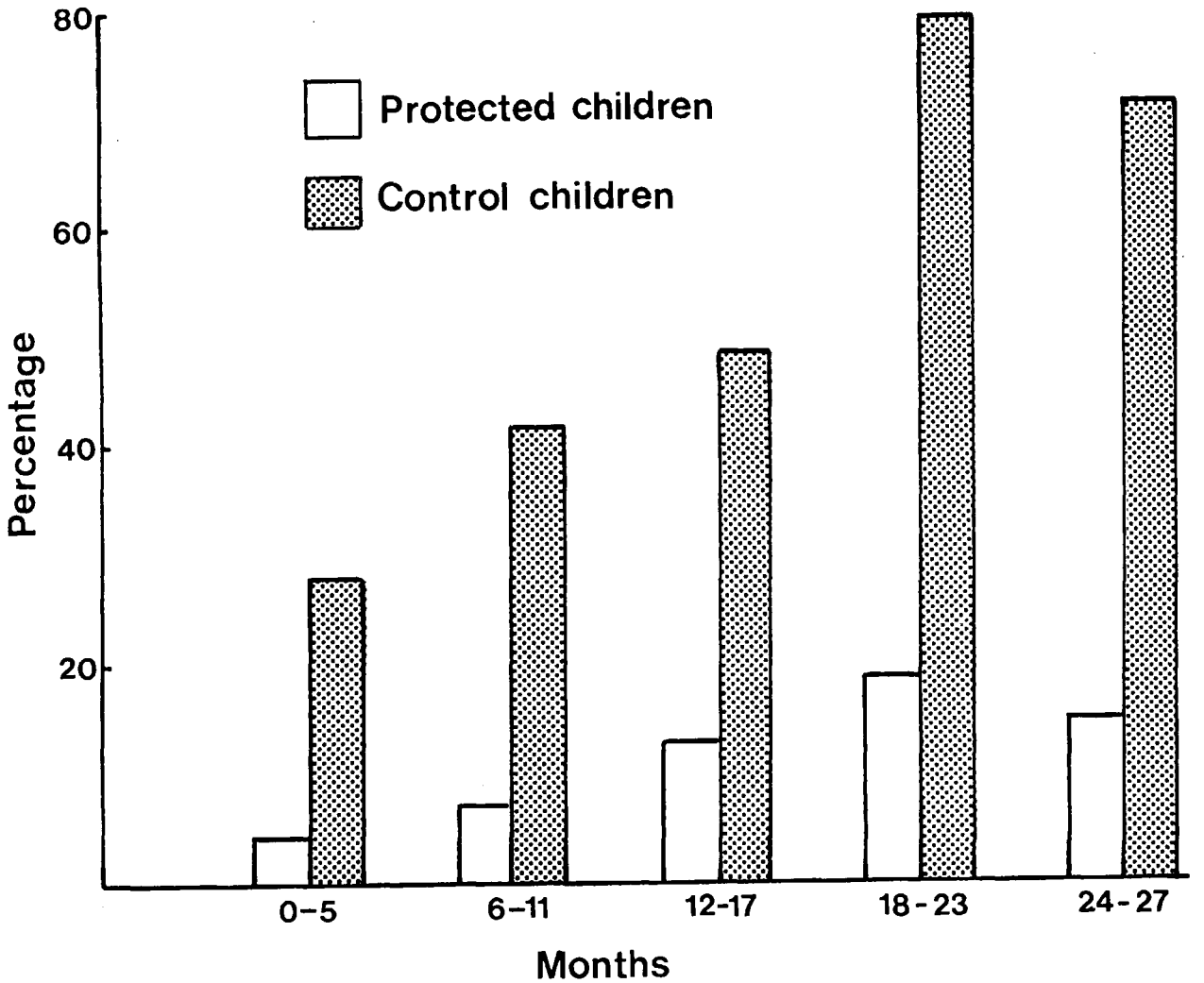


Fig.3c. Figure showing the percentage of children with malaria antibodies detected by the ELISA technique at different ages in the protected and control group of children.

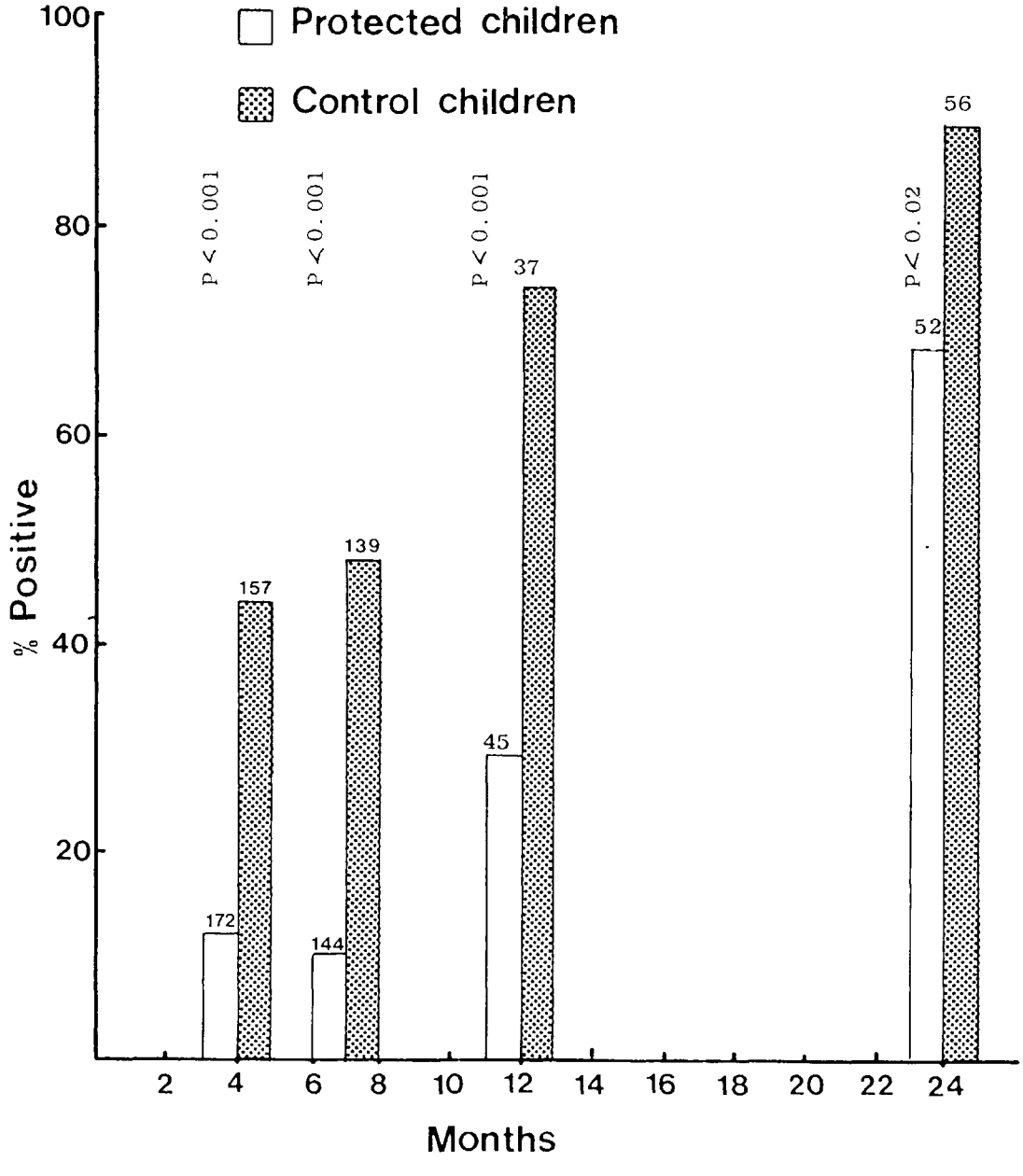


Fig.3a. Figure showing the relationship between THE titre of malaria antibodies measured by the ELISA absorbance and the age of the children in the protected group and the control group.

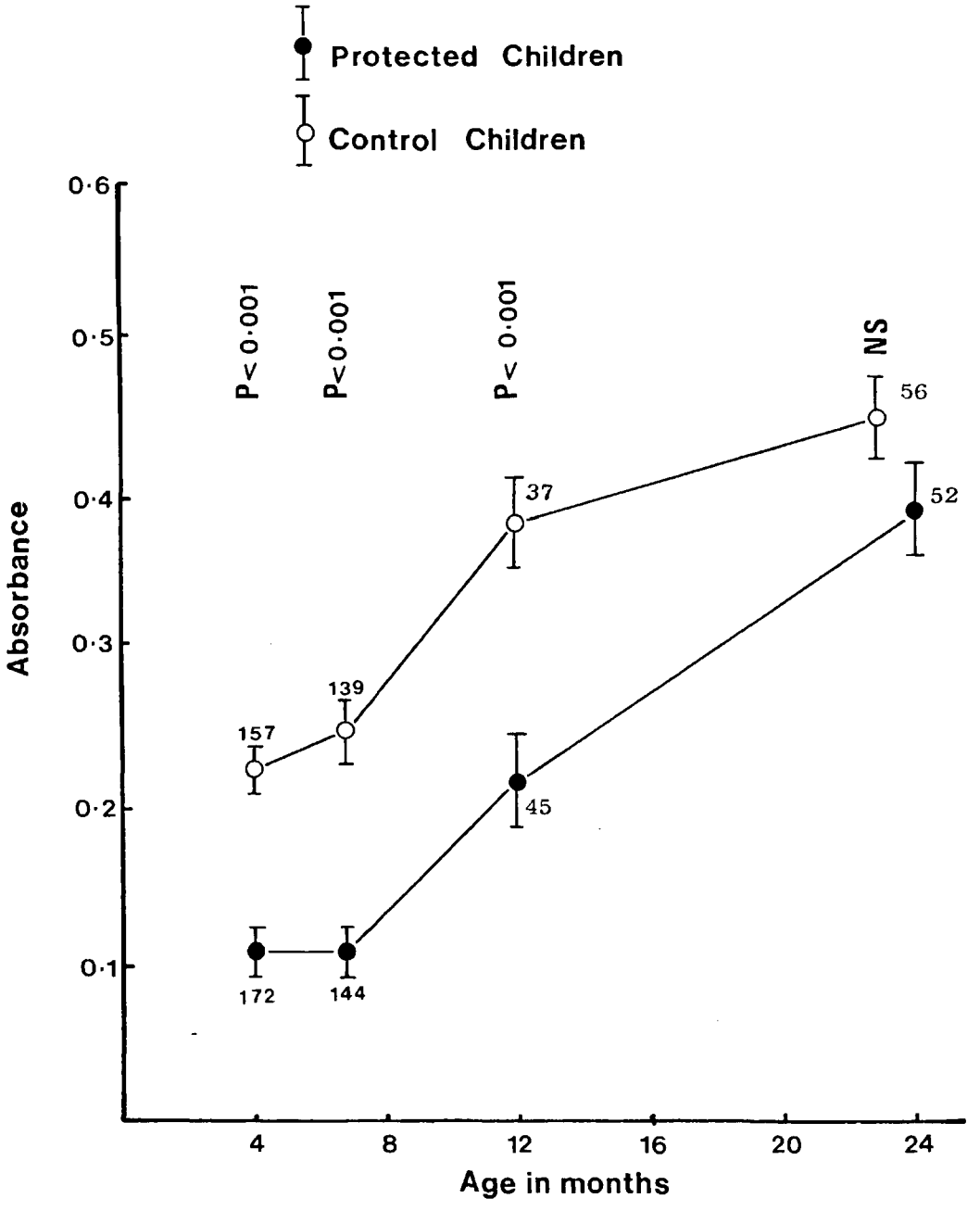
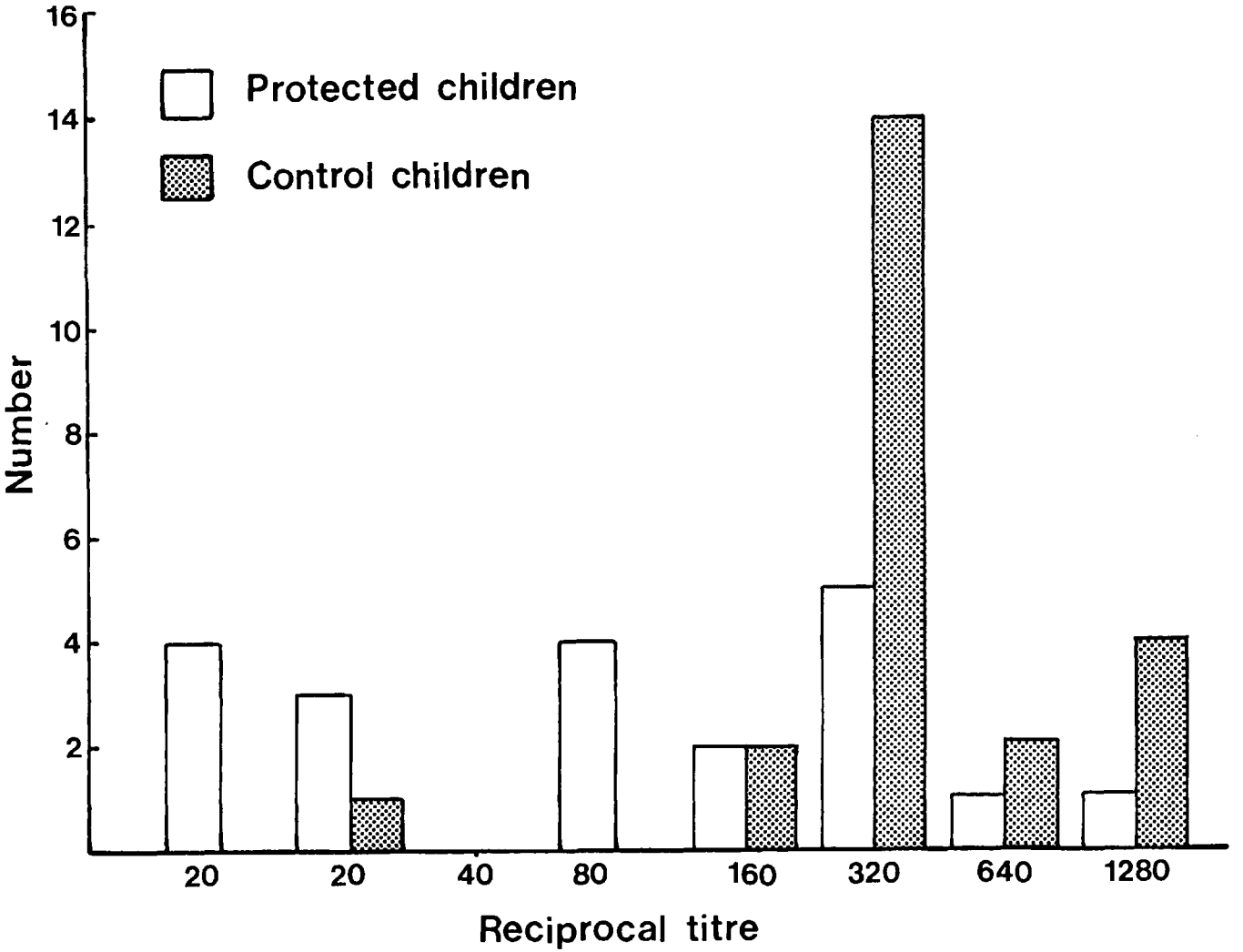


Fig.3e. Comparison of the immunofluorescent malaria antibodies in the two groups of children.



CHAPTER 4 - THE EFFECT OF MALARIA ON THE IMMUNE RESPONSE TO VACCINATIONS

I. INTRODUCTION

A small number of studies, reviewed in chapter I, have shown that the immune response to some vaccines is diminished when they are given to a child with acute clinical malaria. However, acute malaria is an uncommon event even in areas where the infection is highly endemic and where one half of the population has asymptomatic malaria parasitaemia at any one time. Whether asymptomatic malaria parasitaemia has any effect on the immune response to vaccination is not known. Therefore, the immune response to vaccination of children who received regular malaria chemoprophylaxis has been compared with the immune response of age-matched children exposed frequently to malaria.

2. RESULTS

2.1 Overall antibody response

The overall antibody response of children who received malaria chemoprophylaxis and of control children to immunisation with a series of standard vaccines is shown in Tables 4a - 4e. Table 4a shows the mean antibody levels found before and one month after vaccination. Table 4b shows the number of children with antibody at a level likely to be associated with clinical protection 1 month after immunisation. Tables 4c and 4d indicate the rise of antibody level observed one month after immunisation in all protected and control children and in protected and control children without antibody at the time of vaccination. Table 4e indicates the seroconversion rate in the latter group of children. No significant difference in the antibody response to diptheria toxoid, tetanus toxoid, measles vaccine or *S. typhi* vaccine was found between protected and control children, regardless of the method by which the antibody data was analysed. However, the immune response to meningococcal group A vaccine and to meningococcal group C vaccine was higher in protected children than in control children by all methods of analysis and for several analyses the difference between protected and control children was statistically

significant.

An analysis of the antibody response to vaccination in relation to the presence or absence of malaria parasitaemia at the time of vaccination is shown in Table 4f. The mean rise in diphtheria, tetanus ELISA, group A meningococcal and group C meningococcal antibody levels following vaccination was lower in children with parasitaemia than in those without parasitaemia but the difference between the two groups was statistically significant only for tetanus (E) antibody ($P < 0.01$). The results obtained in protected and in control children with and without parasitaemia were analysed separately. Differences between the antibody response of children with or without parasitaemia were more marked among control children than among protected children and were statistically significant for diphtheria and tetanus ELISA antibodies ($P < 0.05$ and $P < 0.01$ respectively). The mean rise in group A and group C meningococcal antibody levels among control children with parasitaemia was only about one half of the values recorded in children without parasitaemia (0.59 ± 1.69 compared with 1.32 ± 1.04 for group A meningococcal antibody and 0.86 ± 1.66 compared with 1.23 ± 1.54 for group C meningococcal antibody but, because of large standard deviations, these differences are not statistically significant.

Because possession of the haemoglobin genotype AS protects against severe malaria the immune response to vaccination was assessed separately in children with the haemoglobin genotype AS and in those with the genotype AA. As a comparable picture was obtained in protected and in control children the results from the two groups are presented together in Table 4g. The mean rise in antibody level following vaccination was similar in AA and AS children for all antibodies except for meningococcal antibodies. Children with the haemoglobin genotype AS showed a greater rise in titre of group A and group C meningococcal antibodies than children with genotype AA but large standard deviations

were recorded and these differences were not statistically significant.

The effect of season on the immune response to vaccination was investigated; no significant differences in antibody response were found regardless of whether vaccination was given during the wet season or during the dry season.

In order to determine whether malaria prophylaxis might delay the decline in antibody level from the peak reached shortly after immunisation tetanus, diphtheria and measles antibody levels were measured in protected and in control children 18 months after immunisation. The results are shown in Table 4h. Antibody levels were slightly higher in protected than in control children 18 months after vaccination and for tetanus ELISA antibody this difference was statistically significant ($P<0.05$).

2.2 Antibody response to individual vaccines

a. Triple vaccine

An excellent antibody response to the diphtheria and tetanus toxoid components of triple vaccine was observed in both control and in protected children. One month after vaccination nearly all children had antibody levels high enough to be associated with protective immunity. Tetanus ELISA antibody levels declined more rapidly than haemagglutinating antibody levels in both protected and in control children (Table 4h); this decline was significantly more rapid in control children than in protected children ($P<0.05$).

Antibodies to *B. pertussis*, the third component of triple vaccine, were not measured because of shortage of serum. No cases of whooping cough were detected among protected or control children following immunisation. No serious side-effects were noted following immunisation with triple vaccine.

b. Poliomyelitis

Attempts to develop an ELISA test to measure poliomyelitis antibodies

were unsuccessful so that a conventional virus neutralisation test had to be used to measure the immune response to this vaccine. As measurement of antibody to each of 3 polio viruses required at least 25 µl of serum these measurements could be made on only a limited number of samples. The results of poliomyelitis antibody determinations have, therefore, not been included in Tables 4a - 4g and they are presented separately in Tables 4i and 4j and in figure 4a. A number of children possessed poliomyelitis antibody before vaccination at the age of 3 months as a result of passive immunisation or as a consequence of early natural infection. Following immunisation only a poor seroconversion rate was obtained in both protected and in control children (Table 4j). Eighteen months after vaccination a number of children who had not had antibody 1 month after vaccination had become seropositive (Fig. 4a).

One month after immunisation the mean antibody level to type I poliomyelitis virus was significantly higher in control children than in protected children. However the high mean level observed in control children was due, in a large part, to the inclusion of 2 sera which had very high antibody levels. When logarithmically transformed data was analysed the differences between control and protected children was no longer statistically significant. In contrast to the results obtained with measurements of tetanus, diphtheria and measles antibodies, antibody levels to type I and to type 3 poliomyelitis virus continued to rise during the 18 months following vaccination.

One patient developed clinical poliomyelitis 2 weeks after he had received his first dose of oral poliomyelitis vaccine and he subsequently died. No other cases of clinical poliomyelitis were identified.

c. Measles

Fifty protected children (33.5%) and 39 control children (27.2%) had detectable measles antibody when they were immunised at the age of 7

months. Antibody levels were low in most of these children suggesting that the antibody had been acquired by passive immunisation from the mother. However, 8 protected children (5.4%) and 10 control children (7.0%) had an antibody titre of $\log_2$ 8 or greater at the time of immunisation suggesting that they had already had an attack of measles, although this had been noted clinically in only 6 of these children, one of whom died. No clinical cases of measles were detected after immunisation among protected or among control children although measles was endemic in study villages during the follow-up period. Overall an excellent immune response to measles vaccination was observed in both protected and in control children; a 99% seroconversion rate was recorded in children without antibody at the time of vaccination and nearly all children had a raised antibody level one month after immunisation. The initial antibody level had an important influence on the immune response to measles vaccine (Fig. 4b) for children with a high pre-vaccination antibody titre showed little rise in titre following immunisation.

d. Meningococcal antibodies

Before vaccination the mean group A meningococcal antibody level was higher than the mean group C meningococcal antibody level. The mean pre-vaccination levels of both antibodies were higher in 2 year old children than in 1 year old children indicating natural immunisation with meningococci during the second year of life (Tables 4k & 4l). The mean pre-vaccination group A meningococcal antibody level of children aged 1 year or 2 years combined was significantly higher in protected than in control children ($P < 0.05$) (Table 4a) suggesting that natural immunisation had been more effective in children receiving chemoprophylaxis than in controls.

Only a modest increase in antibody level followed immunisation with a combined group A + group C meningococcal polysaccharide vaccine. The response was significantly better in protected than in control children

(Tables 4k & 4l). The beneficial effect of chemoprophylaxis was observed both in children vaccinated at the age of 1 year and in those vaccinated at the age of 2 years.

A close correlation was found between the rise in antibody level following vaccination and the pre-vaccination antibody level (Fig. 4c).

e. S. typhi vaccine

High pre-vaccination antibody levels to the O antigen of S. typhi were found in both protected and in control children. These increased only slightly after vaccination.

2.3 Cellular immunity

a. Natural immunisation with C. albicans and M. tuberculosis

The response of protected and of control children to natural immunisation with C. albicans and mycobacteria was assessed by skin testing with an extract of C. albicans and with PPD (5 units of tuberculin) at the age of 1 year or 2 years. Table 4m shows the results obtained. Many candida skin tests were positive; no significant difference was observed between protected and control children. A positive Mantoux test was obtained in 18% of children aged 1 year and in 20% of children aged 2 years; once again no difference was found between protected and control children. Figure 4d shows the size of positive candida skin tests obtained in children in the 2 groups; the mean diameter of positive skin tests in 57 protected children was 9.0 $\pm$ 2.7 mm compared with a mean diameter of 9.3 $\pm$ 3.0 mm observed in 63 control children.

b. Response to BCG vaccination

Children who had a negative Mantoux reaction at the age of 1 year or 2 years were immunised with BCG vaccine and retested with PPD 5 weeks later. Table 4n shows the conversion rates obtained. No difference was observed in the response of protected or of control children. The mean diameter of induration observed in 53 protected children who became Mantoux positive after vaccination was 9.3 $\pm$ 3.4 mm, a figure

similar to the mean diameter of 9.6 ± 2.6 mm observed in 52 control children.

3. DISCUSSION

3.1 The overall antibody response to vaccination and the effects of malaria

The effect of malaria prophylaxis on the response of a young child to a schedule of immunisation that he or she might have expected to receive in a routine mother and child clinic in a tropical African country was investigated. Because Malumfashi lies within the African meningitis belt (Lapeyssonnie, 1963), where outbreaks of meningococcal infection are frequent and severe, a combined group A and group C meningococcal vaccine was given at the age of 1 year or 2 years. Vaccination with BCG was delayed until the age of 1 year or 2 years to allow an assessment to be made of the effect of malaria chemoprophylaxis on the development of natural immunity to tuberculosis.

Overall an excellent antibody response to the tetanus and diphtheria components of triple vaccine and to measles vaccine was obtained among both protected and control children. Only a modest rise in antibody level followed immunisation with a combined group A and group C meningococcal polysaccharide vaccine. The antibody response to immunisation with 3 doses of oral poliomyelitis vaccine was poor.

Comparison, by several methods, of the immune response to immunisation of protected children and of control children showed consistent differences between the two groups only for the immune response to meningococcal polysaccharide vaccines. It is possible that other differences might have emerged had better malaria control been achieved among the protected children but it is unlikely that these would have been marked or clinically important. Children with malaria parasitaemia at the time of vaccination had a lower antibody response to immunisation with meningococcal group A and group C polysaccharide

vaccine than children without parasitaemia. Furthermore children with the haemoglobin genotype AS had a higher antibody response to these vaccines than children with the haemoglobin genotype AA, an effect not apparent with any of the other vaccines studied. A wide range of antibody response to meningococcal polysaccharide vaccines was observed and the difference in antibody response between children with the genotype AS and those with the genotype AA was not statistically significant. However, in a previous investigation undertaken in a village 10 miles south of the present study area, a significant difference in the antibody response to group C meningococcal polysaccharide vaccine was found between subjects with the genotype AS and those with the genotype AA (Greenwood et al., 1980). Thus, it appears that asymptomatic malaria parasitaemia does impair the immune response to meningococcal polysaccharide vaccines whilst having no effect on the immune response to triple, measles or poliomyelitis vaccines. It is likely that the considerable antigenic stimulus provided by the administration of 3 injections of adjuvant containing triple vaccine or by the administration of poliomyelitis or measles vaccines containing live virus can overcome any impairment of immune function induced by asymptomatic malaria.

The finding that malaria chemoprophylaxis influences the immune response to meningococcal polysaccharide vaccines but not the response to other vaccines is in agreement with the observation of Williamson and Greenwood (1978) that the immune response of children with acute clinical malaria to immunisation with a meningococcal polysaccharide vaccine was more severely impaired than the immune response to S. typhi vaccine. Why meningococcal vaccine should be especially susceptible to the suppressive effects of malaria is not certain. However, meningococcal (and pneumococcal) polysaccharide vaccines have certain characteristics which differentiate them from more conventional protein vaccines. Polysaccharide vaccines are poorly

immunogenic in the young, they induce little immunological memory and the immune response that they induce is probably independent of the participation of T lymphocytes. Malaria induces both specific and non-specific proliferation of B lymphocytes and it is possible that these changes interfere with the immune response to antigens which are dependent predominantly on the participation of B lymphocytes.

Investigation of antibody levels in protected and in control children 18 months after vaccination showed that for each antibody studied the rate of decline in mean antibody level was less for protected than for control children. However, the differences between the 2 groups were small and statistically significant only for tetanus (E) antibody. It is likely that the more rapid decline in antibody levels observed in children frequently experiencing malaria resulted from the enhanced rate of immunoglobulin turn-over present in subjects with this infection (Cohen et al., 1961). It would be of interest to follow changes in antibody levels over a longer period of prophylaxis.

3.2 The antibody response to individual vaccines

a. Triple vaccine

This study has confirmed that the diphtheria and tetanus toxoid components of triple vaccine are safe and effective vaccines which produce high levels of antibody in nearly all immunised subjects, even when the recipients have been exposed frequently to malaria and other infections. Much more controversy surrounds the use of B. pertussis antigen, the third component of triple vaccine. Stewart (1977) has suggested that this vaccine should no longer be used in the United Kingdom because of doubts about its efficacy and because of its propensity to induce serious side-effects. Stimulated by these criticisms a number of studies have recently been undertaken in the United Kingdom to investigate the efficacy of B. pertussis vaccine (Church, 1979, Department of Health and Social Security, 1981). These have demonstrated conclusively that B. pertussis vaccine is effective in

preventing whooping cough, although the vaccine does not give complete protection. It is now generally agreed that vaccines containing B. pertussis antigens can occasionally cause serious side-effects, including permanent brain damage (Berg, 1958; Ström, 1967; Kulenkamff et al., 1974), but there is some disagreement as to how frequently these serious side-effects occur. A recent, very detailed survey carried out in the United Kingdom indicated that the risk is less than 1:100,000 (Miller et al., 1981). Thus, most impartial observers would agree that the continued use of B. pertussis vaccine in industrialised countries is still justified. The case for the continued use of B. pertussis vaccine in developing countries is even stronger than the case for its use in industrialised countries because in developing countries whooping cough often occurs at an early age when mortality from the infection is high. Thus, in Nigeria Morley et al. (1966) found that nearly all village children had contracted whooping cough by the age of 5 years and that the mortality from the infection was 6.5%. In Kenya Mahieu et al. (1978) recorded a mortality from whooping cough of 2.5% in rural children under the age of 5 years.

No serious side-effects were recorded following the administration of triple vaccine during the present study and the vaccine appeared to be clinically effective. Whooping cough was prevalent in study villages during the period of the survey and several children from the area required admission to Malumfashi Hospital because of this infection. However, whooping cough was not seen among any vaccinated children.

b. Poliomyelitis vaccine

A poor serological response to immunisation with oral poliomyelitis vaccine was observed among both protected children and among control children as has been noted on many previous occasions when this vaccine has been given to children in tropical developing countries (Greenwood and Whittle, 1981). Various suggestions have been made as

to why the serological response to oral poliomyelitis is so poor in developing countries. It has been suggested that the high prevalence of enterovirus infection in such communities interferes with the establishment of polio vaccine virus in the gut and some evidence to support this view has been obtained (Dömök, 1974). It has been suggested also that breast feeding may interfere with colonisation of the gut by vaccine virus (Plotkin et al., 1966). However, attempts to improve the results of vaccination by stopping breast feeding for a period before the vaccine is given have not been successful (Peradze et al., 1968; John et al., 1976). Dömök et al. (1974) have demonstrated an inhibitor to poliomyelitis vaccine in saliva which can be neutralised by gamma globulin and this may be another important inhibitory factor. Our finding that the antibody response to oral poliomyelitis was equally poor in children receiving malaria chemoprophylaxis and in those who were not indicates that malaria does not play any part in bringing about the poor response to oral poliomyelitis vaccine that has been recorded in the tropics.

A heated debate is in progress as to whether oral poliomyelitis vaccine or killed poliomyelitis vaccine should be used routinely in the tropics. The relative advantages and disadvantages of the two types of poliomyelitis vaccine are summarised by Melnick (1978). Two findings relevant to this debate were noted during the course of the present study. A fatal, paralytic illness developed in a 3 month old child approximately two weeks after the child was given a first dose of oral poliomyelitis vaccine. It is possible that this child died from vaccine-induced poliomyelitis, a recognised complication of oral poliomyelitis vaccine but one which is thought to be rare. Some proponents of oral poliomyelitis vaccine maintain that serum antibody measurements are a misleading method of analysing the response to this vaccine and that many of the children without serum antibodies are immune to the infection because they have local gut immunity. Our

finding that 18 months after vaccination approximately one-half of the children without antibody 1 month after the administration of the last of 3 doses of oral vaccine had become seropositive is against this view and suggests that these children were not immune and that they were colonised by wild virus. However, this observation could also be accounted for by a prolonged vaccine effect or by the induction of some form of anamnestic response by another gut virus.

c. Measles vaccine

Measles occurs at an early age in many parts of tropical Africa including northern Nigeria. Thirty per cent of children admitted to Ahmadu Bello University Hospital, Zaria with measles are under the age of 1 year (Whittle, personal communication) and 6% of the children in the present study had high measles antibody levels at the age of 7 months, suggesting previous clinical infection. Thus, if vaccination is to be effective in preventing measles in a tropical developing country it must be given at an early age. However, if vaccine is given to a child who still has more than a small amount of maternally derived antibody the vaccine will not take (Fig. 4b). Both of these factors must be considered when the optimum age for measles vaccination in any community is determined. A detailed serological study in Kenya (W.H.O. 1977) indicated that the optimum age for measles vaccination in that country is 9 months and a similar conclusion was drawn from an analysis of the measles antibody response to a combined measles/meningococcal/tetanus vaccine given to children in another part of the Malumfashi area (Greenwood et al., 1981a). However, the results of the present study show that a satisfactory response can be obtained in this community when measles vaccine is given at an earlier age. Although 6% of children had raised measles antibody levels at the time that they were vaccinated at the age of 7 months some of these children responded to vaccination and one month after vaccination 94% of vaccine recipients had a measles antibody at a level likely to be associated

with clinical immunity.

d. Meningococcal vaccine

Only a modest rise in antibody level was obtained after immunisation with a combined group A and group C meningococcal polysaccharide vaccine at the age of 1 year or 2 years. This finding was expected as previous studies undertaken in industrialised countries (Goldschneider et al., 1973; Gold et al., 1975; Mäkelä et al., 1977) and in Nigeria (Greenwood et al., 1980) have shown that the magnitude of the immune response to meningococcal polysaccharide vaccine is strongly influenced by age. Only a poor and transient meningococcal antibody response was obtained when a combined meningococcal/measles/tetanus vaccine was given to Nigerian children under the age of 18 months (Greenwood et al. 1981a). In the United States it has been shown that raised meningococcal antibody levels can be maintained throughout childhood by the administration of 3 carefully spaced doses of vaccine (Gold et al., 1979). It is uncertain whether such a regimen would be equally effective in a tropical developing country where children are exposed frequently to malaria and other infections and such a regimen would be beyond the financial resources of many tropical developing countries. Fortunately, within countries of the African meningitis belt meningococcal disease is infrequent in children under the age of 2 years (Greenwood et al., 1979) and the condition is seen most frequently in children aged 5-14 years (Lapeyssonie, 1963). Thus, in this region it may be wise to delay routine immunisation with meningococcal polysaccharide vaccines until children enter primary school at the age of about 5 years, for a much better antibody response will be obtained in children of this age than will be obtained in infants. Alternatively, meningococcal vaccination can be restricted to the control of epidemics.

3.3 Cell mediated immunity

Regular administration of malaria chemoprophylaxis had no influence on

tests of cell mediated immunity among the study children. Natural immunisation with C. albicans and with M. tuberculosis occurred at an almost identical rate in both protected and control children and no difference was observed between children in the two study groups in the rate of Mantoux conversion following BCG vaccination. These observations are in keeping with the previous finding that in vitro tests of cell mediated immunity are little affected in children with acute clinical malaria (Greenwood et al., 1971a; Moore et al., 1974). Therefore, it is very unlikely that administration of malaria chemoprophylaxis will have any effect on the response to a vaccine which depends for its beneficial effect upon the induction of a cell mediated immune response.

3.4 Conclusions

From the results obtained from the Malumfashi study and described in this chapter it is clear that a subclinical infection with malaria had little or no effect on the antibody response to routine immunisations. The only exception to this statement was found with meningococcal polysaccharide vaccines when there was an improved response to immunisations if malaria parasitaemia had been cleared before, or at the time that, the vaccine was administered to the children.

Consequently malaria should not be held responsible for the failure of vaccination schedules in countries with endemic malaria, and other causes must be identified and controlled to improve the efficacy of these programmes.

TABLE 4a

Antibody levels before and 1 month after vaccination in protected and in control children. In this table, and in subsequent comparable tables in this chapter, antibody levels are expressed as a mean titre ± 1 standard deviation. Diphtheria and tetanus (E) (ELISA) antibody levels are expressed as an absorbance. Tetanus haemagglutinating antibody (HA), measles, meningococcal and S. typhi 0 antibodies are expressed as a reciprocal of the mean $\log_2$ titre. Figures in brackets indicate the number of observations made.

Antibody	Protected children		Control children	
	Pre-vaccination	Post[1] vaccination	Pre-vaccination	Post-vaccination
Tetanus (E)	0.09 ± 0.06 (169)	1.54 ± 0.39 (146)	0.09 ± 0.07 (155)	1.43 ± 0.46 (140)
Tetanus (HA)	0.17 ± 0.67 (156)	10.91 ± 1.71 (147)	0.37 ± 1.15 (148)	10.73 ± 2.41 (140)
Diphtheria (E)	0.04 ± 0.02 (169)	1.07 ± 0.32 (142)	0.04 ± 0.02 (152)	1.00 ± 0.33 (140)
Measles	1.04 ± 2.08 (149)	6.25 ± 2.15 (136)	0.99 ± 2.26 (143)	6.36 ± 2.05 (133)
Meningococcal Group A	*2.05 ± 1.64 (74)	**3.11 ± 1.61 (73)	*1.53 ± 1.46 (69)	**2.30 ± 1.50 (69)
Meningococcal Group C	0.58 ± 1.06 (74)	**2.25 ± 1.61 (73)	0.54 ± 1.09 (68)	**1.54 ± 1.44 (69)
S. typhi 0	3.28 ± 2.00 (72)	3.90 ± 1.82 (72)	3.13 ± 2.14 (68)	3.87 ± 1.78 (69)

Notes:
1. Post vaccination tetanus, diphtheria and poliomyelitis antibodies were measured 1 month after administration of the last of 3 doses of vaccine.

* $P < 0.05$.

** $P < 0.01$.

TABLE 4b

The number of protected and control children with antibody levels likely to be associated with protective immunity one month after vaccination.

Antibody	Cut-off titre	Protected children		Control children	
		Number	%	Number	%
Diphtheria (E)	0.4 or >	130/134	97	126/132	95
Tetanus (E)	0.4 or >	134/135	99	126/128	98
Tetanus (HA)	3.0 or >	140/140	100	133/135	99
Measles	3.0 or >	124/132	94	125/127	98
Meningococcal Group A	3.0 or >	* 52/72	72	* 28/68	41
Meningococcal Group C	3.0 or >	** 32/72	44	** 17/68	25
S. typhi O	3.0 or >	52/70	74	56/67	83

* $P < 0.001$; ** $P < 0.02$.

TABLE 4c

The mean rise in antibody titre (post-vaccination titre - pre-vaccination titre) ± 1 standard deviation in all protected and in all control children one month after immunisation.

Antibody	Protected children		Control children	
	No.	mean titre	No.	mean titre
Diphtheria (E)	121	1.45 ± 0.24	118	1.45 ± 0.25
Tetanus (E)	123	1.31 ± 0.25	119	1.25 ± 0.31
Tetanus (HA)	127	10.76 ± 1.80	119	10.53 ± 2.15
Measles	121	5.15 ± 2.81	116	5.57 ± 2.36
Meningococcal Group A	72	1.08 ± 1.42	68	0.77 ± 1.60
Meningococcal Group C	72	*1.71 ± 1.92	66	*0.97 ± 1.60
S. typhi O	70	0.66 ± 1.77	67	0.73 ± 1.36

* $P < 0.02$

TABLE 4d

The mean rise in antibody titre (post vaccination titre - pre-vaccination titre) ± 1 standard deviation in protected and in control children one month after immunisation excluding children with detectable antibody at the time of immunisation.

Antibody	Protected children		Control children	
	No.	mean titre	No.	mean titre
Diphtheria (E)	121	1.46 ± 0.23	114	1.46 ± 0.23
Tetanus (E)	121	1.32 ± 0.24	111	1.30 ± 0.24
Tetanus (HA)	114	11.08 ± 1.55	102	10.78 ± 2.04
Measles	81	6.52 ± 1.86	84	6.54 ± 1.43
Meningococcal Group A	13	2.62 ± 1.12	13	2.39 ± 0.87
Meningococcal Group C	41	2.61 ± 1.39	28	2.25 ± 1.08
S. typhi O	3	3.00 ± 1.73	3	2.67 ± 1.53

TABLE 4e

The number and percentage of protected children and control children without antibody at the time of vaccination who had seroconverted[1] one month later.

Antibody	Protected children		Control children	
	Number	%	Number	%
Diphtheria (E)	134/135	99	127/130	98
Tetanus (E)	134/135	99	127/128	99
Tetanus (HA)	126/126	100	116/116	100
Measles	90/91	99	90/91	99
Meningococcal Group A	13/18	72	15/18	83
Meningococcal Group C	*41/48	85	*30/49	61
S. typhi O	3/3	100	3/3	100

Notes: 1. For ELISA antibody determinations seroconversion was defined as a change in absorbance from <0.2 before vaccination to >0.2 after vaccination. For other antibody measurements seroconversion was defined as an increase in antibody titre following vaccination of at least 2 $\log_2$ doubling dilutions.

* $P<0.01$.

TABLE 4f

The antibody response to vaccination (post vaccination titre - pre-vaccination titre) in relation to the presence or absence of parasitaemia at the time that the vaccine was given. In the case of triple vaccine the analysis has been made on the basis of the presence or absence of parasites on the day that the first dose of vaccine was given.

	Parasitaemia	
	Present	Absent
Diphtheria (E)	1.39 ± 0.24 (39)	1.46 ± 0.25 (194)
Tetanus (E)	*1.12 ± 0.33 (41)	1.32 ± 0.26 (200)
Tetanus (HA)	10.80 ± 1.97 (40)	10.70 ± 2.31 (201)
Measles	6.03 ± 2.12 (30)	5.35 ± 2.66 (171)
Meningococcal Group A	0.74 ± 1.56 (54)	1.11 ± 1.36 (84)
Meningococcal Group C	1.06 ± 1.69 (54)	1.56 ± 1.76 (84)
S. typhi O	0.65 ± 1.24 (54)	0.64 ± 1.72 (81)

* $P < 0.01$

TABLE 4g

The immune response to vaccination (post-vaccination titre - pre-vaccination titre) in relation to haemoglobin genotype.

Antibody	Genotype AA	Genotype AS
Diphtheria (E)	1.45 ± 0.25 (166)	1.46 ± 0.25 (66)
Tetanus (E)	1.28 ± 0.29 (168)	1.31 ± 0.21 (67)
Tetanus (HA)	10.57 ± 2.00 (173)	10.81 ± 1.89 (66)
Measles	5.26 ± 2.66 (169)	5.61 ± 2.34 (62)
Meningococcal Group A	0.87 ± 1.38 (99)	1.30 ± 1.86 (35)
Meningococcal Group C	1.26 ± 1.77 (99)	1.49 ± 1.85 (35)
S. typhi O	0.61 ± 1.66 (98)	0.68 ± 1.27 (34)

TABLE 4h

Mean antibody levels (± 1 standard deviation of the mean) in protected and control children followed for 2 years before, 1 month and 18 months after vaccination.

Antibody	Protected children			Control children		
	Pre-vaccination	1/12 post vaccination	18/12 post vaccination	Pre-vaccination	1/12 post vaccination	18/12 post vaccination
Diphtheria (E)	0.05 ± 0.03 (110)	1.08 ± 0.32 (94)	0.24 ± 0.17 (55)	0.04 ± 0.02 (99)	0.99 ± 0.35 (95)	0.21 ± 0.13 (51)
Tetanus (E)	0.09 ± 0.04 (111)	1.52 ± 0.35 (99)	*0.42 ± 0.26 (53)	0.10 ± 0.08 (102)	1.39 ± 0.48 (95)	*0.33 ± 0.15 (53)
Tetanus (HA)	0.06 ± 0.23 (102)	11.02 ± 1.60 (99)	9.09 ± 1.36 (44)	0.28 ± 1.05 (96)	11.04 ± 2.30 (96)	8.45 ± 1.97 (43)
Measles	2.00 ± 2.25 (101)	6.35 ± 2.15 (98)	5.22 ± 1.93 (41)	1.36 ± 2.65 (97)	6.30 ± 2.21 (96)	4.62 ± 1.53 (42)

* $P < 0.05$

TABLE 4i

Mean poliomyelitis antibody titres (reciprocal of serum dilution giving neutralisation) ± 1 standard deviation in protected and control children before, 1 month and 18 months after vaccination with 3 doses of oral poliomyelitis vaccine.

Antibody	Protected children			Control children		
	Pre-vaccination	1/12 post vaccination	18/12 post vaccination	Pre-vaccination	1/12 post vaccination	18/12 post vaccination
	n = 29	n = 29	n = 22	n = 19	n = 19	n = 19
Type 1	36 ± 28	*410 ± 113	663 ± 219	< 32	*1344 ± 522[1]	540 ± 204
Type 2	< 32	137 ± 37	*394 ± 183	< 32	145 ± 55	*196 ± 91
Type 3	< 32	227 + 69	*126 + 27	< 32	205 + 74	* 66 + 26

Notes: 1. Includes 2 samples with very high titres of 4096.

* $P < 0.001$.

TABLE 4j

The seroconversion rate to 3 doses of oral poliomyelitis vaccine in children receiving malaria chemoprophylaxis and in controls. Only subjects without antibody before vaccination have been included.

Antibody	Protected children		Control children	
	Number	%	Number	%
Type 1	13/27	48	11/19	58
Type 2	13/28	46	10/19	53
Type 3	13/26	50	9/17	53

TABLE 4k

Group A meningococcal antibody levels before and 1 month after immunisation with group A + group C meningococcal polysaccharide vaccine at the age of 1 or 2 years in protected and control children. Mean titres ± 1 standard deviation are shown.

	Initial		Post immunisation	
	1 year	2 years	1 year	2 years
Protected	1.84 ± 1.66 (n = 31)	2.21 ± 1.63 (n = 43)	*2.94 ± 1.63 (n = 31)	**3.24 ± 1.61 (n = 42)
Control	1.13 ± 1.33 (n = 24)	1.75 ± 1.50 (n = 44)	*2.08 ± 1.74 (n = 24)	**2.42 ± 1.36 (n = 45)

* $0.1 > P < 0.05$; ** $P < 0.02$

TABLE 41

Group C meningococcal antibody levels before and 1 month after immunisation with group A + group C meningococcal polysaccharide vaccine at the age of 1 or 2 years in protected and control children. Mean titres ± 1 standard deviation are shown.

	Initial		Post immunisation	
	1 year	2 years	1 year	2 years
Protected	0.36 ± 0.66 (n = 31)	0.74 ± 1.26 (n = 43)	2.52 ± 1.90 (n = 31)	*2.05 ± 1.34 (n = 42)
Control	0.50 ± 0.98 (n = 24)	0.57 ± 1.15 (n = 44)	1.79 ± 1.25 (n = 24)	*1.40 ± 1.53 (n = 45)

* $P < 0.05$

TABLE 4m

The number and percentage of protected and control children with a positive skin test to C. albicans antigen or to PPD at the age of 1 year or 2 years.

Age	C. albicans				PPD			
	Protected children		Control children		Protected children		Control children	
	Number	%	Number	%	Number	%	Number	%
1 year	27/42	64	27/39	69	8/42	19	6/36	17
2 years	30/60	50	36/56	64	11/57	19	11/52	21

TABLE 4n

The Mantoux conversion rate in protected and in control children 1 month after BCG vaccination.

Age	Protected children		Control children	
	Number	%	Number	%
1 year	23/26	88	29/35	83
2 years	30/34	88	32/37	87
Combined	53/60	88	61/72	85

Fig.4a. Figure showing the percentage of protected and control children with antibodies greater than 1:32 before, one month after and eighteen months after vaccination with three doses of oral poliomyelitis vaccine

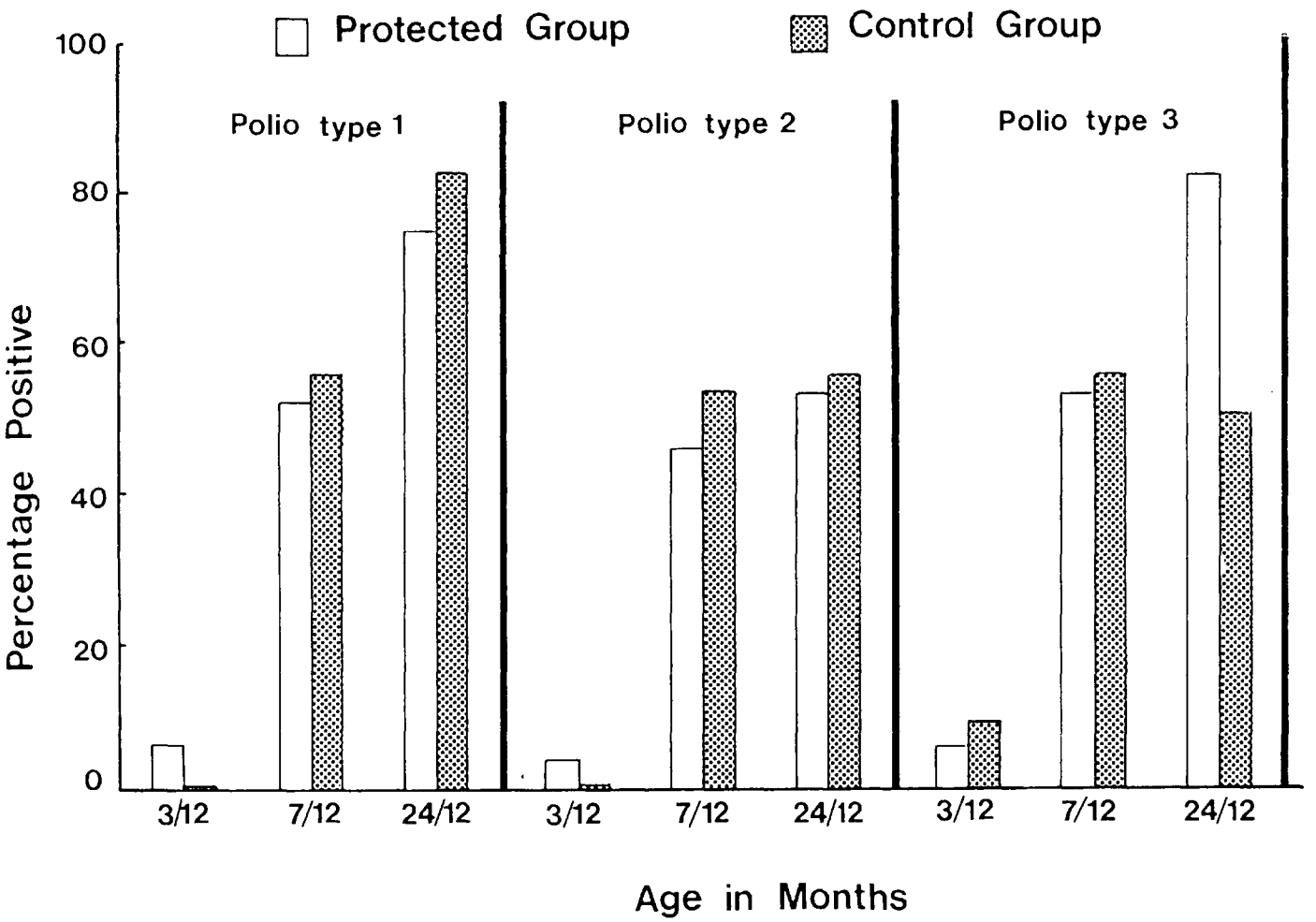


Fig. 4b. The rise in measles antibody titre in relation to initial antibody levels. The vertical lines represent standard errors of the mean

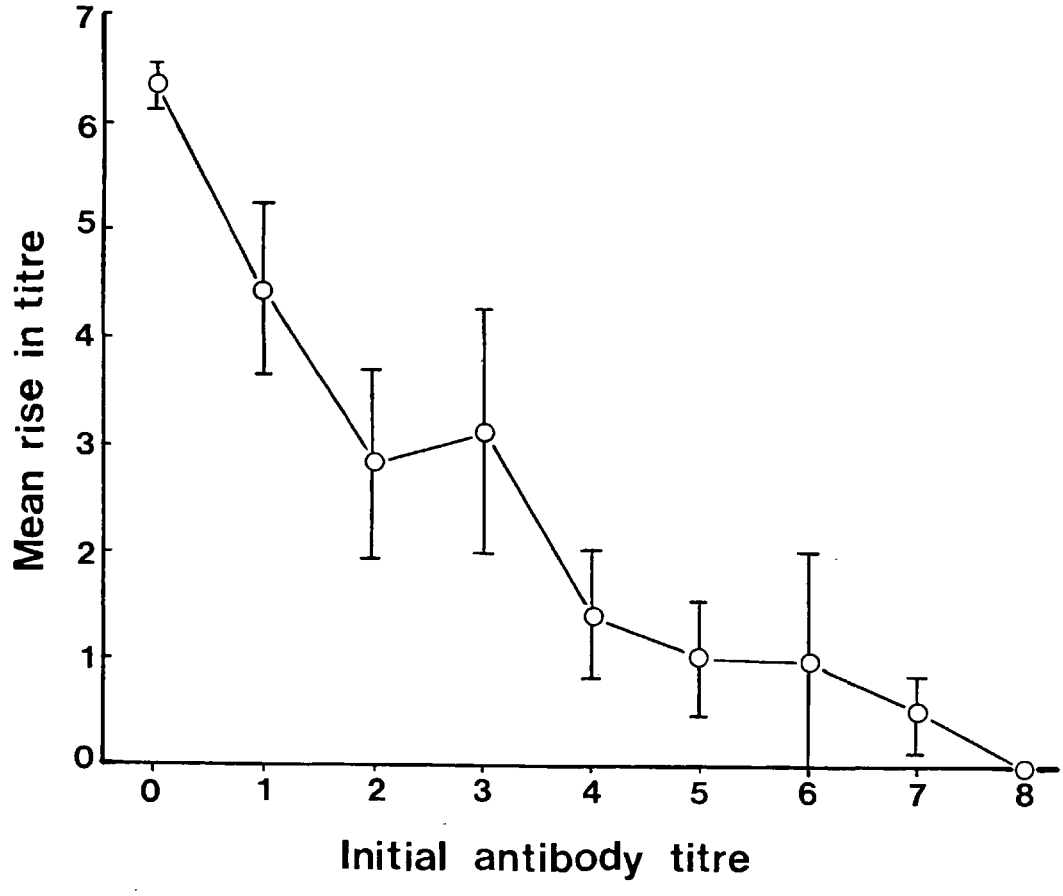


Fig.4c. The mean rise of titre in relation to the initial titre of antibody to meningococcal vaccine groups A and C. The vertical lines represent the standard error of the mean.

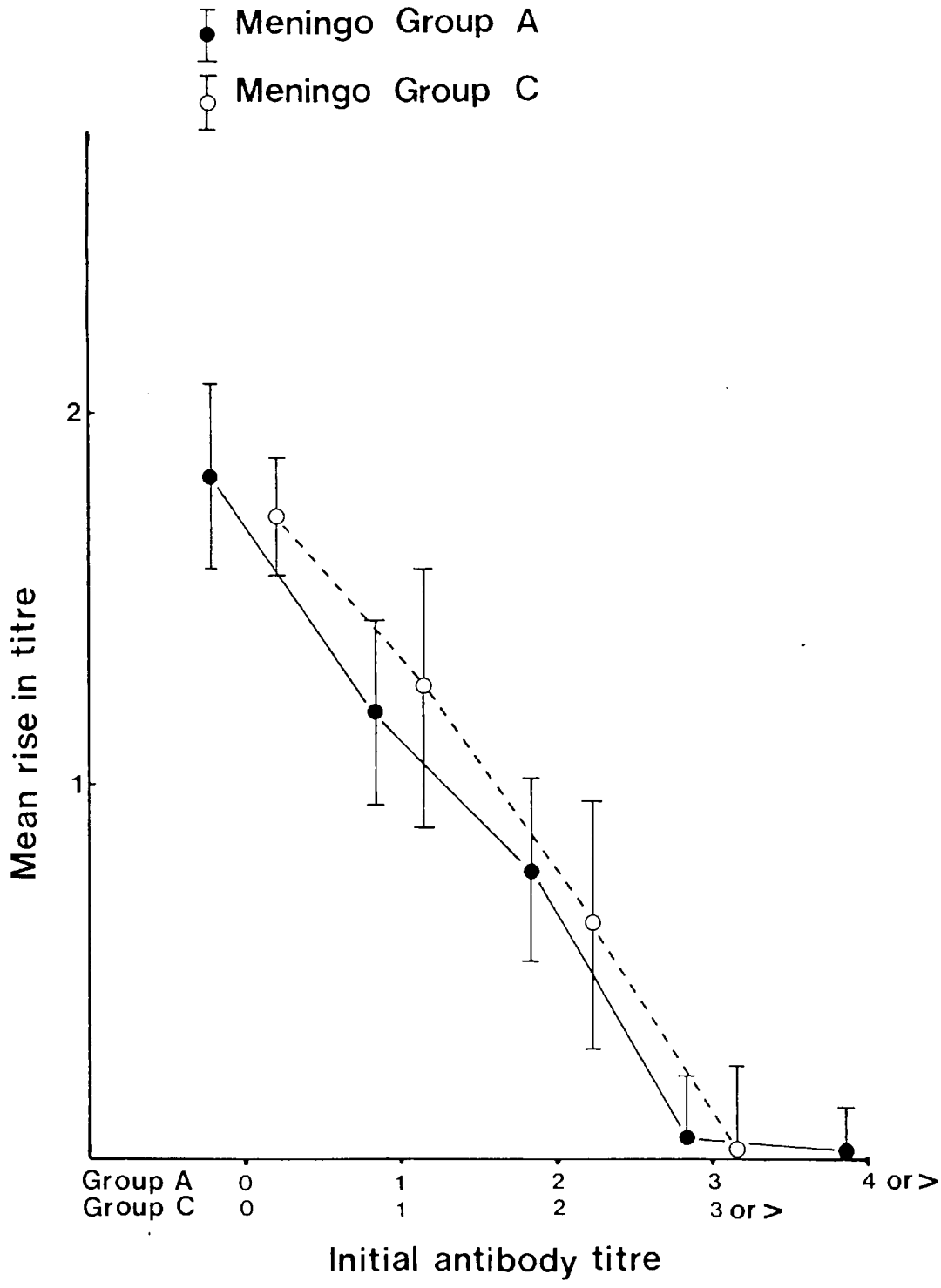
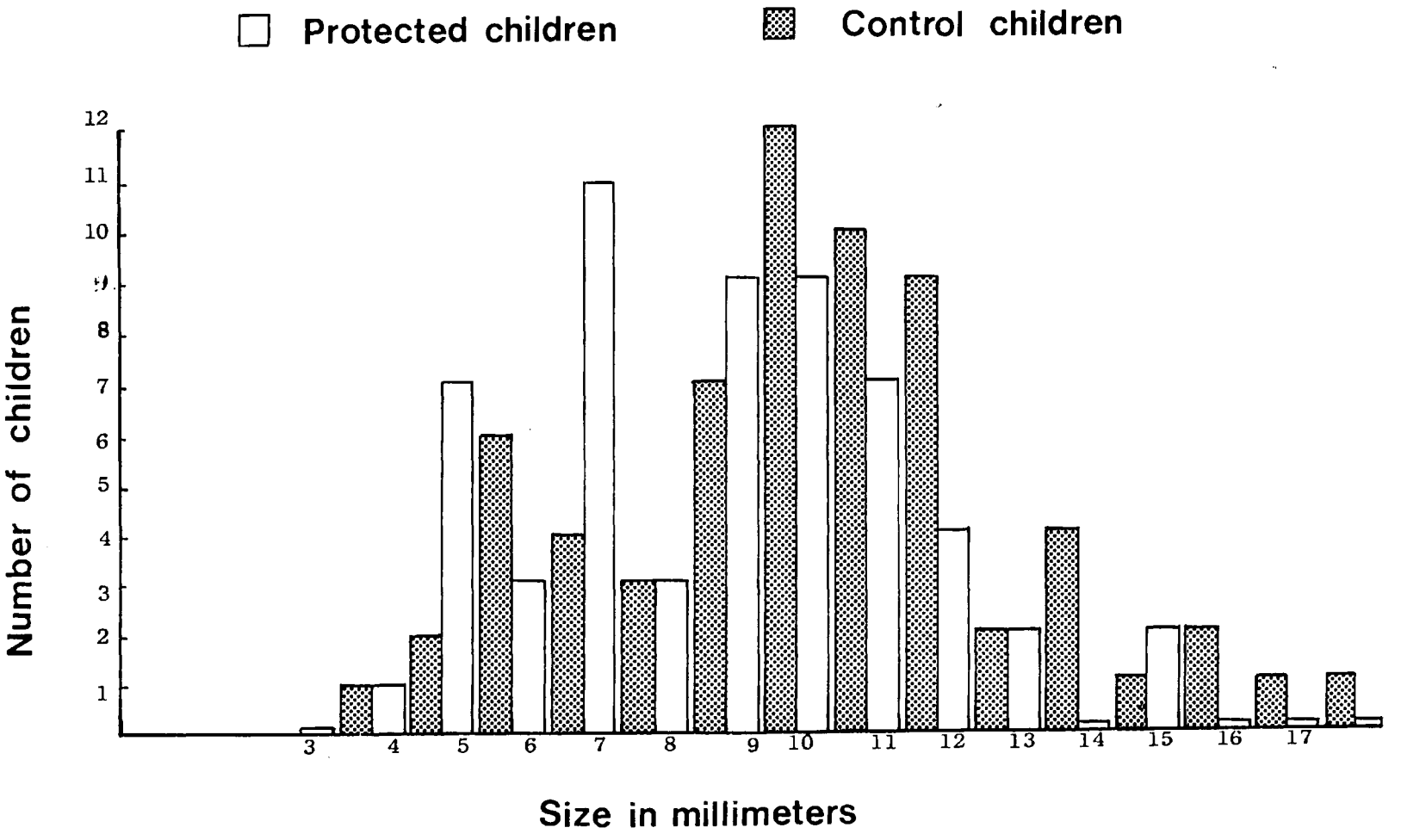


Fig. 4d. The comparison of the size of the response to testing with Candida antigen in the protected and the control groups of children. (2mm or less was taken as a negative response)



CHAPTER 5 - MALARIA CHEMOPROPHYLAXIS, NUTRITION AND THE IMMUNE RESPONSE TO VACCINATION.

1. INTRODUCTION

The deleterious effects of infection on the nutrition of young children growing up in tropical developing countries has been clearly demonstrated (Scrimshaw et al., 1968; Mata, 1975; Chandra and Newberne, 1977). An episode of acute gastroenteritis can cause transitory faltering of growth and recurrent episodes of diarrhoea may lead to persistent failure to thrive (Morley, 1962; Frood et al., 1971; Martorell et al., 1975; Rowland et al., 1977). Measles can cause a sudden loss of weight and a fall in serum albumin (Poskitt, 1971) and Garnham (1949) noted a transient loss of weight in young African children during their first attack of malaria. This latter finding was confirmed by Bruce-Chwatt (1952) who showed that approximately one half of young Nigerian babies showed a fall in weight during their first attack of malaria and that in 15% of babies weight loss was severe. Frood et al. (1971) found that malaria was one of the infections that precipitated weight loss and hypoalbuminaemia among Ugandan children. At Keneba, in The Gambia, Rowland et al. (1977) demonstrated that malaria contributed significantly to the poor weight gain of children brought up in this village but they found that malaria was not as important an infection in this respect as gastroenteritis.

There have been few reports from Africa of the effects of malaria control on nutrition. McGregor et al. (1956) showed that the mean weight of a small number of Gambian children who received regular chemoprophylaxis with chloroquine was slightly, but not significantly, greater than the mean weight of a group of control children. Draper and Draper (1960) studied the nutrition of Tanzanian children before and after the introduction of a malaria control programme. No significant improvement in the weight of children aged 2-18 months was found despite successful control of malaria transmission with insecticide. However, at Garki in northern Nigeria malaria control by a combination of insecticide spraying and chemoprophylaxis resulted in

a small, but significant, improvement in the nutrition of children living in the study area (Molineaux and Gramiccia, 1980).

Severe malnutrition impairs the immune response to some, but not all, vaccines (Chandra and Newberne, 1977). Therefore, the effects of chemoprophylaxis on the nutrition of children in the present study was carefully followed in case chemoprophylaxis had a beneficial effect on the immune response to vaccination by improving the nutritional state of the children receiving prophylaxis rather than by exerting a more direct effect on parasite mediated immunodepression. The results of these nutritional observations are presented in this chapter.

2. RESULTS

a. Clinical Malnutrition

A clinical diagnosis of marasmus or kwashiorkor was made at some stage during the course of the study in 12 of 184 control children but in only 4 of the 199 children who received malaria chemoprophylaxis ($P = 0.02$, Fisher's exact test). Six of the 12 control children with malnutrition died whilst only 1 death from malnutrition occurred among children receiving regular malaria chemoprophylaxis ($P = 0.05$, Fisher's exact test).

b. Anthropometry

Anthropometric surveys were carried out every 3 months on all available study children. At each survey measurements of height, weight, mid-arm circumference, chest circumference and head circumference were made. During preliminary surveys measurements of skin fold thickness were found to give inconsistent results and skin fold thickness was not measured subsequently.

Overall analysis of the anthropometric results obtained showed that boys were significantly taller and heavier than girls ($P < 0.001$ for each measurement) and that boys had a significantly greater mid upper arm circumference than girls ($P < 0.002$). However, combined data from boys

and girls has been used for comparisons between protected and control children because the proportions of boys and girls in each group was very similar.

The mean height, weight and mid upper arm circumference of protected and of control children aged from 1-24 months are shown in figures 5a-5c. Among children of both groups faltering of weight was observed from about the age of 3 months and faltering of height from about the age of 6 months. At the age of 2 years the mean weight of control children had fallen to 75% of the Harvard standard. Protected children tended to be taller and heavier than control children (Figs. 5a and 5b) and to have a larger mid upper arm circumference than control children (Fig. 5c). Differences between the two groups were more marked during the second year of life than during the first. When the results presented in figures 5a-5c were studied by an analysis of variance it was found that protected children were significantly taller and heavier than control children ($P < 0.05$ and $P < 0.01$ respectively) and that protected children had a significantly larger mid upper arm circumference than control children ($P < 0.001$). However, when a comparison of first and last measurements in individual children was made no significant difference was found between the two groups. No significant differences in measurements of head circumference or chest circumference were found between protected and control children.

The results of anthropometric measurement in protected and in control children have been analysed in a different way in figure 5d which shows the proportion of underweight children (< 80% NCHS standard) in each age group in relation to age. During the first few months of life more protected than control children were underweight but this difference was reversed by the age of 6 months and from the age of 18-26 months fewer protected children than control children were underweight. However, the differences between the two groups are not

statistically significant.

Anthropometric measurements were continued for 6 months after the administration of chloroquine or placebo was stopped. An insufficient number of observations were made for this data to be analysed on a month to month basis. Therefore, a comparison has been made between children in the protected and control groups of all observations made in the final 6 month following-up period, expressing the results obtained as a percentage of the Harvard standard (Table 5a). The mean of measurements of mid upper arm circumference was higher in protected than in control children but the difference between the two groups was not statistically significant. Mean measurements for height and weight were similar in the two groups of children.

In order to see whether malaria caused a seasonal variation in nutritional status that could be altered by chemoprophylaxis the effect of season on anthropometric measurements was investigated among both protected and control children. Measurements of weight by month of the year and by season showed no consistent seasonal pattern in either group of children. The season of birth did not influence the subsequent growth rate of either protected or control children.

c. Albumin and Pre-albumin

Serum albumin and pre-albumin levels were measured in protected and in control children on 3 occasions. Both albumin and pre-albumin levels fell with increasing age (Fig. 5e). Serum albumin and serum pre-albumin levels were higher in protected children than in control children at the ages of 8, 12 and 24 months but the difference in mean values between the 2 groups was statistically significant only for pre-albumin levels at the ages of 8 and 24 months.

To investigate further the relationship between malaria and serum albumin and pre-albumin levels a comparison was made of mean albumin and pre-albumin levels in children with or without malaria parasitaemia

at the time that the blood sample was collected. Children with malaria parasitaemia had significantly lower albumin and pre-albumin levels than children without parasitaemia (Tables 5b and 5c).

d. Nutrition and the antibody response to vaccination

The relationship between the antibody response to vaccination and the nutritional state of a child, as assessed by both anthropometric and biochemical measurements, at the time of immunisation was investigated. The weight pattern of protected and of control children at the time that they were immunised is shown in figure 5f. At the age of 4 months, when first immunisations with triple vaccine and with poliomyelitis vaccine were given, only a small proportion of protected or control children were less than 80% of the Harvard standard of weight for age. However, by the age of 12 months or 24 months, when meningococcal vaccine, typhoid vaccine and BCG were given, approximately half the children in each group were underweight.

The results of correlations between the antibody response to immunisation and anthropometric measurements made at the time of vaccination are shown in Table 5d. (In the case of triple vaccine correlations have been made with anthropometric measurements made at the time that the first dose of vaccine was given.) Overall no signficant correlations between antibody response and measurement of height, weight and arm circumference were found. The results obtained in protected and in control children were analysed separately (data not shown). No significant correlations between antibody response and nutritional state were found among children receiving chemoprophylaxis. Among control children a significant correlation was found between the antibody response to group A meningococcal polysaccharide and height and weight ($P < 0.01$ and $P < 0.05$ respectively) when vaccination was given at the age of 1 year but not when vaccination was given at the age of 2 years. These correlations are, therefore, of doubtful clinical significance. The relationship between the antibody response to

vaccination and weight at the time of vaccination is expressed in a different way in Table 5e which shows the mean rise in antibody titre following vaccination in relation to weight at the time of immunisation. Good antibody responses were obtained in children < 70% weight for age. Similar patterns were obtained on analysis of the antibody response to vaccination in relation to height or weight for height at the time of immunisation. Correlations between antibody response to vaccination and serum albumin and pre-albumin levels measured at the time of vaccination (measles, meningococcal and S. typhi vaccines) or at the time of collection of post immunisation blood samples (triple vaccine) are shown in Table 5f. Weak, but statistically significant, correlations were found between the immune response to meningococcal group A vaccine and serum pre-albumin levels and between the immune response to group C meningococcal vaccine and serum albumin levels. Separate analysis of the results obtained in protected and in control children and in children vaccinated at the age of 1 year or 2 years did not reveal any other positive correlations.

e. Nutritional state and cellular immunity

The relationship between nutritional state and the response to skin tests with PPD and with candida antigen was investigated. Table 5g shows the correlation coefficients obtained on comparison of the size of skin test responses with various measurements of nutritional state. Only one correlation, that between the response to candida antigen and serum pre-albumin in 2 year old children, approached a level of statistical significance. Subjects with negative skin tests were included in the analysis presented in Table 5g. Reanalysis of the data excluding subjects with negative skin tests did not give any significant positive correlations. An analysis of mean albumin and pre-albumin levels in skin test responders and in non-responders was made. No significant differences were found between responders and non-responders except for the mean pre-albumin level of 2 year old

children which was significantly higher in 83 responders to candida skin test antigen than in 42 non-responders (65.5% ± 23.1% compared with 55.6% ± 22.2%, $P < 0.05$).

Overall a good response to BCG immunisation was obtained but 14% of children were still Mantoux test negative when tested 4-6 weeks after immunisation with BCG. Table 5h compares the nutritional state of these non-responders to that of children who converted following immunisation with BCG. No significant differences between the two groups were found. The relationship of nutritional state to BCG conversion is illustrated in a different way in Table 5i which shows the number of responders to vaccination, and the mean diameter of their challenge Mantoux text, in relation to their weight measured during the month before their immunisation. Although there is a trend for well-nourished children to convert more frequently than under-nourished or marasmic children there are no signficant differences between the 3 groups.

3. DISCUSSION

Children who received weekly chemoprophylaxis with chloroquine developed severe malnutrition less frequently than control children and overall they were slightly taller and heavier than control children and they had a slightly larger mid upper arm circumference. When the results of all anthropometric observations made in children in the two groups were tested by an analysis of variance protected children were found to be significantly heavier and taller than control children and they were found to have a significantly larger mean mid upper arm circumference. Analysis of the results in this way does not take into account the length of time that individual children spent in the study and it regards repeated measurements from the same child as independent variables. The latter effect tends to exaggerate the statistical significance of any differences found between the two groups. A second

method of analysis was, therefore, employed in which first and last anthropometric measurements made in individual children in the two groups were compared. When this method of analysis was used no statistically significant differences in the nutritional state of children in the two groups could be shown. However, this second method of analysis disregards a large number of observations. Thus it seems probable that protected children were slightly better nourished than control children but that the difference between the two groups was not as marked as suggested by an analysis of variance. This view is supported by the fact that serum albumin and pre-albumin levels, sensitive biochemical indicators of nutritional status (Ingenbleek et al., 1972), were consistently higher in protected than in control children. Insufficient observations were collected for a detailed comparison of the nutritional findings in the 2 groups of children during the 6 months after administration of chloroquine or placebo was stopped. However, a crude analysis suggested that during this period the differences in weight and height between the two groups of children observed earlier in the study were lost.

The anthropometric results of this study are thus very similar to those recorded by McGregor et al. (1956) in a small group of Gambian children who received weekly chemoprophylaxis with chloroquine. McGregor and his colleagues observed that the mean weight of protected children was consistently higher than that of control children but that the difference between the two groups never became statistically significant. In this early study in The Gambia the difference in weight between the two groups of children was maximal during the second year of life; the difference between the groups narrowing subsequently. At Garki, in northern Nigeria, where malaria control was achieved by a combination of insecticide spraying and chemoprophylaxis, a small, but statistically significant, difference in weight was observed between protected and control children up to the age of 5 years and

difference in mid upper arm circumference were observed up to the age of 10 years (Molineaux and Gramiccia, 1980).

Thus, it appears that malaria control by chemoprophylaxis, with or without associated control of mosquitoes, can result in improvement of the nutrition of protected children. Although such an improvement is likely to be only modest it occurs at a time when nutritional stresses on a child are at a maximum as a result of weaning and associated gastroenteritis and other infections. Any measure which can produce improvement in nutrition at this time, however modest, may be worthwhile.

The detrimental effect of severe protein-energy malnutrition on the immune system has been repeatedly demonstrated (Chandra and Newberne, 1977; Chandra, 1980; Greenwood and Whittle, 1981). Assays of cell mediated immunity are markedly depressed in severely malnourished children and the antibody response of such children to some, but not to all, vaccines is impaired. However, much less is known about the effects of milder degrees of malnutrition on the immune response. A few studies of the antibody response to vaccination of underweight children have been reported. Matthews et al. (1972) measured the antibody response to flagellin in a group of underweight New Guinean school children and showed that protein supplementation of their diet resulted in a modest, but significant, increase in antibody response. Edsall et al. (1975) found no difference in the antibody response to tetanus toxoid between well nourished and inadequately nourished Gambian children. Similarly, McMurray et al. (1979) showed a normal response to measles vaccine in underweight Colombian children. Greenwood et al. (1980) did not find any correlation between nutritional status and the immune response to immunisation with group C meningococcal polysaccharide vaccine. The results of the present study are in keeping with these earlier findings. With one possible exception no significant correlations were found between the antibody

response to immunisation and anthropometric measurements made at the time of vaccination. Although only a few children were underweight at the time of administration of their first dose of triple and poliomyelitis vaccines a much greater proportion of children were underweight at the age of 1 year or 2 years when meningococcal, typhoid and BCG vaccines were given. Significant correlations were found between the antibody response to group A and group C meningococcal polysaccharide vaccines and serum pre-albumin and albumin levels respectively. A poor antibody response to these vaccines is observed in subjects who have malaria parasitaemia at the time of immunisation (chapter 4). As malaria depressed serum albumin and pre-albumin levels a relationship to the presence of parasitaemia at the time of vaccination is the probable explanation for these correlations.

With the exception of a weak correlation between pre-albumin level and a response to candida antigen no significant correlations were found between nutritional state and the response to initial skin tests with candida antigen or PDD. Neither could any difference be demonstrated between the nutritional state of responders and of non-responders to BCG immunisation. The latter was an unexpected finding as Heyworth (1977) has shown previously that the immune response to BCG of Gambian children is influenced by their immune state at the time of vaccination. He found conversion rates of 56%, 80% and 86% in marasmic, under-nourished and normally nourished children respectively. When the data from the present study was analysed in this way (Fig. 5i) a similar trend was observed but differences between the groups were not statistically significant. The different results obtained in these two studies may have been due in part to the fact that the proportion of marasmic children in the Malumfashi series (4%) was less than that in the series from The Gambia (10%).

Thus, this study has shown that the humoral and cellular immune response of a group of poorly nourished children is little influenced

by their nutritional state. Under-nutrition (<80% but >60% Harvard standard) is, therefore, unlikely to interfere with the success of routine immunisation schemes undertaken in developing countries.

TABLE 5a

Anthropometric measurements in protected and in control children made during the 6 month period after the administration of chloroquine or placebo was stopped at the age of 1 or 2 years. Mean values ± 1 standard deviation, expressed as a percentage of the Harvard standard, are shown.

	AGE			
	12-18 months		25-30 months	
Measurement	Protected children	Control children	Protected children	Control children
	n = 25	n = 16	n = 28	n = 22
Height	93 ± 7	92 ± 8	92 ± 4	93 ± 5
Weight	76 ± 13	77 ± 16	80 ± 16	80 ± 11
Arm circumference	86 ± 10	83 ± 10	87 ± 7	84 ± 5

TABLE 5b

Mean serum albumin levels ± 1 standard deviation expressed as a percentage of a normal adult Nigerian standard in children with or without malaria parasitaemia. Figures in brackets indicate the number of observations made.

Parasitaemia	Age		
	8 months	12 months	24 months
Present	85 ± 7 (27)	76 ± 12 (13)	66 ± 9 (25)
Absent	92 ± 9 (76)	77 ± 11 (13)	73 ± 8 (26)
	$P < 0.001$	NS	$P < 0.01$

TABLE 5c

Mean serum pre-albumin levels ± 1 standard deviation expressed as a percentage of a normal adult Nigerian standard in children with or without malaria parasitaemia. Figures in brackets indicate the number of observations made.

Parasitaemia	Age		
	8 months	12 months	24 months
Present	67 ± 18	63 ± 20	57 ± 19
	(46)	(18)	(53)
Absent	81 ± 21	77 ± 21	68 ± 27
	(197)	(33)	(36)
	$P < 0.001$	$P < 0.05$	$P < 0.05$

TABLE 5d

Correlation coefficients for the relationship between the antibody response to vaccination (post vaccination titre - pre-vaccination titre) and anthropometric measurements made at the time of immunisation.[1]

Antibody	Number of observations	Weight	Height	Mid arm circumference
Diphtheria (E)	117	- 0.02	0.00	- 0.03
Tetanus (E)	121	- 0.06	- 0.12	- 0.11
Tetanus (H)	119	- 0.03	- 0.08	- 0.07
Measles	126	- 0.10	- 0.10	- 0.15
Meningococcal A 1 year	37	+ 0.13	+ 0.27	- 0.13
Meningococcal A 2 years	53	+ 0.08	+ 0.16	+ 0.02
Meningococcal C 1 year	37	0.00	+ 0.15	- 0.04
Meningococcal C 2 years	53	+ 0.01	- 0.03	+ 0.07
S. typhi O 1 year	36	+ 0.24	+ 0.24	+ 0.23
S. typhi O 2 years	52	+ 0.25	+ 0.16	+ 0.24

Note: 1. For diphtheria and tetanus antibodies correlations have been determined for antibody response and the nutritional state at the time of administration of the first dose of triple vaccine.

TABLE 5e

The mean rise in antibody titre following vaccination ± 1 standard deviation in relation to weight at the time of vaccination. Figures in brackets indicate the number of observations made. Antibody titres are expressed as in Table 4a.

Antibody	Weight for age (% NCHS standards)					
	<60	60-	70-	80-	90-	100+
Diphtheria (E)	1.6 ± 0.4 (3)	1.7 ± 0.2 (7)	1.4 ± 0.3 (15)	1.4 ± 0.3 (25)	1.5 ± 0.2 (38)	1.5 ± 0.2 (29)
Tetanus (E)	1.6 ± 0.2 (3)	1.4 ± 0.3 (7)	1.1 ± 0.4 (14)	1.2 ± 0.2 (24)	1.3 ± 0.2 (40)	1.4 ± 0.3 (33)
Tetanus (HA)	12.7 ± 1.2 (3)	10.9 ± 1.6 (7)	10.5 ± 1.6 (15)	11.0 ± 1.4 (23)	11.4 ± 2.0 (39)	11.2 ± 1.7 (32)
Measles	6.0 ± 0.0 (2)	5.1 ± 2.8 (9)	5.5 ± 2.3 (24)	5.3 ± 2.6 (51)	5.0 ± 2.8 (32)	4.7 ± 2.0 (7)
Meningococcal Group A	1.3 ± 0.6 (3)	- 0.1 ± 1.5 (11)	0.9 ± 1.6 (31)	0.9 ± 1.5 (33)	0.5 ± 2.1 (10)	2.0 ± 1.4 (2)
Meningococcal Group C	2.0 ± 0.0 (3)	1.0 ± 2.2 (11)	1.0 ± 1.9 (31)	1.5 ± 2.2 (33)	1.2 ± 1.6 (10)	2.5 ± 0.7 (2)
S. typhi O	- 3.0 ± 4.4 (3)	0.7 ± 1.6 (11)	0.7 ± 1.0 (29)	0.9 ± 1.4 (33)	0.9 ± 1.6 (10)	1.0 ± 1.4 (2)

TABLE 5f

Correlation co-efficients for the relationship between the antibody response to vaccination (post vaccination titre - pre-vaccination titre) and serum pre-albumin and albumin levels measured at the time of, or shortly after immunisation. Figures in brackets indicate the number of observations made.

Antibody	Pre-albumin	Albumin
Diphtheria (E)	0.04 (276)	0.05 (118)
Tetanus (E)	0.06 (282)	0.13 (116)
Tetanus (H)	0.03 (288)	0.02 (119)
Measles	-0.10 (219)	0.13 (107)
Meningococcal A	0.06 (159)	0.21* (102)
Meningococcal C	0.16* (159)	-0.07 (102)
S. typhi O	0.02 (155)	-0.06 (100)

* $P < 0.05$

TABLE 5g

Correlation coefficient obtained on comparison of nutritional state with the response to skin tests with PPD and candida antigen.

	1 year old children		2 year old children	
	Mantoux	Candida	Mantoux	Candida
Weight	0.14	- 0.04	0.15	0.10
Height	0.09	0.05	0.15	0.04
Mid upper arm circumference	0.13	- 0.07	0.07	0.10
Albumin	0.14	0.13	- 0.10	0.05
Pre-albumin	0.11	0.09	- 0.05	0.17*

* P = 0.06

TABLE 5h

Mean values ± 1 standard deviation of height, weight, mid upper arm circumference, albumin and pre-albumin in responders and in non-responders to immunisation with BCG. Figures in brackets indicate the number of observations made.

Measurement	Unit	1 year old children		2 year old children	
		Responders*	Non-responders	Responders*	Non-responders
Weight	kg	8.2 ± 1.1 (44)	8.1 ± 1.2 (8)	9.5 ± 1.3 (43)	10.0 ± 2.1 (2)
Height	cm	72.5 ± 3.4 (44)	71.9 ± 2.7 (8)	79.3 ± 3.7 (43)	81.0 ± 0.0 (2)
Mid upper arm circumference	cm	13.8 ± 1.4 (44)	14.0 ± 1.6 (8)	13.9 ± 1.3 (43)	14.5 ± 0.7 (2)
Albumin	%	73.0 ± 9.7 (37)	80.2 ± 11.2 (6)	68.0 ± 9.5 (39)	48.0 ± 0.0 (1)
Pre-albumin	%	74.9 ± 21.0 (49)	63.8 ± 22.1 (8)	63.8 ± 22.3 (75)	61.3 ± 39.2 (3)

Note: * Induration 5 mm or greater on challenge with 5 T.U. of PPD 5 weeks after BCG vaccination.

TABLE 5i

The response to BCG immunisation in relation to weight at the time of vaccination.

	% NCHS standard		
	<60	60-80	80+
	n = 4	n = 86	n = 20
Number showing conversion (%)**	3 (75%)	76 (88%)	19 (95%)
Mean diameter in mm of second Mantoux test	8.0 ± 4.3	*8.2 ± 3.7	*9.9 ± 4.1

Notes: * $0.1 > P > 0.05$

** Induration 5 mm or greater on challenge with 5 T.U. PPD 5 weeks after BCG vaccination.

Fig.5a. Weight for age in protected and in control children. Dotted lines indicate Harvard standards (Sexes combined)

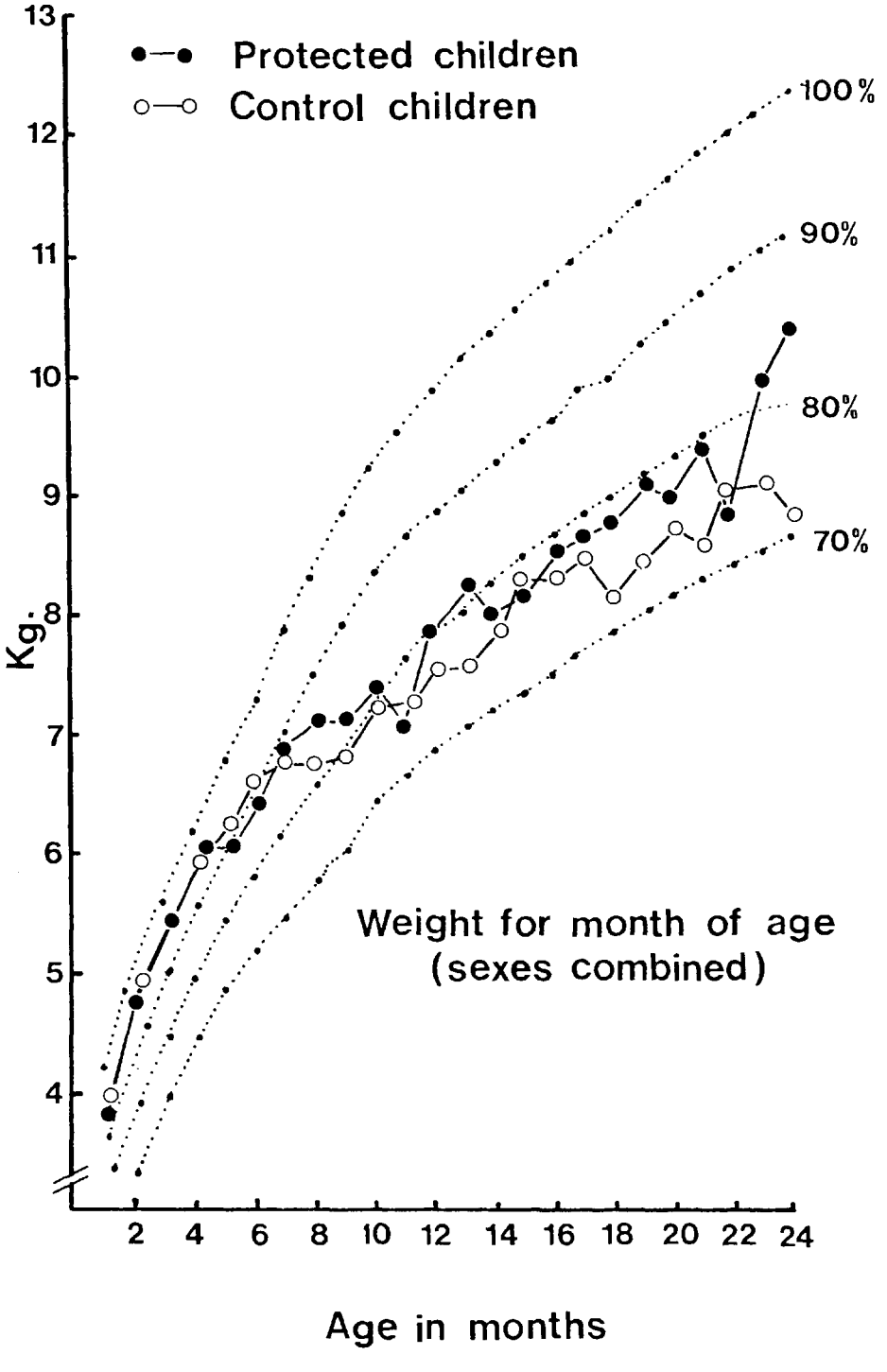


Fig.5b. Height for age in protected and in control children. Dotted lines indicate Harvard standards (Sexes combined)

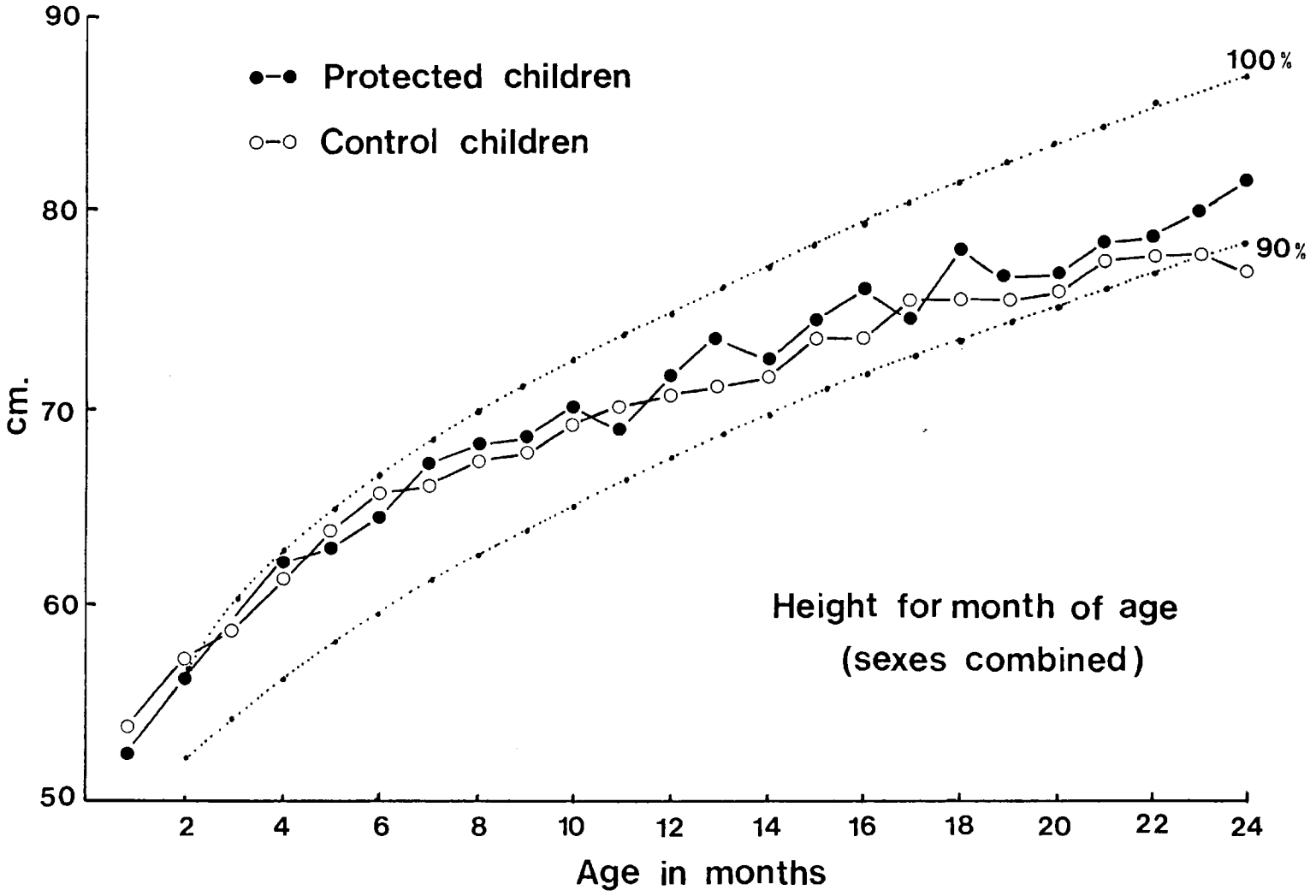


Fig.5c. Mid upper arm circumference in protected and in control children. Dotted lines indicate normal standards (Jeliffe, 1966) (Sexes combined)

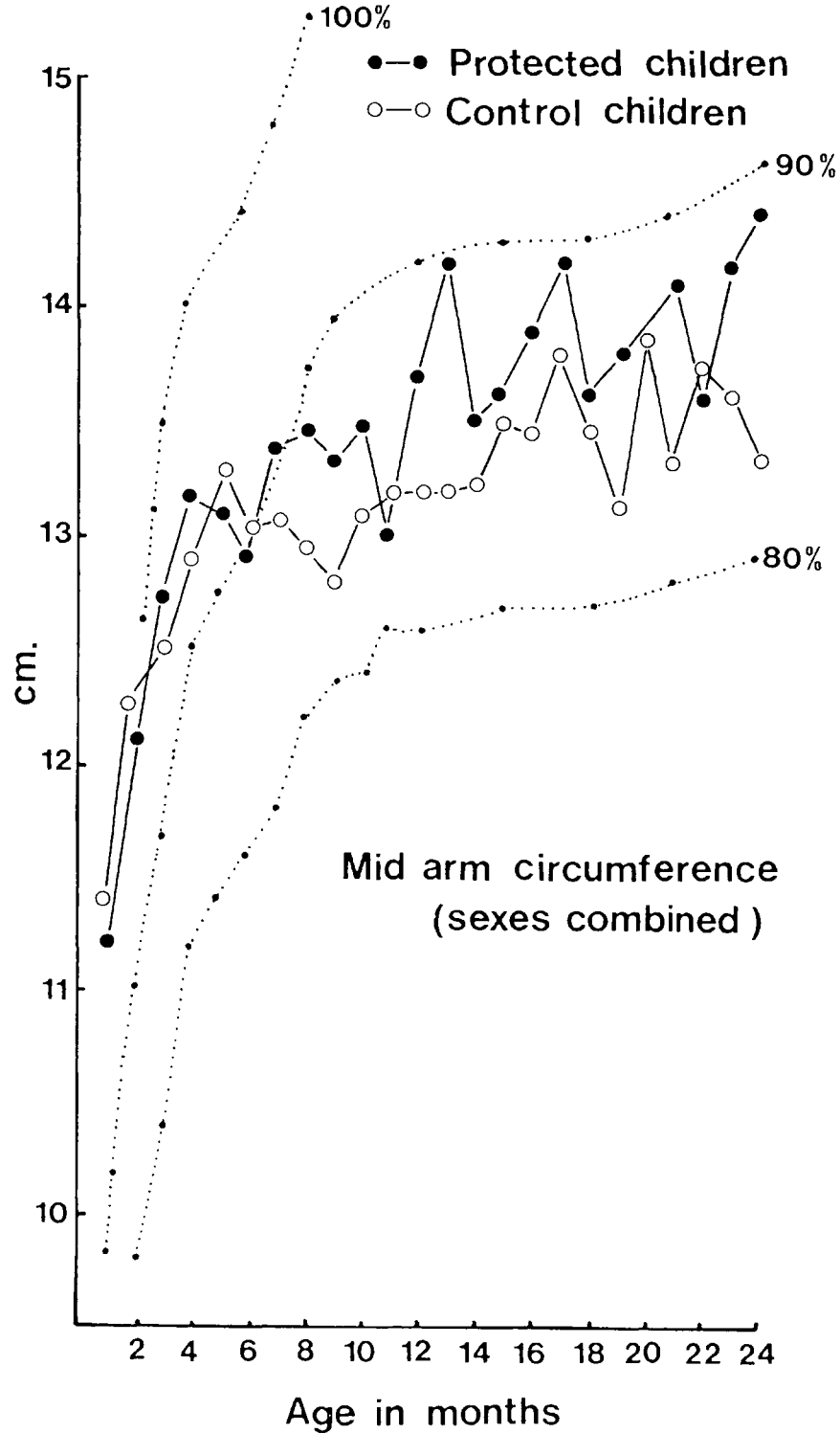


Fig.5d. The percentage of protected and control children with a weight of < 80% Harvard standard by age in months

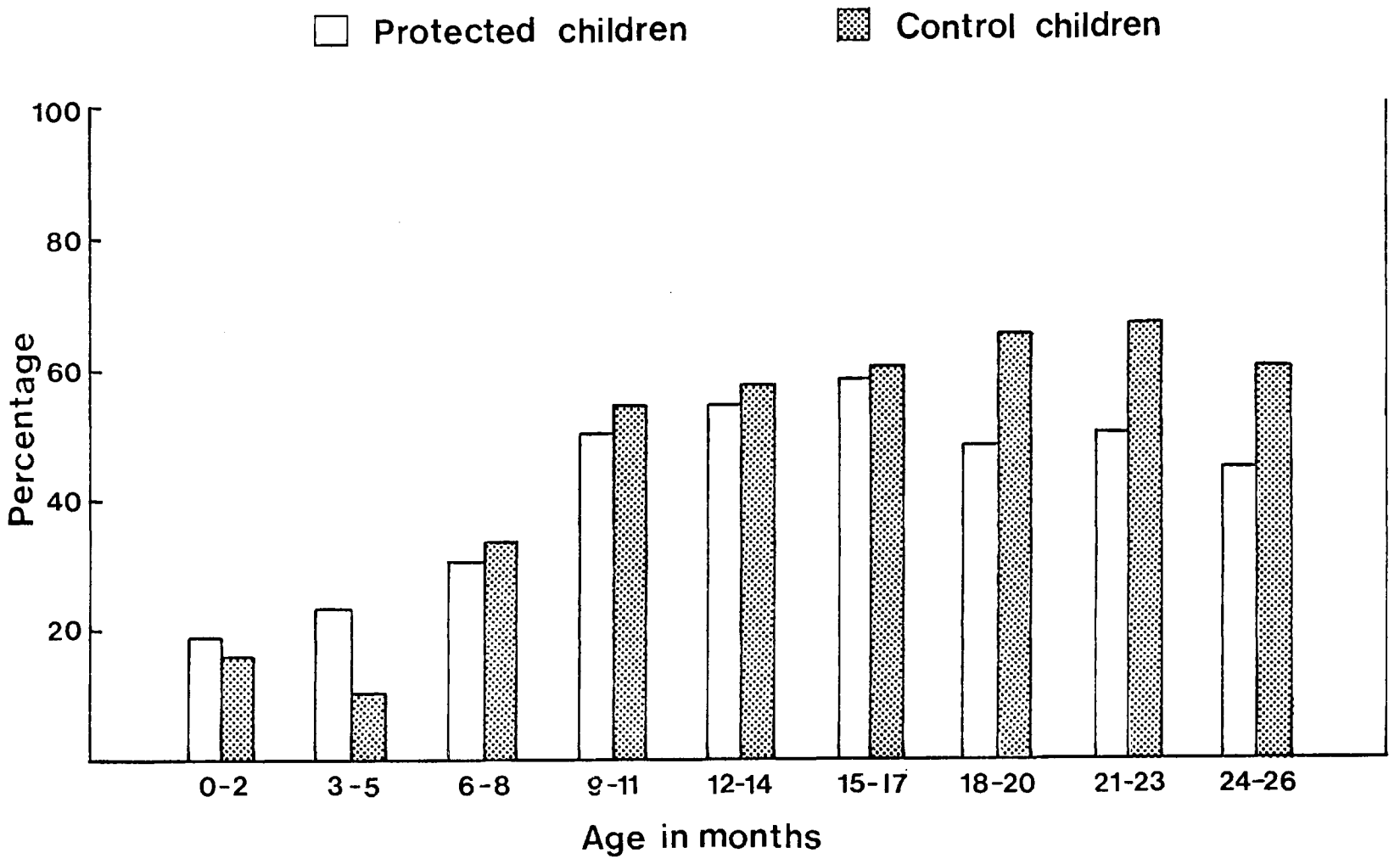


Fig.5e. Mean serum albumin and pre-albumin levels (± 1 standard error of the mean) in protected and control children of different ages.

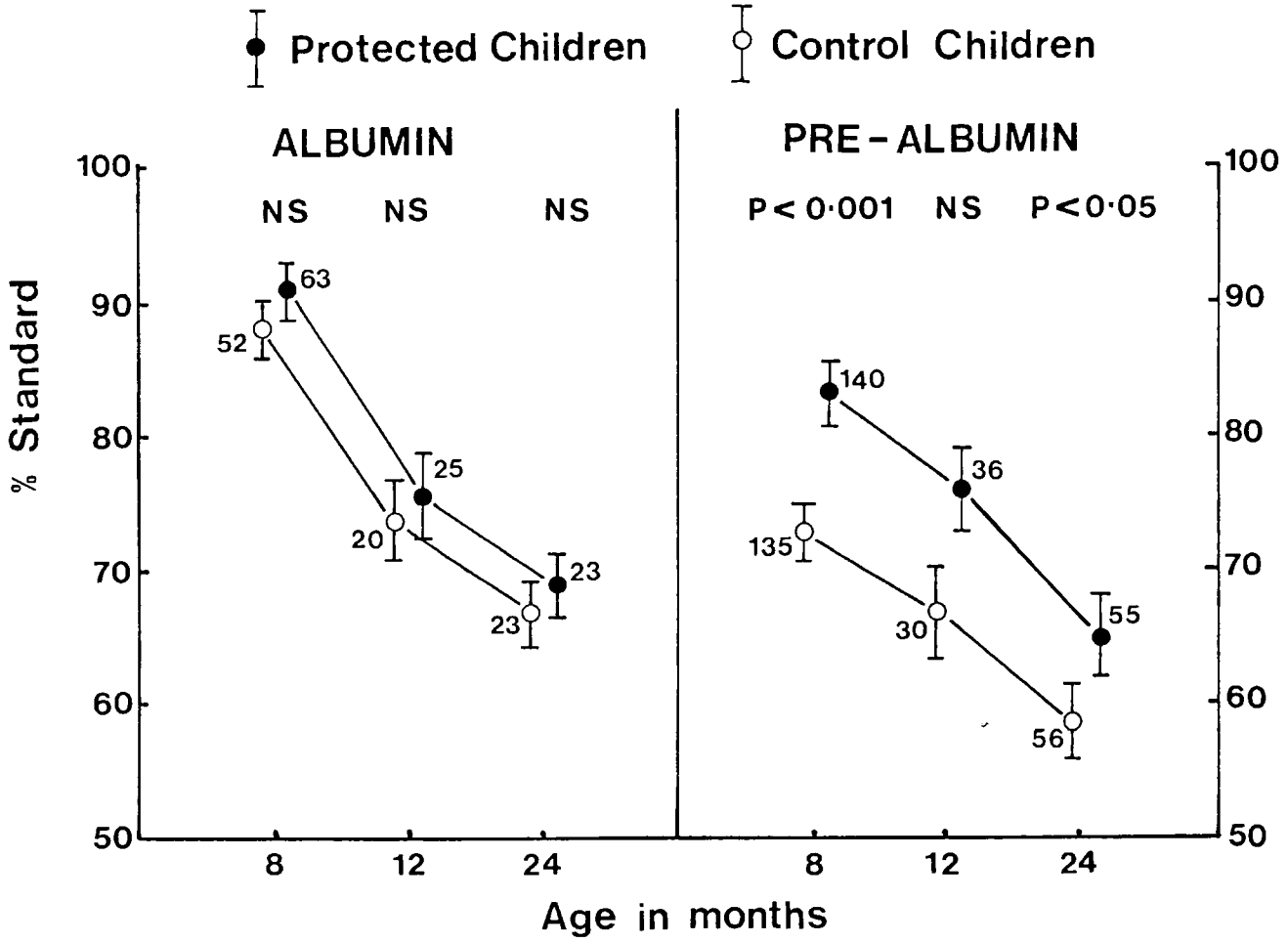
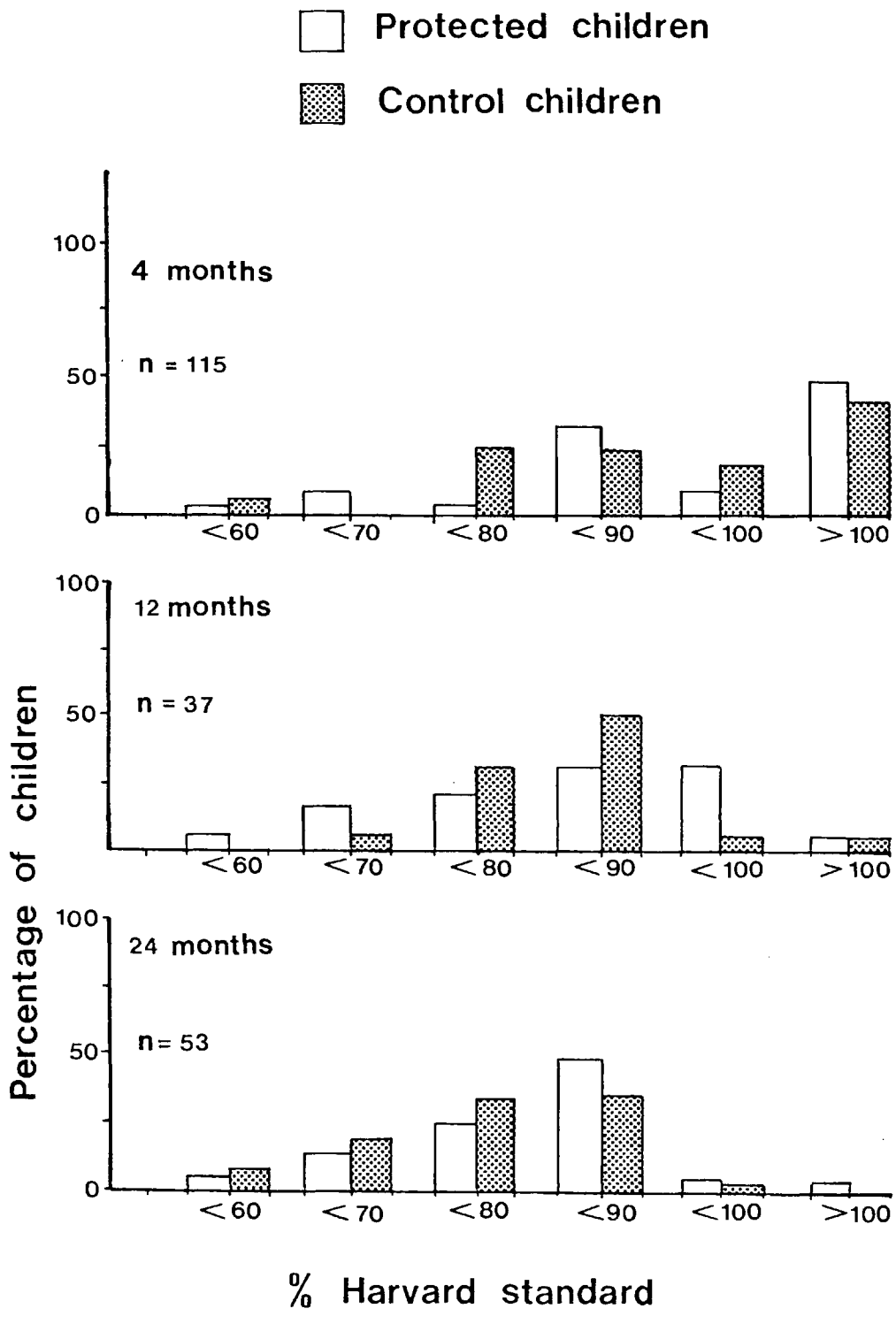


Fig. 5f. The distribution of weights as a percentage of the Harvard standard among protected and control children at the times of vaccination.



CHAPTER 6 - HAEMATOLOGICAL CHANGES AND MALARIA CHEMOPROPHYLAXIS.

1. INTRODUCTION

In tropical developing countries young children are often anaemic as a result of dietary deficiencies and as a consequence of infection with hookworm, malaria and other parasites. Episodes of acute malaria associated with high parasitaemia can cause severe anaemia as a result of direct damage to a large number of red blood cells by the parasite. Less severe malarial infections may also be associated with anaemia. The mechanisms of the anaemia seen in such patients is complex. Malaria causes hyperplasia of the reticuloendothelial system and this may lead to enhanced destruction of both normal and parasitised red blood cells (Sheagren et al., 1970). Immunoglobulin G and complement components can be demonstrated on the surface of the red cells of many subjects with malaria (Facer et al., 1979; Woodruff et al., 1979; Greenwood et al., 1979) and such coated cells are probably more susceptible to phagocytosis by reticuloendothelial cells which possess immunoglobulin and complement receptors than normal red blood cells. Severe dyserythropoetic changes may be present in the bone marrow of anaemic children with low levels of malaria parasitaemia (Abdalla et al., 1980). As low levels of malaria parasitaemia can be associated with anaemia it would be expected that successful malaria control campaigns would lead to an increase in the mean haemoglobin (Hb) level of the protected subjects. A limited number of studies undertaken in Africa suggest that this is the case.

In The Gambia McGregor et al. (1956) showed that the mean packed cell volume (PCV) of a small group of children aged 0-36 months who received malaria chemoprophylaxis with chloroquine was approximately 3% higher than the mean PCV of age matched control children. However, Archibald and Bruce-Chwatt (1956) found little difference between the Hb level of Nigerian schoolchildren taking pyrimethamine weekly during term time and the Hb level of control children. Red blood cell (RBC) and white blood cell (WBC) counts were also similar in the

two groups. Following a successful malaria control programme in Tanzania Draper and Draper (1960) found that the mean Hb of both children and adults increased by 1-2 g/dl over the mean values obtained before the start of the control programme. The most marked difference between pre- and post-intervention values was observed in children aged 3-24 months. Some improvement in Hb level was also observed in a control population living in a nearby area where no malaria control was in operation so that it is possible that part of the increase in Hb observed in the study population was due to an overall improvement in living standards throughout the study area as well as to the effects of the malaria control programme. During a trial of pyrimethamine and pyrimethamine combinations in Nigerian schoolchildren Lucas et al. (1969) noted a fall in PCV of 3% or more in approximately twice as many children in the control group as among children receiving one or other of the pyrimethamine combinations. More recently Björkman et al. (1980) have reported a significant rise in mean PCV among Liberian children aged 2-9 years given chlorproguanil for 2 years but no increase in PCV was observed in children given chemoprophylaxis with pyrimethamine.

In the present study these observations on the effects of malaria chemoprophylaxis on Hb and PCV levels have been extended to include an assessment of the impact of chemoprophylaxis on other haematological variables including red cell indices, total and differential white cell counts, plasma folate and serum ferritin levels. Haematological findings in children with and without malaria parasitaemia have been contrasted.

2. RESULTS

a. Haemoglobin, packed cell volume and red blood cell abnormalities

A clinical diagnosis of severe anaemia was made in only 2 children; one child had received chemoprophylaxis whilst the other had not. Both

of these children died.

The results of Hb and PCV determinations in protected and in control children at different ages are shown in Figs. 6a and 6b and in Table 6a. Protected children maintained a consistently higher mean Hb (1-1.5 g/dl) and mean PCV (2%-4%) than control children throughout the period of observation. An analysis of variance showed that the differences between the two groups is highly significant for both Hb and PCV ($P < 0.001$ in each case). In both groups the mean Hb, but not the mean PCV, increased with age. In order to determine whether administration of malaria chemoprophylaxis might prevent the occurrence of severe anaemia, as well as resulting in a modest overall increase in Hb, children in whom a haemoglobin of less than 6.6 g/100 ml or in whom a PCV of less than 22% had been obtained on any occasion were identified. (These arbitary values are the mean of all Hb and PCV determinations less two standard deviations of the mean.) Eight such anaemic children were identified among control children and only three among children receiving chemoprophylaxis but this difference is not statistically significant ($P > 0.05$). As anticipated children with malaria parasitaemia had significantly lower mean Hb, PCV and RBC counts than children without parasitaemia (Table 6b). The results of measurements of various red cell indices in protected and in control children are shown in Table 6a. Mean values for mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) did not differ significantly between the two groups. Neither did these indices differ between children with or without malaria parasitaemia (data not shown). Thin blood films obtained from protected and from control children were examined for red blood cell abnormalities. If these were found they were recorded as mild, moderate or severe. Table 6c notes the number and percentage of films showing moderate or severe abnormalities. With the exception of poikilocytosis all abnormalities were found more

frequently in control children than in children receiving malaria chemoprophylaxis; for anisocytosis, macrocytosis, microcytosis, polychromasia and hypochromasia, the differences between the two groups are statistically significant. Macrocytosis, microcytosis and polychromasia were also noted significantly more frequently in films showing parasitaemia than in those without parasitaemia ($P<0.001$ in each case).

b. White cell counts

The total white cell counts recorded in protected and in control children at different ages are shown in Fig. 6c. The mean total white cell count increased with age among children in both groups. No significant differences in total white cell count were observed between protected and control children at any age. The mean value of all white cell counts made in protected children was $10.6 \pm 4.8 \times 10^9/L$ whilst the mean value of all observations made in control children was $10.9 \pm 4.0 \times 10^9/L$. The differential white cell count altered with age (Table 6d); children aged 2 years had a higher mean percentage of neutrophils and a lower mean percentage of lymphocytes than children under the age of 6 months. This age related change was observed among both protected and control children. Mean differential white cell counts recorded in protected and in control children are shown in Table 6e. No significant differences in differential white cell counts were observed between the two groups. However, children with malaria parasitaemia had a significantly higher percentage of neutrophils and monocytes than children without parasitaemia and a significantly lower percentage of lymphocytes and eosinophils (Table 6f).

c. Plasma and red blood cell folate levels

Plasma folate measurements in protected and in control children are shown in Table 6g. Plasma folate levels were at the upper limit of the normal range in both groups of children. Control children had a slightly higher mean plasma folate level than protected children but

the difference between the two groups is not statistically significant ($P > 0.05$).

Red blood cell folate levels calculated according to the formula described in chapter 2 are shown in Table 6h. Mean red blood cell folate levels were significantly higher in control than in protected children at the ages of 1 year and 2 years. Both plasma and red blood cell folate levels were slightly, but not significantly, lower in 2 year old children than in 1 year old children. Children with malaria parasitaemia had a similar mean plasma folate level to children without parasitaemia (Table 6i) but children with parasitaemia had a significantly higher red cell folate level than children without parasitaemia.

d. Serum ferritin

Serum ferritin levels in protected and in control children are shown in Table 6j. A wide range of values was observed among both protected and control children but in both groups mean values were on the low side of normal. Mean values in protected children were slightly higher than those in control children but differences between the two groups are not statistically significant. Among both groups of children samples collected at the age of 2 years had significantly higher mean values than samples collected at the age of 1 year. Children with malaria parasitaemia had a significantly higher mean serum ferritin level than children without parasitaemia (Table 6i).

3. DISCUSSION

The findings reported in this chapter are in keeping with the results of the 3 studies reviewed in the introduction (McGregor et al., 1956; Lucas et al., 1969; Björkman et al., 1980) which showed that successful control of malaria with chemoprophylaxis resulted in a modest increase in the mean Hb or PCV of the protected population. In the present study it was shown that the difference between protected and

control children was apparent within a few weeks of starting prophylaxis and that morphological abnormalities of red blood cells were present less frequently in protected children than in control children. Overall mean Hb and PCV levels among protected and control children were just within the normal range for European children of a comparable age. It might be anticipated that in such a population malaria chemoprophylaxis would exert its most important clinical effect by preventing severe anaemia in a small number of children rather than by producing a modest increase in haemoglobin levels in a larger proportion of the protected population. Thus, an assessment was made of the number of children noted to have very low Hb or PCV levels at any time during the course of the study. Such children were encountered less frequently in the protected group than in the control group but the difference observed was not statistically significant.

Africans living in Africa tend to have lower total white cell counts than Europeans and healthy Africans have a lower proportion of neutrophils and a higher proportion of lymphocytes in their differential white blood cell count than Europeans. Various theories have been put forward to explain these differences. Shaper and Lewis (1971) suggested that the neutropenia of Africans was primarily genetic but Ezeilo (1972; 1978) has shown that Zambians living in Europe have higher white cell counts than Zambians living at home and he believes that the neutropenia of Africans is predominantly due to environmental factors such as diet. In order to test whether malaria might play a part in producing the low total white cell counts and relative neutropenia frequently encountered in healthy northern Nigerians total and differential white cell counts were performed in protected and control children throughout the study period. As expected, total white cell counts were higher in these young children than those found in Nigerian adults but no difference in total or in differential white cell count was observed between children in the two groups. These observations

are in agreement of those of McGregor et al. (1956) who found that young Gambian children protected with chloroquine had a similar mean total white cell count to that of control children and with the findings of Archibald and Bruce-Chwatt (1956) in older Nigerian children. However, Draper and Draper (1960) noted a small, but significant, fall in total white cell count among Tanzanian children after the introduction of a successful malaria control programme.

Chronic haemolytic anaemia enhances erythropoiesis and can result in folate deficiency in subjects with a poor dietary intake of this vitamin. Thus, some paediatricians recommend that a child who is recovering from an attack of acute malaria should be given folic acid supplements. However, there is little direct evidence that malaria leads to folic acid deficiency in children. In a recent study undertaken in The Gambia Abdalla et al. (1980) found normal serum folate levels in anaemic children associated with either high or low levels of malaria parasitaemia. Therefore, it was not surprising that we found that the mean plasma folate level of control children was no lower than those of protected children and that children with parasitaemia had a similar mean plasma folate level to those without parasitaemia. However, it is very difficult to understand why control children had a significantly higher mean red blood cell folate level than protected children and why children with parasitaemia had a higher red blood cell folate level than children without parasitaemia. These findings could not be due to a seasonal effect as blood samples for folate estimation were collected from both protected and control children at the same time of the year. Although some antimalarials have an antifolate activity this is not a property of chloroquine.

Serum ferritin is a valuable indicator of iron status but as ferritin also shows some of the characteristics of an acute phase protein interpretation of serum ferritin levels may be difficult in subjects with infections (Munro and Linder, 1978). In a previous study under-

taken in northern Nigeria Leyland et al. (1979) showed a good correlation between serum iron and serum ferritin levels but some patients with hookworm infection had high serum ferritin levels despite features of iron deficiency. Serum ferritin levels were measured in a small number of protected and control children. A wide range of values was obtained but no difference in mean values was found between the two groups of children. Mean values for both groups were at the lower end of the normal range obtained in adult non-elite Nigerians (Leyland et al., 1979) and within the normal range described for European children. Flynn et al. (personal communication) have found very high serum ferritin levels in European patients with acute malaria which fall on treatment. In the present study children with malaria parasitaemia were found to have a higher mean serum ferritin level than children without parasitaemia, perhaps reflecting the role of serum ferritin as an acute phase protein.

TABLE 6a

Haemoglobin, packed cell volume and red blood cell indices recorded in children receiving malaria chemoprophylaxis and in control children over a 2 year period. Mean values ± 1 standard deviation are shown. Figures in brackets indicate the number of observations made.

Measurement	Units	Normal range*	Protected children	Control children
Haemoglobin (Hb)	g/dl	10.5 - 14.0	**10.8 ± 2.0 (140)	10.0 ± 1.7 (99)
Packed cell volume (PCV)	%	32.0 ± 42.0	**35.9 ± 5.5 (197)	33.7 ± 6.5 (172)
Red blood cell count (RBC)	$x10^{12}/L$	3.5 - 5.6	4.4 ± 0.8 (144)	4.2 ± 0.8 (113)
Mean corpuscular haemoglobin (MCH)	pg	24.0 - 30.0	25.0 ± 2.6 (25)	26.1 ± 2.4 (19)
Mean corpuscular haemoglobin concentration (MCHC)	g/dl	30.0 - 36.0	32.2 ± 1.8 (27)	32.0 ± 2.4 (21)
Mean corpuscular volume (MCV)	fl	76.0 - 88.0	82.0 ± 10.5 (144)	79.7 ± 9.7 (115)

* Normal range for European children aged 6 months - 6 years (Belton, 1978)

** $P < 0.001$.

TABLE 6b

Haematological findings in children with or without malaria parasitaemia. Measurements made in protected and in control children have been combined. Mean values ± 1 standard deviation are given. Figures in brackets indicate the number of observations made.

Measurement	Units	Parasitaemia present	Parasitaemia absent	Significance (Student's 't' test)
Haemoglobin (Hb)	g/dl	9.3 ± 2.2 (70)	11.0 ± 1.6 (165)	$P<0.001$
Packed cell volume (PCV)	%	30.9 ± 5.9 (86)	36.0 ± 5.2 (203)	$P<0.001$
Red blood cell count (RBC)	$x10^{12}/L$	3.9 ± 0.9 (73)	4.5 ± 0.7 (178)	$P<0.001$
White blood cell count (WBC)	$x10^{9}/L$	10.7 ± 3.6 (73)	10.7 ± 4.4 (174)	NS

TABLE 6c

The number of thin blood films obtained from protected or control children which showed moderate or severe red blood cell abnormalities.

Abnormality	Protected children n = 447		Control children n = 384		Significance (X^2 test)
	Number	%	Number	%	
Anisocytosis	139	31.8	161	41.9	$P < 0.01$
Poikilocytosis	61	13.8	39	10.2	NS
Microcytosis	89	19.9	111	29.0	$P < 0.01$
Macrocytosis	60	13.4	80	20.9	$P < 0.01$
Polychromasia	8	1.8	27	7.1	$P < 0.001$
Hypochromia	28	6.3	39	10.2	$P < 0.05$
Target cells	30	6.7	35	9.2	NS

TABLE 6d

The influence of age on the differential white cell count. Data from 684 observations in both protected and control children are presented. Mean percentage values ± 1 standard deviation are shown.

	3-5	6-11	12-17	18-23	24-36
Neutrophils	23 ± 11	27 ± 12	31 ± 15	35 ± 9	35 ± 13
Eosinophils	5 ± 7	4 ± 4	3 ± 3	3 ± 1	5 ± 6
Basophils	1	1	1	1	1
Lymphocytes	70 ± 13	67 ± 12	62 ± 15	58 ± 15	55 ± 12
Monocytes	3 ± 3	3 ± 3	5 ± 3	5 ± 3	5 ± 4

TABLE 6e

The differential white cell count observed in blood films obtained from protected and control children. Mean percentage values ± 1 standard deviation are shown.

Cell type	Protected children n = 424	Control children n = 355	Significance (Student's 't' test)
Neutrophil	27.0 ± 12.3	27.7 ± 13.1	NS
Eosinophil	4.5 ± 6.0	4.1 ± 5.1	NS
Basophil	0.1 ± 0.3	0.1 ± 0.4	NS
Lymphocytes	65.1 ± 13.2	64.5 ± 13.5	NS
Monocytes	3.2 ± 2.9	3.6 ± 3.3	NS

TABLE 6f

Differential white cell counts in children with or without malaria parasitaemia. Measurements made in protected and in control children have been combined. Mean values, expressed as a percentage, ± 1 standard deviation are given.

	Parasitaemia present n=136	Parasitaemia absent n=524	Significance (Student's 't' test)
Neutrophils	32.8 ± 13.7	26.1 ± 12.3	$P < 0.001$
Eosinophils	2.8 ± 3.3	4.7 ± 6.1	$P < 0.001$
Basophils	0.1 ± 0.5	0.1 ± 0.3	NS
Lymphocytes	59.8 ± 13.7	65.9 ± 13.2	$P < 0.001$
Monocytes	4.5 ± 3.9	3.2 ± 2.8	$P < 0.001$

TABLE 6g

Plasma folate levels in protected and in control children aged 1 or 2 years. Mean values ± 1 standard deviation, expressed as ng/ml are shown. Figures in brackets indicate the number of observations made. The normal range of values for European children is 5-15 ng/ml (Belton, 1978) and for adult northern Nigerians 5-12 ng/ml (Leyland et al., 1979).

Age	Protected children	Control children	Combined
1 year	14.76 ± 4.47 (13)	16.19 ± 4.33 (8)	15.30 ± 4.42 (21)
2 years	12.72 ± 5.45 (22)	15.66 ± 4.78 (21)	14.15 ± 5.12 (43)

TABLE 6h

Red blood cell folate levels in protected and in control children aged 1 or 2 years. Mean values ± 1 standard deviation, expressed as ng/ml are shown. Figures in brackets indicate the number of observations made. The normal range of values for adult Nigerians is 117-400 ng/ml (Fleming et al., 1971).

Age	Protected children	Control children	Combined
1 year	*360.0 ± 106.0 (11)	603.1 ± 156.8 (8)	462.6 ± 127.4 (19)
2 years	*305.0 ± 84.3 (20)	567.1 ± 108.4 (19)	410.0 ± 154.5 (39)

*$P < 0.001$ for comparisons between protected and control children.

TABLE 6i

Plasma and red blood cell folate levels and serum ferritin levels in children aged 1 year or 2 years with and without malaria parasitaemia. Measurements made in protected and control children have been combined. Mean values ± 1 standard deviation, expressed as ng/ml are shown. Figures in brackets indicate the number of observations made.

	Parasitaemia present	Parasitaemia absent	Significance (Student's 't' test)
Plasma folate			
1 year	16.9 ± 4.0 (7)	14.7 ± 4.5 (13)	NS
2 years	14.7 ± 5.5 (25)	13.8 ± 4.9 (17)	NS
Red cell folate			
1 year	652.6 ± 201.9 (7)	381.7 ± 116.8 (12)	$P < 0.01$
2 years	492.4 ± 224.7 (24)	291.3 ± 76.1 (15)	$P < 0.01$
Serum ferritin			
1 year	45.3 ± 44.7 (7)	23.0 ± 17.9 (13)	NS
2 years	104.3 ± 66.7 (26)	45.1 ± 45.3 (15)	$P < 0.01$

TABLE 6j

Plasma ferritin levels in protected and in control children aged 1 or 2 years. Mean values ± 1 standard deviation, expressed as ng/ml are shown. Figures in brackets indicate the number of observations made. The normal range of values for adult Nigerians is 20-280 ng/ml (Leyland et al., 1979).

Age	Protected children	Control children	Total
1 year	26.0 ± 19.9	35.8 ± 43.7	*29.7 ± 29.0
	(13)	(8)	(21)
2 years	77.3 ± 66.1	86.0 ± 67.0	81.7 ± 66.6
	(20)	(21)	(41)

*$P < 0.01$, 1 year versus 2 years.

Fig. 6a. Haemoglobin values in protected and in control children by age. Mean values ± 1 standard error of the mean are shown. Figures indicate the number of observations made.

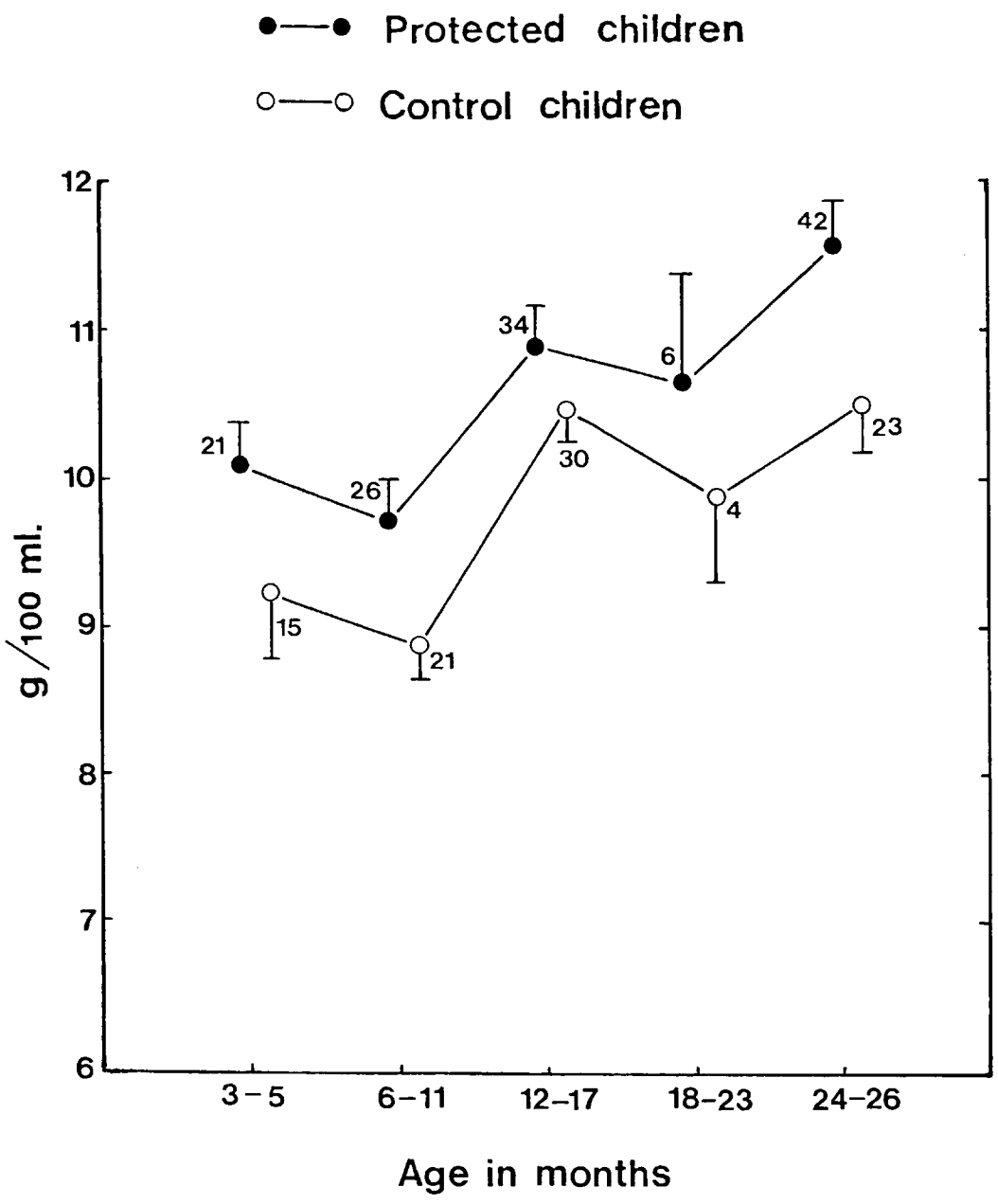


Fig. 6b. Packed cell volumes in protected and in control children by age. Mean values ± 1 standard error of the mean are shown. Figures indicate the number of observations made.

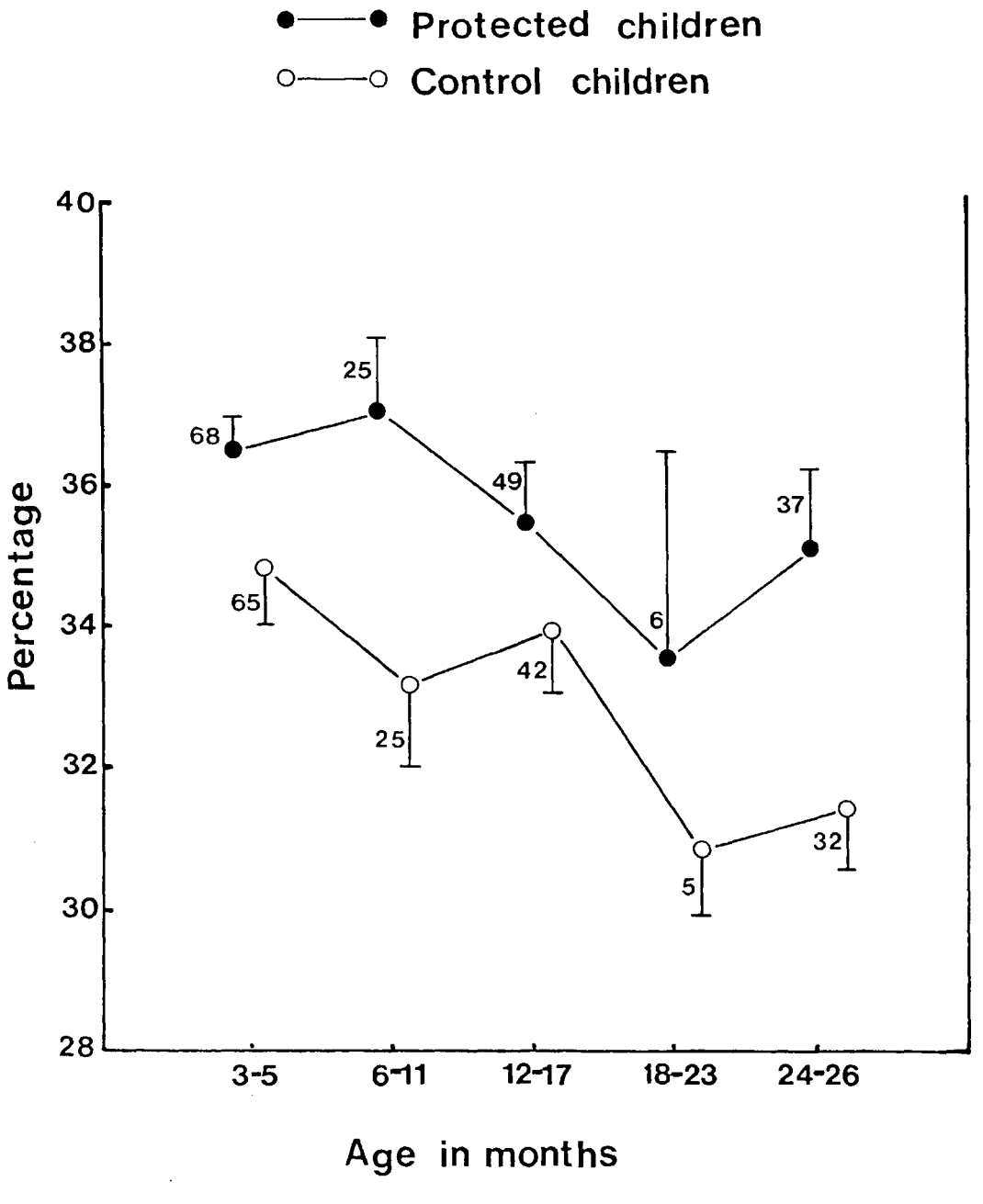
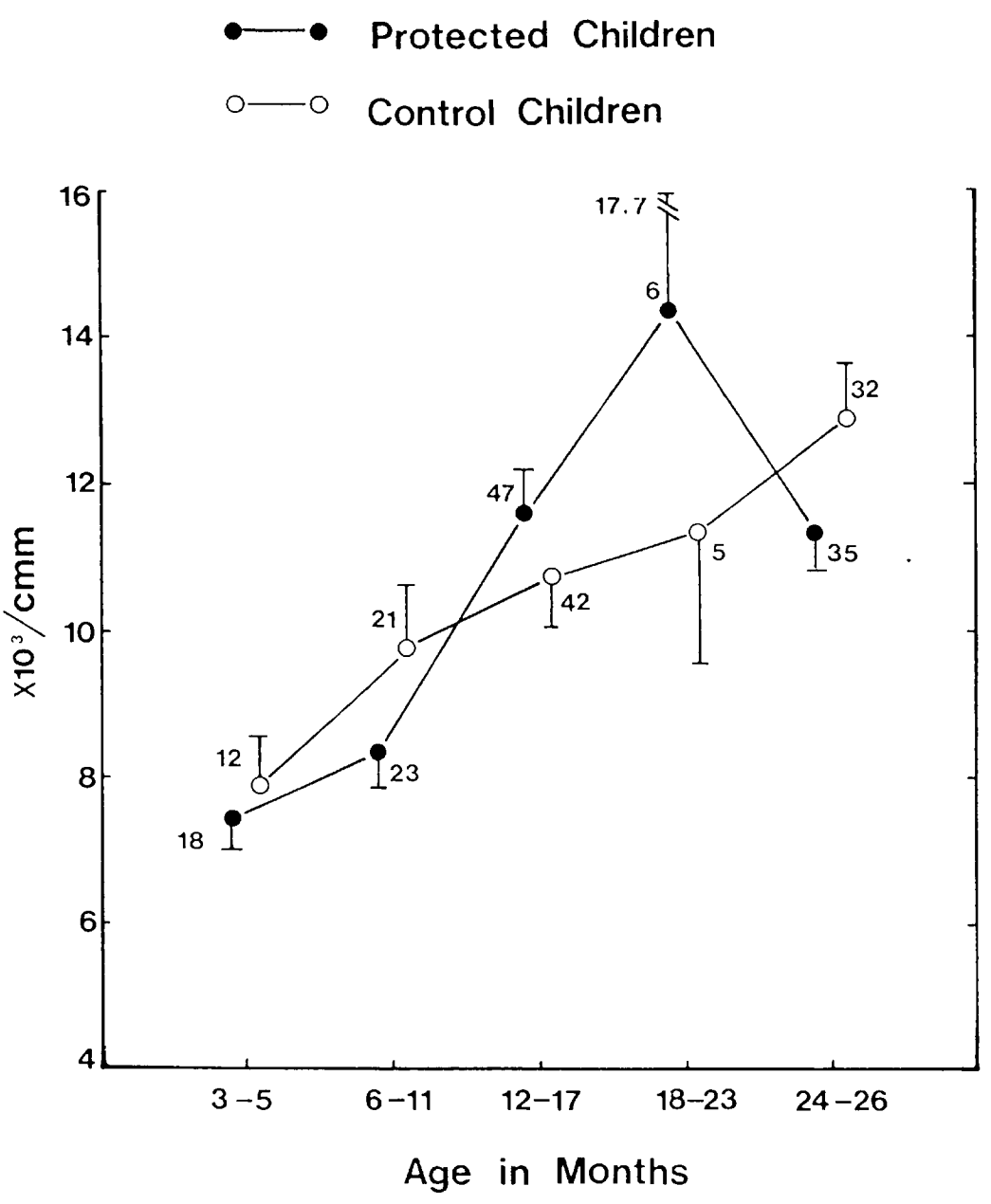


Fig. 6c. Total white cell counts in protected and in control children by age. Mean values $\pm$ 1 standard error of the mean are shown. Figures indicate the number of observations made.



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Addendum

DIALLO, S., COULIBALY, A. et KONATÉ, L. (1976). Bulletin de Société de Médicine Afrique Noire, Langue Français, 21: 398-401. Effets de l'administration de l'association sulfadoxine - pyriméthamine sur l'accès palustre en zone d'hyperendémie.

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Enhancement of the immune response to meningococcal polysaccharide vaccine in a malaria endemic area by administration of chloroquine

Acute, clinical malaria suppresses the immune response to tetanus, typhoid and meningococcal vaccines (McGregor and Barr, 1962; Greenwood *et al.*, 1972; Williamson and Greenwood, 1978) and the antibody response to meningococcal polysaccharide vaccines is diminished in subjects with asymptomatic parasitaemia (Greenwood *et al.*, 1980). We have therefore investigated whether, in a malaria endemic area, the antibody response to vaccination can be enhanced by the administration of chloroquine at the time of vaccination.

Two hundred and six Nigerian infants aged three to 17 months were studied during the course of a trial of a combined meningococcal, measles and tetanus vaccine. Details of the epidemiological and serological methods employed in this study are given elsewhere (Greenwood *et al.*, 1981). On entry into the trial, children were allocated randomly into one of two groups. Those in one group each received 5 ml chloroquine syrup (50 mg of chloroquine base) one week before immunization, when households were being informed of the coming vaccination campaign, and immediately before immunization. Children were watched for ten to 20 minutes after chloroquine was given to see if any vomiting occurred; if it did, the drug was readministered. A few children vomited persistently and so did not receive an adequate dose of chloroquine. Children in the second group received no anti-malaria treatment. All children received a single injection of combined vaccine containing 50 μg of group A and group C meningococcal polysaccharides, 30 Lf of tetanus toxoid and at least 1000 $TCID_{50}$ of measles vaccine virus. One month after vaccination half the children in each group received a second injection of group A and group C meningococcal polysaccharide vaccine whilst the remainder received an injection of tetanus toxoid. The mean age of the children in the group receiving chloroquine (8·7 months ± 4·4 months) was similar to the mean age of the group of children who did not (9·2 months ± 4·3 months).

At the time of vaccination, one week after the initial chloroquine treatment, only six of the 90 treated children had a positive thick blood film whilst 50 of the 85 control children showed malaria parasitaemia.

The antibody levels to the four components of the combined vaccine found in chloroquine treated and in control children before, one month after and six months after vaccination are shown in Table 1. Children who received chloroquine had significantly higher group A and group C meningococcal haemagglutinating antibody levels one month after vaccination than did control children, but six months after vaccination this difference was no longer apparent. One month after vaccination the proportion of children with no detectable group A meningococcal antibody was lower among those who had received chloroquine than among those who had not (21/93 compared with 31/91) but this difference is not statistically significant. A similar trend was found for meningococcal group C antibodies; nine of 93 children who had received chloroquine had no detectable group C meningococcal antibody compared with 15 of 91 children who had not received chloroquine. This difference is not statistically significant.

Among the group of children who did not receive chloroquine the rise in both group A and group C meningococcal HA antibody titres following vaccination was significantly less in those with malaria parasitaemia than in those without parasitaemia (Table 2).

There were no significant differences in the antibody response to the tetanus toxoid and measles components of the vaccine between children who had received chloroquine and

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TABLE 1

Antibody levels in two groups of children before, one month after and six months after immunization with a combined meningococcal, measles and tetanus vaccine. One group of children received chloroquine at the time of vaccination and one week previously while the other group did not. Mean antibody titres (log_2) ± 1 standard error of the mean are shown

	Chloroquine given			*Chloroquine not given*		
	Pre	*1/12 post*	*6/12 post*	*Pre*	*1/12 post*	*6/12 post*
Meningococcal group A	1·6 ± 0·2	*2·7 ± 0·2	1·8 ± 0·2	1·4 ± 0·1	1·9 ± 0·2	1·6 ± 0·1
Meningococcal group C	1·6 ± 0·2	*3·0 ± 0·2	1·6 ± 0·1	1·4 ± 0·1	2·4 ± 0·2	1·6 ± 0·1
Measles	0·6 ± 0·2	4·0 ± 0·2	3·5 ± 0·4	0·7 ± 0·2	4·2 ± 0·3	2·9 ± 0·3
Tetanus	0·6 ± 0·1	6·6 ± 0·2	5·7 ± 2·6	1·0 ± 0·1	6·7 ± 0·2	5·9 ± 0·4

*P < 0·01.

TABLE 2

A comparison of the antibody response to the meningococcal components of a combined meningococcal, measles and tetanus vaccine in children with or without malaria parasitaemia at the time of vaccination. The mean rise in antibody titre (log_2) following vaccination ± 1 standard error of the mean is shown

	Malaria parasitaemia	*No malaria parasitaemia*
No. of children	50	35
Meningococcal group A	*0·2 ± 0·04	0·9 ± 0·05
Meningococcal group C	*0·5 ± 0·04	1·2 ± 0·06

*P < 0·01.

between those who had not. The antibody responses to these components of the vaccine were similar in children with or without malaria parasitaemia.

Our results show that in a malaria endemic area prior treatment with chloroquine enhances the antibody response to meningococcal vaccines but not the response to tetanus toxoid or measles vaccine. This difference probably depends upon the fact that meningococcal vaccines are purified polysaccharides which have a number of unusual characteristics. They are weakly immunogenic in the very young and are poor at inducing immunological memory. The response to the vaccines can be influenced by malaria, and possibly by other environmental factors (Greenwood *et al.*, 1980). Pneumococcal polysaccharide vaccines possess many of the same characteristics as meningococcal vaccines and it is possible that in a malaria endemic area the response to pneumococcal polysaccharide vaccines might also be enhanced by administration of chloroquine at the time of vaccination. Our preliminary findings in young children require confirmation in older children who generally make a much better response to polysaccharide vaccines.

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