Impact of asymptomatic malaria parasitaemia on cognitive function and school achievement of schoolchildren in the Yemen Republic

by

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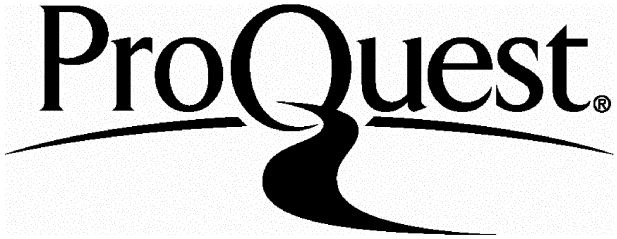

Abstract

Asymptomatic malaria parasitaemia is highly prevalent among schoolchildren especially in hyperendemic and holoenedemic areas where up to 90% of school-children are affected. We hypothesized that parasitaemia may have a direct but transient effect on cognition through cytokine production. The impact of asympto-matic parasitaemia on cognitive functions and school achievement was examined in two separate studies, with different designs, that were conducted in two hyperendemic areas in Yemen. We first conducted a randomized controlled trial of the effect of treatment of asymptomatic parasitaemia on cognitive functions of schoolchildren. Due to unforeseen rapid clearance of parasitaemia among the placebo group we abandoned the trial after half the children had been enrolled and ran a second study designed to be a natural trial. A group (n= 445) of asymptomatic parasitaemic boys and a group of non-parasitaemic boys (n=142) matched for grade and school were first compared on their performance on a battery of cognitive and school achievement tests. Two weeks later the parasitaemic children were re-screened and 150 children of those who remained parasitaemic were matched for grade and school with 150 children who were previously parasitaemic but no longer parasitaemic. These children were then re-tested and compared on their performance on the cognitive tests.

The results showed a high prevalence of anaemia and malnutrition among school-children. The parasitaemic children were significantly more anaemic, had a higher prevalence of splenomegaly, were more likely to come from fathers who were illiterate and farmers. After controlling for age, socioeconomic background and nutritional status it was found that the parasitaemic children performed worse than the

non-parasitaemic children in fine motor function tests, but not on the other cognitive tests. The parasitaemic children in the clinical trial also had worse performance only in these tests.

The results from the natural experiment showed no difference in performance in cognitive tests between those who naturally cured and those who remained parasitaemic. However, children who initially had the highest parasite density improved the most in a fine motor tests and a picture memory test.

C-reactive proteins (CRP) concentrations were determined on enrollment and at follow-up. On both occasions they were significantly higher among parasitaemic children than non-parasitaemic children. The CRP concentrations at follow-up of those who were parasitaemic on enrollment and became non-parasitaemic at follow-up returned to normal concentration, while the CRP concentrations of children who remained parasitaemic remained high. The CRP concentaration was highly positively correlated with parasite density and splenomegaly, and negatively correlated with haemoglobin and picture memory test.

In both the clinical trial and natural trial, parasitaemia was associated with low performance in fine motor function. In addition, initial parasite density predicted changes in scores of the same tests and picture memory. Therefore there is limited evidence that parasitaemia may affect performance in these tests. However, we were unable to show a benefit from loosing parasitaemia over a two weeks period. The need for more research in this area and the ideas for future studies are highlighted.

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Dedication

To the future, the schoolchildren in the Yemen and all over the developing world

wishing them better health and brilliant future

Acknowledgment

"All praise is due to Allah, the Lord of the worlds. The Beneficent, the Merciful"

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List of acronyms

BMI	Body mass index
°C	Degree Celsius
CI	Confidence interval
CNS	Central nervous system
CFS	Chronic fatigue syndrome
CRP	C-reactive protein
df	Degrees of freedom
dL	Decilitre
DQ	Development quotient
EEG	Electroencephalogram
fL	Femtolitre
g	Gram
HAZ	Height-for-age Z-score
Hb	Haemoglobin
Hct	Haematocrit
HPF	High power field
kg	Kilogram
IDA	Iron deficiency anaemia
IL-1	Interleukin-1
IL-6	Interleukin-6
INF-α	Interferon –alpha

INF-γ	Interferon –gamma
IQ	Intelligence quotient
MCV	Mean corpuscular volume
MFFT	Matching Familiar Figures Test
mo	month
M	mollar
n	Number of observations
ng	nanogram
nm	nanometre
NMCP	National Malaria Control Programme
P	Probability
PCR	Polymerase chain reaction
PEM	Protein energy malnutrition
Pl	Placebo
OR	Odds ratio
r	Correlation coefficient
R	Coefficient of multiple correlation
RBC	Red blood corpuscle
Rx	Treatment
SBP	School breakfast programme
SD	Standard deviation
SE	Standard error
SES	Socioeconomic status

TDS	*Trichuris* Dysentery Syndrome
TNF	Tumour necrosis factor
μL	microlitre
UNESCO	United Nations Educational, Scientific, and Cultural Organization
UNICEF	United Nations Children's Fund
WAZ	Weight-for-age Z-score
WHZ	Weight-for-height Z-score
WRAT	Wide Range Achievement Test
WISC	Wechsler's Intelligence Scale for Children
y	year

Definitions of the main terms used in this study

For the purpose of this research and throughout this study the following terms was used according to the definitions that have been given below:

Parasitaemia: is the presence of *Plasmodium falciparum* in a stained thick blood smear.

Non-parasitaemic children: children who have no *Plasmodium falciparum* seen in 100 high power fields in a stained thick blood smear.

Parasite density: is the number of the *Plasmodium falciparum* parasite per μL of peripheral blood.

Asymptomatic malaria (or asymptomatic parasitaemia): is presence of the *Plasmodium falciparum* parasite in the peripheral blood without any symptoms.

Clinical malaria: is presence of the *Plasmodium falciparum* parasite in the peripheral blood with clinical symptoms e.g. fever

Chapter One

Background information

1. Introduction

This study concerns the impact of asymptomatic malaria parasitaemia on cognitive functions and school achievement of schoolchildren in the Yemen Republic. Some other factors related to cognition such as nutritional status, hunger and breakfast history, and socioeconomic status (SES) have been investigated also.

Recently, Basic Education for All initiative was launched jointly by UNESCO, UNICEF, and the World Bank, and aims to provide primary school education to all children in developing countries. In 1990, at the World Summit for Children, many nations agreed to expand and improve the educational facilities in primary schools. They set a target of achieving basic primary education for at least 80% of the children by the year 2000. As a response to these initiatives, more and more children are enrolling in school in developing countries. However, their achievement levels are generally disappointing. Some studies have shown that the first-grade repetition rate was 42 % and the repetition rate for primary school is 29% (Alleyne, 1998). For the Region of Americas only, the cost of this was estimated to be four billion US$, a cost that will be a great burden to the weak economic structure of many developing countries (Alleyne, 1998). It has been also shown that fewer than 60 % of children complete primary schools, and those do who usually fail to perform at the desired standard. Lockheed and Verspoor (1991) reported that only 10% of 14-year olds are as literate as their fellows in industrialised countries. Figures from some other developing countries may be even worse but data are not available.

It is well known that deficient schooling and poor family conditions are partly responsible for children's poor achievement levels, but health and nutritional factors have usually been ignored. Currently, there is increasing concern that poor health and nutrition may hinder children's cognitive and social development and consequently might detrimentally affect their ability to learn in school (Pollitt, 1990; Grantham-McGregor, Walker, 1998).

UNESCO has started a ten-year special project. The aims of this project are to facilitate the development of aptitudes and abilities involved in school learning. The outcomes that UNESCO and the Ministries of Education would like to improve through health programs are therefore educational ones. These include school performance, alertness, information processing, absenteeism, under-enrolment, dropout rates, and repetition of classes.

Two research priorities have been identified to help in achievement of these aims, mainly in nutrition and infection. The nutritional areas of interest are protein energy malnutrition, iron deficiency anaemia and iodine deficiency (Pollitt, 1990; Ramalingaswami, 1992). Infections mainly with geohelmenthis are also attracting a lot of interest (Pollitt, 1990; Halloran, Bundy, Pollitt, 1989).

I am unaware of any study on the effect of asymptomatic malaria on school achievement and there are only two studies that investigated the impact of asymptomatic malaria on cognitive function. Through the present study about the impact of asymptomatic parasitaemia on cognitive function and school achievement, I hope to throw some light on this unexplored area.

In this section, I will review the relevant literature on the effect of asymptomatic malaria on children's nutritional status, and available literature about malaria and its relation to cognition and schooling.

2. Malaria

2.1. Introduction:

Malaria is a disease caused by sporozoa of the genus *Plasmodium* transmitted by a species of *Anopheles* mosquitoes. There are four recognised species of human *Plasmodium*: *P. falciparum* (malignant tertian, subtertian or falciparum malaria), *P. vivax* (benign tertian or vivax malaria), *P. ovale* (ovale tertian or ovale malaria), and *P. malariae* (quartan or malariae malaria). *Plasmodium falciparum* malaria is the most common and the most deadly species. The genus *Plasmodium* undergoes asexual multiplication in the vertebrate host (schizogony) and sexual multiplication (sporogony) in the *Anopheles* species of mosquito.

Malaria is characterised by fever, which is often periodic; varying degrees of anaemia; splenic enlargement; and various syndromes resulting from the physiological and pathological involvement of certain organs including the brain, the liver, and the kidneys. However, in endemic areas only a small proportion of malaria parasitaemia reach a high enough parasite density to cause an acute illness and the remainder produce a low parasite density that lasts for weeks or months without any apparent symptoms; this is known as asymptomatic parasitaemia or asymptomatic malaria (Greenwood, 1987a).

Malaria is widely distributed throughout the tropics and subtropics and in some temperate zones. According to WHO, the overall world malaria situation has not improved in the last 15 years. It remains a major public health problem throughout

most of the developing world. Of the total world population of about 5.4 billion people, it is estimated that 2.2 billion people are exposed to malaria in some 90 countries. There are approximately 300 to 500 million clinical cases a year and 1.4 to 2.6 million deaths worldwide (WHO, 1995a). One in 15 children on the Kenyan coast will have experienced an episode of severe malaria before the age of five, and 1% of Gambian children less than five years of age will die of malaria (Newton, 1998).

In 1988, WHO estimated that in Africa alone there are 250 million parasite carriers (Sexton, 1994). Prevalence depends on several factors, the most important are the level of endemicity and of acquired immunity. Different levels of prevalence have been described, even within the same country, in different age groups and in different seasons varying from three per cent to 90% (Trape *et al.* 1993; Smith *et al.* 1994; Trape, Rogier, 1996).

There is a vast and continuously expanding literature on malaria which is mostly far beyond the scope of this short literature review. Brief reference will be made mainly to aspects which are relevant to asymptomatic malaria.

2.2. Epidemiology of Asymptomatic malaria:

As a disease, malaria is relatively rare compared with malaria as an infection. In endemic areas, very young infants rarely contract the disease. This protection has been attributed to trans-placental acquired malarial antibodies (Chizzolini *et al.* 1991).

However, above six months of age, unprotected infants suffer repeated severe attacks that become milder as they grew older. From about five years of age and as the children become immuno-protected, the frequency of clinical disease declines and malaria deaths become very rare, though the children frequently have parasitaemia but are apparently well (Marsh, 1992). Colbourne (1955) noticed that many schoolchildren with parasitaemia suffered no disability from malaria. Two recent studies have confirmed this well-known epidemiological feature of malaria. In an endemic area in Tanzania, Shiff and colleagues found that although over 82% of children were parasitaemic, less than 7% had the clinical disease (Shiff *et al.* 1996). In another study in Mali, it was found that out of 78 % carrying the parasites in their blood only 13% had fever (Bouvier *et al.* 1997).

The precise magnitude of the public health problem posed by asymptomatic malaria has always been unclear and under-estimated. Currently, asymptomatic parasitaemia is largely ignored. However, it is possible that it may affect the health status in different ways.

2.3. Prevalence of asymptomatic malaria among schoolchildren:

In spite of the high prevalence of asymptomatic parasitaemia among schoolchildren in endemic areas (Table 1), schoolchildren are not considered the main priority group for treatment or control programs. However, it is possible that parasitaemia may affect their cognitive function and hinder their ability to learn in school (Halloran, Bundy, Pollitt, 1989).

Table 1. Prevalence of asymptomatic parasitaemia among schoolchildren

In

some developing countries

Country	*Prevalence*	*Reference*	*Comments*
Accra, Ghana	20-68%	Colbourne, 1955	According to the season and rural or urban
Tanzania	84%	Pringle, Avery-Jones, 1966	
Nigeria	26-81 %	Fasan, 1969	According to urban and rural
Southern Sudan	63%	Taha, Broadhead 1986	
Nigeria	74 %	Salako *et al.* 1990	
Senegal	3.6-7.5 %	Trape *et al.* 1993	Hypoendemic area
Senegal	83-93 %	Trape *et al.* 1994	Hyperendemic area
Papua New Guinea	41-46%	Smith *et al.* 1994	
Mozambique	68 %	Hogh *et al.* 1995	
Zanzibar	61%	Albonico *et al.* 1997	

2.4. Asymptomatic malaria and anaemia:

Childhood anaemia is increasingly recognised as a major health problem in malarious areas. Many studies have identified clinical malaria as a major cause for anaemia (e.g. Hedberg *et al.* 1993; Binda-ki-Muaka, Nzita, Eeckels, 1995; Zucker *et al.* 1996; Newton *et al.* 1997). It has been shown also, that asymptomatic parasitaemia contributes to the anaemia found frequently in children in areas where malaria is endemic (Greenwood, 1987a). Even low parasite density can generate long-term severe anaemia as result of haemolysis of parasitized cells (Gravenor, McLean, Kwiatkowski, 1995).

Many observational and experimental field studies have identified asymptomatic parasitaemia as a risk factor for anaemia in endemic areas.

2.4.1. Observational studies:

Many cross-sectional and longitudinal surveys have shown that asymptomatic malaria is associated with anaemia. Some of these studies are summarized in table 2.

As a part of the National Food and Nutrition programme in Zambia, a total of 7479 people of both sexes and all age groups were surveyed in rural areas. It was found that among the parasitaemic group, 87% had low haemoglobin (Hb) and 58% had a low haematocrit (Hct) compared with 60% and 21% respectively among the non-parasitaemic group ($P<0.001$) (Wenlock, 1979).

In another cross-sectional survey, Kandiah and colleagues (1984) studied 96 pre-school children one to 72 months old and 61 women of childbearing age whose mean age was 29 years. The results showed that the prevalence of anaemia was higher among those who had parasitaemia (56%) compared with the non-parasitaemic group (41%). The Hb was found to be significantly lower among the subjects who were infected with malaria compared with controls with a mean Hb ± SD of 10.53 ± 1.3 g/dL *versus* 11.41 ± 1.2 g/dL respectively ($P<0.05$).

In Liberia, children two to nine years old from two holoendemic villages were compared with children from a neighbouring hypoendemic town. The parasite and spleen rates of the holoendemic villages were 91% and 99% compared with 13% and 11% respectively in the hypoendemic town. The mean Hct values were significantly ($P < 0.001$) lower among those from the two holoendemic villages: 34.8% and 35.6% compared with 37.9% in the hypoendemic town (Willcox, Bjorkman, Brohult, 1985). However, other possible differences between a village and town which may have accounted for such differences were not properly addressed.

In a longitudinal mortality and morbidity survey in rural Gambia, 600 children under the age of seven years were kept under close surveillance for one year. At the end of the dry and rainy seasons, children were examined clinically and blood samples were collected. It was found that the mean Hct of 214 children with parasitaemia during the post-rainy season survey was 30.6% ± 5.1%, which was significantly less than that of 359 children without parasitaemia: 33% ± 5.2%, ($P < 0.001$) (Greenwood *et al.* 1987b).

In Malawi, a longitudinal study was conducted to investigate the risk factors for anaemia in young children. 252 children delivered in the hospital were seen between 20 and 120 days after birth and their Hct was measured. Parasitaemic infants had a median Hct value of 28% compared with a value of 31% among those without parasitaemia ($P < 0.05$). Also, being parasitaemic at the time of the follow-up visit was associated with having Hct value less than 28% (Relative Risk = 1.55, $P < 0.05$) (Reed, Wirima, Steketee, 1994).

In another community-based study which looked at the risk factors for anaemia in holoendemic malarious areas in Tanzania, 338 children between the ages of 6-40 months were recruited randomly from seven villages in the study area. At enrolment 74% of the children were anaemic and 2.5% were severely anaemic. Of the 2.5 % found severely anaemic, all were parasitaemic. Univariate analysis showed strong association between anaemia and parasitaemia ($P < 0.01$). A multiple regression analysis of Hct on parasitaemia, fever, and splenomegaly showed a strong evidence of association between anaemia and parasitaemia ($P < 0.01$). Both parasitaemia and fever were statistically significant predictors of anaemia (Premji *et al.* 1995).

In another longitudinal study, bimonthly surveys were carried out for 12 months to investigate the dynamics of the acquisition of malaria parasitaemia in relation to development of anaemia in the first year of life among 117 new-born recruited at birth. During the first year of life, the mean Hct level of malaria-positive infants was significantly lower than that of malaria-negative infants. The mean Hct for

parasitaemic group aged 4, 6, 8, and 10 months were 29 ± 3.01, 27 ± 0.78, 28 ± 1.1, 27 ± 1.7% respectively, compared with 32 ± 0.48, 31 ± 0.76, 31 ± 0.53, and 34 ± 1.0% among the non-parasitaemic group. No clear explanation was found for the non-significant difference found among those at 2 and 12 months of age. The difference in the Hct levels of highly parasitized and non-parasitized children was also found to be as distinct (Achidi *et al.* 1996).

Newton and colleagues (1997) assessed the contribution of falciparum malaria to anaemia in 559 Kenyan children under five in the community and in 2412 children admitted to Kilifi district hospital during a 2-years period. In the community, children with parasitaemia had significantly lower Hb concentrations than those without parasitaemia. The mean Hb was 87.5 ± 19.5 and 100.9 ± 20.1 g/L respectively (P< 0.001). Parasitaemia was found to be an important determinant of Hb concentration.

More recently, risk factors for anaemia were investigated in Cameroon. Two cohorts of children were followed for a three-year period. The first cohort was composed of 122 children from 0 to 36 months of age and the second cohort of 84 children from 24 to 60 months of age. The two cohorts were followed weekly for symptomatic malaria, monthly for both symptomatic and asymptomatic malaria, and every six months for haematological data. It was found that concurrent parasitaemia is a risk factor for anaemia in the 12-month and 18-month age groups. The adjusted odds ratio (OR) and 95% confidence interval (CI) were 8 (2.2-29) and 4.5 (1.4-14.7) and the P values were less than 0.01 and 0.05 respectively. However, among the 6-months age group this is only approaching significance (OR: 4.3, CI: 0.9-19, P= 0.06) (Cornet *et al.* 1998).

Table 2. Asymptomatic malaria and anaemia I- Observational studies		
Author	***Study type and sample***	***Conclusion***
1- Wenlock, 1979	n= 7479 - CS All age groups	Parasitaemic had lower haemoglobin (Hb) and lower haematocrit (Hct) than non-parasitaemic ($P < 0.001$).
2- Kandiah *et al.* 1984	n= 157 - CS Pre-school children and women of childbearing period	Hb was found to be significantly lower amongst subjects who were infected with malaria ($P < 0.05$).
3- Willcox, Bjorkman, Brohult, 1985	n=236 - CC Children 2-10 years (y)	Children from holoendemic villages had lower Hct than those from neighboring hypoendemic town ($P<0.001$).
4- Greenwood *et al.* 1987b	n= 573 - LC Children <7 y	The mean Hct of parasitaemic children was significantly lower than non-parasitaemic ($P< 0.001$).
5- Reed, Wirima, Steketee, 1994	n= 252. – LC Children 1-12 months (mo)	Infants who had parasitaemia had lower Hct than infants without parasitaemia ($P <0.05$).
6- Premji *et al.* 1995	n= 338. – CS Children 6-40 months.	Strong association between anaemia and parasitaemia has been found ($P <0.01$).
7- Achidi *et al.* 1996	n= 117 - LC Children <1y.	The mean Hct level of parasitaemic infants was significantly lower than that of non-parasitaemic ($P<0.01$).
8- Newton *et al.* 1997	n= 595 - CC Children under five y	Children with parasitaemia has lower Hb than those without ($P <0.001$).
9- Cornet *et al.* 1998	n= 206 - LC Children under five y	Parasitaemia was significantly associated with anaemia and a strong risk factor for it
10- Akenzua *et al.* 1985	n= 552 - CS Children from birth to 15 y	The difference between the mean Hct of those with and without parasitaemia was not statistically different

CS: cross-sectional survey CC: case control LC: longitudinal cohort

2.4.2. Intervention studies:

The strongest evidence for a causal relationship between asymptomatic parasitaemia and anaemia comes from treatment trials, a few of which were carried out in Africa. Some of these trials are summarized in table 3.

To assess the effect of repeated malaria infection in African children, 26 infants who were protected from birth up to the age of three years by weekly chemoprophylaxis were compared with an unprotected control group of equal size from the same population. Children were assigned randomly to each group and the control group received weekly placebo. After one year of the intervention, the mean Hb of the protected children was significantly higher than that of the unprotected: 12.1 ± 1.06 *versus* 9.5 ± 1.22 g/dL. After 3 years, the mean Hb concentration showed no difference but the mean Hct values of the unprotected children were lower than those protected: 31.96 ± 2.54 g/dL compared with 34.37 ± 1.61 g/dL respectively but no statistical analysis were provided. However, the small sample size and the high dropout rate especially at the three years investigation were strong limitations of this study (McGregor *et al.* 1956).

Following a successful malaria control program by use of residual insecticide in Tanzania, Draper (1960) found that the mean Hb of both children and adult increased by one to two grams per cent over the values obtained before the start of the control programme. However, there was no control group.

In Nigeria, schoolchildren (n=289) aged eight to 17 years were enrolled for chemo-prophylaxis placebo-controlled trial. A fall in Hct of 3% was observed in twice as many children in the placebo group compared with other groups received chemoprophylaxis, but no statistical analysis was provided (Lucas *et al.* 1969)

In another Nigerian study, 198 children aged three months to two years who received weekly malaria chemoprophylaxis with chloroquine from shortly after birth until the age of one or two years were compared with 185 control children matched for sex and ethnic group and who received weekly vitamin C. Successful control of malaria with chemoprophylaxis resulted in a significant increase in Hb and Hct. The mean Hb and Hct for the protected children were 10.9 ± 1.8 g/dL and 36.3% ± 4.5% respectively compared with 9.8 ± 1.6 g/dL and 33.6 ± 5.7 among the control group (Bradley-Moore *et al.* 1985a).

In Liberia, children aged between two and 10 years in two holoendemic villages were assigned either to monthly chloroquine chemoprophylaxis or to treatment of cases with overt clinical malaria. After two years, the Hct of children who received the chemoprophylaxis rose from 34.8% to 38.2% ($P < 0.001$) while the mean Hct of children in the control village was not significantly different ($P > 0.05$). However, no placebo was giving to the control group and there was no proper matching (Willcox, Bjorkman, Brohult, 1985).

In the Gambia, Greenwood colleagues (1988) found that fortnightly chemoprophylaxis with 1/4 strength tablet of Maloprim (25 mg dapsone + 6.25 mg pyrimethamine) given to children 3-59 months resulted in a rise in the mean Hct compared with the placebo group. The mean post intervention Hct of the chemoprophylaxis and placebo groups were 33.9% and 31.1% respectively ($P < 0.001$). Thirty per cent of children resident in the villages which received a placebo had a haematocrit <30% compared with 16% among those who received chemoprophylaxis ($P < 0.05$).

Recently two bed net trials investigated the impact of impregnated bed net programmes on morbidity. In the Gambia, five areas were chosen as sentinel sites for evaluation of National Impregnated Bed net Program (NIBP). During the first year of the intervention, data from four intervention sites showed that there was a statistically significant reduction of about 50% in the prevalence of malaria, high parasite density rates, and an increase of 0.9% in mean Hct. However, when data from the fifth intervention site was added this significant difference was no longer found. A subsequent observation has shown a low usage of nets by children in this the fifth site (D'Alessandro *et al.* 1995).

In another bed net trial, Shiff and colleagues (1996), investigated the effect of malaria on haematocrit in Tanzanian children aged 6-40 months following a combined malaria control scheme that used impregnated bed nets with chloroquine chemotherapy on demand. A significant deficit in Hb in children unprotected by impregnated bed nets was found. Children not protected were twice as likely to be anemic as children protected by bed nets (Relative risk: 2.0, CI: 1.4-2.7). The authors underlined the importance of

malaria in causing anaemia among children in endemic areas even when their infection is asymptomatic.

Asymptomatic malaria has also been identified as an important cause of anaemia among pregnant women. In a sample of 275 women attending the antenatal clinic at Kilifi district hospital, Kenya, it was found that among primigravidae, malaria infection was strongly associated with moderate and severe anaemia ($P<0.01$). Severe anaemia was more than twice as common in women with peripheral parasitaemia as in those who were non-parasitaemic, and parasitaemia was associated with a 2.2 g/dL decrease in mean Hb concentration ($P<0.001$) (Shulman *et al.* 1996). On the other hand, improvement in the prevalence of anaemia has been found in pregnant women who received chemoprophylaxis (Greenwood *et al.* 1989, Cot *et al.* 1998) and following a bed nettrial (Dolan *et al.* 1993).

Very few studies have failed to show an association between malaria and anaemia in the malaria patients or asymptomatic carriers in endemic areas. In a series of 50 anaemic patients aged between four months and 10 years admitted to the children's ward in a Nigerian hospital, Azubuike and colleagues found that poor nutrition was much more important than malaria as a risk factor for anaemia among children under two years of age while hookworm infestation was a more common cause in children over two years (Azubuike, Izuora, Obi, 1977). However, this study was conducted among symptomatic patients.

Finally, in a survey among children in Nigeria, a random sample of 552 children representing 50% of those on the clinic register of children from birth to 15 years were selected. It was found that the frequency of a Hct less than 33% among infected children was not significantly different from those without infection. The differences between the mean Hct of children with and without parasitaemia were not statistically significant except among the two-to-four and five-to-nine years age groups (Akenzua *et al.* 1985).

Negative correlation between haemoglobin concentration and parasite density was found in some studies (e.g. Achidi *et al.* 1996; Kitua *et al.* 1997). However, others did not find such correlations although they identified malaria as a risk factor for anaemia (Beales, 1997; Cornet, *et al.* 1998). There is no obvious reason for such inconsistency.

2.4.3. Conclusions of studies of asymptomatic malaria and anaemia:

While some of the observational studies have design problems such as comparing different populations (e.g. town and villages by Willcox, Bjorkman, Brohult, 1985), recent studies were better designed (e.g. Premji *et al.* 1995, Cornet *et al.* 1998). Nonetheless, most of observational studies have shown consistent differences between parasitaemic and non-parasitaemic groups. Observational studies due to design limitations cannot prove causality and hence experimental trials are needed.

Table 3. Asymptomatic malaria and anaemia II- Intervention studies		
Author	***Study type and sample***	***Conclusion***
1- McGregor *et al.* 1956	Chemoprophylaxis RCT n=52 Children followed from birth to 3 y	After one year: Hb of the protected children was significantly higher than the unprotected (P <0.05). Three years later, the mean Hct values of the unprotected children were lower than those protected.
2- Draper, 1960	Malaria control programme in 3 villages - no control	The mean Hb of both children and adults in the programme area increased by 1-2 g/dL.
3- Lucas *et al.* 1969	Chemoprophylaxis trial RCT N=289 Schoolchildren 8 to 17 y	A fall in Hct of 3% was observed in twice as many children in the placebo group compared with other groups received chemoprophylaxis.
4- Bradley-Moore *et al.* 1985a	Chemoprophylaxis RCT. N=198 Children 3 months to 2 y	Protected children had higher mean Hb and Hct than control (P<0.05).
5- Willcox, Bjorkman, Brohult, 1985	Chemoprophylaxis CT n= 358 Children 2-10 y	Mean Hb and Hct of protected children rose progressiv-ely and significantly more than the initial values (P<0.001).
6- Greenwood *et al.* 1988a	Chemoprophylaxis CT n= 248 Children 3-59 mo	There was a significant increase in mean Hct in children who were receiving chemoprophylaxis compared with the placebo group (P<0.001).
7- D'Alessandro, *et al.* 1995	Bed net CT n=2300 Children 1-4 y	Mean Hct was higher in children who slept regularly under treated or untreated bed nets than those who did not use a net (P< 0.05).
8- Shiff *et al.* 1996	Bed net CT 7 villages: three intervention and four control Children 6-40 mo	Use of impregnated bed nets produced 54% reduction in the prevalence of anaemia among young children (P<0.05).

RCT: Randomized controlled trial CT: controlled trial but not randomized

On the other hand, some of the early drug trials had design problems such as lack of a control group (Draper, 1960), lack of proper randomisation and placebo group (Willcox, Bjorkman, Brohult, 1985) or small sample size and high dropout rate (e.g. McGregor, 1956). However, recent trials avoided some of these limitations (e.g. Bradley-Moore *et al.* 1985a; Greenwood *et al.* 1988a; Shiff *et al.* 1996). Recent drug and bed net trials have shown an improvement which mostly related to malaria control. The fact that some studies have shown a significant negative correlation between the parasite density and haemoglobin concentration (e.g. Achidi *et al.* 1996; Kitua *et al.* 1997) gives further evidence to the association. Even the studies that did not find such correlation found that parasitaemia was associated with anaemia. The two studies that did not find association between malaria and anaemia have shown a significant association for some age groups (Akenzua *et al.* 1985) or found that poor nutrition, which may be itself related to malaria, is an important cause for anaemia. The high prevalence of hookworms found in one study may also have confounded the results (Azubuike, Izuora, Obi, 1977).

2.4.4. Pathogenesis of anaemia in asymptomatic malaria:

The exact pathogenesis of anaemia in malaria remains unclear (Weatherall, Abdalla, 1982; Fleming, Werblinska, 1982). Some researchers found that there is a disturbed function of the bone marrow (dyserythropoiesis and ineffective erythropoiesis) in a group of children with asymptomatic parasitaemia (Kurtzhals *et al.* 1997, Abdalla *et al.* 1980).

It has been also suggested that anaemia in asymptomatic parasitaemia often results from previous untreated clinical disease (Greenwood, 1987a). Recently, there has been strong evidence to suggest that persistent low parasite density remaining after a poor response to antimalarial treatment may lead to severe and life-threatening anaemia. As drug resistant strains of the malaria parasite proliferate in many endemic countries, this is becoming increasingly important (Greenwood, Marsh, and Snow, 1991; Bloland *et al.* 1993; Schapira, Beales, Halloran, 1993; Gravenor, McLean, Kwiatkowski, 1995; Beales, 1997; Newton *et al.* 1997).

Asymptomatic malaria can precipitate iron deficiency anaemia but the mechanism is uncertain. Since most people in endemic areas are on a marginally sufficient diet, and many also have worm infestations, increasing red cell turnover together with increasing requirements may precipitate deficiencies in haemetinics e.g. iron and folic acid (Fleming and Werblinska 1982; Abdalla, 1990; Editorial, 1991; Premji *et al.* 1995). This, together with reduced iron absorption, reduced iron availability and immobilisation of iron in malaria pigments may exacerbate iron deficiency anaemia following malaria infection (Brabin, 1992).

2.5. Asymptomatic malaria and nutrition:

The interaction between malaria and malnutrition is complex (McGregor, 1982; Fleming, Werblinska, 1982; Greenwood, Marsh, Snow, 1991). Early studies attempted to assess the influence of malaria on nutrition through observational studies. However, the strongest evidences for the influence of malaria on nutrition come from some of the recent intervention studies.

2.5.1. Observational studies:

Early attempts to assess the influence of malaria on nutrition by comparing the nutritional status of closely related communities, one of which affected by malaria and the other was not, produced conflicting results (Faich, Mason, 1975; Sharp, Harvey, 1980). This is perhaps due to design problems and difficulty in controlling for different confounding factors such as socioeconomic background. Some of these studies are summarized in table 4.

In Uganda, a group of children (n=60) were followed up from birth to 3 years in an attempt to characterize the aetiology of kwashiorkor. The results showed that there was a close relationship between the pattern of infections, including malaria, and weight faltering and hypoalbuminaemia. However, not enough details were given (Frood, Whitehead, Coward, 1971).

Faich and Mason (1975) compared school aged children six to 16 years old (n=853) from a high malaria incidence area and an adjacent low malaria incidence area. Weight,

height and arm circumference were compared. It was found that 62% of children from both the high and the low incidence areas had an arm circumference below 90% of the standard. Nine per cent from the high incidence area had weight-for-height lower than 90% of standard compared with 11% of children from the low incidence area. Comparing those with evidence of malaria as indicated by positive blood film, splenomegaly, or positive serology to those without such an evidence revealed no significant difference in the nutritional status between the two groups.

At Keneba, in the Gambia, Rowland and colleagues investigated 152 children between the ages of 6 months to 3 years. Multiple regression analysis demonstrated that malaria contributed significantly to poor weight gain and growth faltering in children and was second only to gastroenteritis (Rowland, Cole, Whitehead, 1977).

Wenlock (1979) in a large survey (n=7479) in rural areas in Zambia, found that malaria parasitaemia was significantly associated with malnutrition in children. Among those who had parasitaemia 30%, 8%, and 60% had weight-for-age, mid-upper arm circumference, and serum albumin below 80% of the standard respectively compared with 23%, 5%, 37% among those who did not have parasitaemia.

In Papua New Guinea, children (n= 166) from a high malarious area were compared with children (n=1084) from a low malarious area. Weight, length, mid-upper arm circumference, and triceps skin fold thickness were measured for children under five years of age. It was found that in the high malarious area there was a greater proportion of stunted children (46%) compared with the low malaria area (36%).

Table 4. Asymptomatic malaria and nutrition I- Observational studies		
Author	***Study type and sample***	***Conclusion***
1- Frood, Whitehead, Coward, 1971	n=60 - LC Children followed up from birth until three y	There was a close relationship between the pattern of infections including malaria and weight faltering and hypoalbuminaemia
2- Faich, Mason, 1975	n=853 - CC School aged children six to 12 y	No difference in nutritional status could be found between those from high malaria incidence area and an adjacent low incidence malaria area
3- Rowland, Cole, Whitehead, 1977	n=152 - LC Children between six months and three y	Malaria contributed significantly to poor weight gain and growth faltering in children
4- Wenlock, 1979	n= 7949 - CS All age groups	Parasitaemic children had lower weight-for-age ($P < 0.01$), lower mid arm circumference($P < 0.02$), and lower mean serum albumin than control ($P < 0.001$).
5- Sharp, Harvey, 1980	n=1250 - CC Children under five y	In a highly malarious area there was a greater proportion of stunted under five children (46%) than in an area with low malaria (36%)
6- Kandiah *et al.* 1984	N= 157 - CS Pre-school children and women in childbearing period	There was tendency for infected children to be more retarded in growth than those without evidence of parasitaemia. However the difference was not significant.

LC: longitudinal cohort CC: case control CS: cross-sectional survey

Also, in the highly malarious area there was a significant association between splenomegaly and stunting. The authors linked splenomegaly in the high malaria area to malaria. However, there was no control for other socioeconomic variables which may be related to both stunting and poor health (Sharp, Harvey, 1980).

Finally, Kandiah and colleagues (1984) in a cross-sectional survey described before (2.4.1), found that there was a tendency for infected children to be more retarded in growth than those without evidence of infection. However, the difference was not significant.

2.5.2. Intervention studies:

The most direct evidence for the influence of malaria on nutrition comes from some of the intervention studies, a few of which has been carried out in Africa. However the small sample size and lack of control for possible confounding factors have influenced the results of some of them. Some of these trials are summarized in table 5.

In Ghana, a group of schoolchildren aged seven years (n=176) were divided into two similar groups regarding spleen size, malaria parasite rate and density, body weight, and intelligence. Amodiaquine was given at the beginning of school term to clear parasitaemia, followed by a weekly dose of pyrimethamine or placebo. The children were followed-up for one year. Children were weighed before the intervention started and again at the end. The treated group showed a slightly higher increase in weight than the control but the difference was not statistically significant (Colbourne, 1955).

McGregor and colleagues (1956) in the study described above (2.4.2) showed that after one year of chemoprophylaxis the unprotected children showed lower gain in weight than their protected fellows. Nevertheless, at no time was the weight difference between groups statistically significant. After three years, the mean weight of protected children was slightly lower than that of the unprotected children, but the protected children were slightly taller. However, neither of these differences were significant.

Draper and Draper (1960) following a successful malaria control programme by residual spraying, found that malaria control was not associated with any significant improvement in the weight of Tanzanian children aged 2 to 18 months.

In another chemoprophylaxis placebo-controlled trial described before (2.4.2), weight and height of the treatment and placebo groups showed no significant difference after one year of the intervention (Lucas *et al.* 1969).

As a part of a malaria control programme, limited anthropometric surveys were conducted in two village clusters, which were undergoing the most intensive control treatment, and in one untreated comparison cluster. Protected infants and children had slightly better nutritional status. They were somewhat heavier and taller and had somewhat higher upper arm circumference. The differences were small to moderate but rather consistent and in several cases were statistically significant. The differences disappeared in the post-intervention phase (Molineaux, Gramiccia, 1980).

Bradley-Moore and colleagues (1985b) in a randomised placebo-controlled trial described before (2.4.2), found that the protected children tended to be taller and heavier than the control children and to have a larger mid-upper arm circumference. However, the only differences that were statistically significant were height-for-age and arm circumference in 12-17 months age group and height-for-age in 18-23 month age group. Six months after the intervention was stopped, protected children tended to maintain a slight advantage over control children but the differences were not statistically significant.

In a bed net trial described above, D'Alessandro and colleagues (1995) reported an improvement in nutritional status of children living in the treated villages. The mean Z-score of weight-for-age and weight-for-height were higher in children protected by impregnated bed nets than those from untreated villages: -1.36; -0.98 *versus* -1.46; and -1.13. The differences were statistically significant after allowing for area, age, bed net use, and sex ($P< 0.01$ for both).

In another bed net trial, Shiff and colleagues (1996) found that the children not protected by impregnated bed nets grew 286 grams (CI: 171- 402) less than the protected children in a 5 month period. The authors stated that:

> " Our data clearly support other studies in demonstrating the importance of malaria in retarding development of children, even when their infections appear asymptomatic".

Table 5. Asymptomatic malaria and nutrition II- Intervention studies		
Author	***Study type and sample***	***Conclusion***
1- Colbourne, 1955	Chemoprophylaxis CT n=176 Seven-year schoolchildren	The treated group showed a slightly higher increase in weight than the control but the difference was not significant
2- McGregor *et al.* 1956	Chemoprophylaxis RCT n=52 children Followed from birth up to 3 y	After one year: unprotected children showed lower weight gain than the protected children but this was not significant. After three years: the mean weight of protected children was slightly lower than that of the unprotected children but the former were slightly taller. However, differences were not significant.
3- Draper, Draper, 1960	Malaria control programme in three villages - children 2-18 mo - no control group	Malaria control has not been associated with any significant improvement in weight of the protected children
4- Lucas *et al.* 1969	Chemoprophylaxis RCT n=289 schoolchildren 8 to 17 y	Weight and height of the treatment and placebo groups were not significantly different
5- Molineaux, Gramiccia, 1980	2 village clusters which were undergoing control programme and one untreated cluster - CT	Protected infants and children were somewhat heavier and taller and had somewhat higher upper arm circumference. The differences were small to moderate but constant and sometimes statistically significant
6- Bradley-Moore *et al.* 1985b.	Chemoprophylaxis RCT n= 198 Children from birth to 2 y	Protected children tended to be taller and heavier than control and to have larger mid-upper arm circumference. However, differences were small and only in few instances were significant.
4- D'Alessandro *et al.* 1995	Bed net CT n= 4014 - Children 1-9 y	The mean Z-score of weight-for-age and weight-for-height were higher in children from treated than those from untreated villages ($P<0.01$).
5- Shiff *et al.* 1996	Bed net CT 7 villages: 3 intervention and 4 control. children 6-40 mo	Children not protected grew 286 g less than the protected children ($P<0.01$).

RCT: Randomized controlled trial CT: controlled trial but not randomized

Some researchers suggested that under-nutrition may be protective against clinical and severe malaria. However, the results are rather inconsistent. While some showed that malnutrition was protective (Hendrickse *et al.* 1971; Murray *et al.* 1978a; Ahmad *et al.* 1985) others did not find an effect (Snow *et al.* 1991) or found that those who were malnourished were at a greater risk (El Samani, Willett, Ware, 1987; Snow, Marsh, 1998).

2.5.3. Conclusions of studies of asymptomatic malaria and nutrition:

Early observational studies on malaria and nutrition have major limitations. Some of these studies compared low and high incidence malaria areas without considering other factors which may cause both malaria and malnutrition (e.g. Faich, Mason, 1975; Sharp, Harvey, 1980). This may explain some of the inconsistency found in those studies.

Also, some of the drug trials had design problems such as lack of control group (e.g. Draper, Draper, 1960) and small sample size and high dropout (e.g. McGregor *et al.* 1956). However, recent drug and bed net trials has shown consistency in showing a difference between protected and unprotected children (Molineaux, Gramiccia, 1980; D'Alessandro *et al.* 1995; Shiff *et al.* 1996). The fact that the differences between the protected and unprotected children tended to disappear after the control has stopped gives another evidence that theses differences were mostly related to malaria (Molineaux, Gramiccia, 1980).

2.5.4. Iron and malaria:

The hypothesis that iron deficiency may provide protection against malaria and that iron supplementation can predispose to malaria infection or enhances its clinical severity has attracted interest especially in the past (Murray *et al.* 1978b; Oppenheimer *et al.* 1986 a, Oppenheimer *et al.* 1986b; Smith *et al.* 1989a). However, recent robust clinical trials did not find such effect (van-Hensbroek *et al.* 1995; van-den-Hombergh, Dalderop, Smit, 1996; Menendez *et al.* 1997).

2.5.5. Micronutrients and malaria:

Interaction between malaria and micronutrients have been reported (Filteau and Tomkins, 1994). In an endemic area in Ghana, Filteau and colleagues (1993) have shown that higher parasite density was significantly associated with low serum retinol. Recently Friis and colleagues found that malaria is a significant predictor of low serum retinol in pre-school children but not in schoolchildren (Friis *et al.* 1997). After a vitamin A supplementation trial, the rates of the clinic attendance and hospital admission were lower by 12 and 38% respectively (Arthur *et al.* 1992). These results - although limited - suggest that malaria may be a contributing factor in the vitamin A deficiency especially with a marginal dietary intake and in non-immune populations. However, this merits further research.

The interaction between malaria and zinc was investigated in few studies. Gibson and colleagues (1991) found that high prevalence of malaria was a contributing factor to the suboptimal zinc status in children in Papua New Guinea. More recently, it has

been shown that zinc deficiency in rural pregnant Malawian women was associated with increased prevalence of malaria (Gibson, Huddle, 1998). Zinc supplemented children had fewer clinic visits for malaria than non-supplemented children (Bates *et al.* 1993). More recently, a zinc supplementation trial in Papua New Guinea resulted in fewer health centre visits, fewer reported fevers, and most importantly lower parasite density (Black, 1998).

2.6. Cytokines and acute phase proteins in malaria:

Cytokines are important immunoregulatory molecules that modulate protective mechanisms and immunopathological changes in infection. Cytokines that are produced by activated mononuclear phagocytes are believed to play an important role in protection and also in disease exacerbation in *Plasmodium falciparum* malaria.

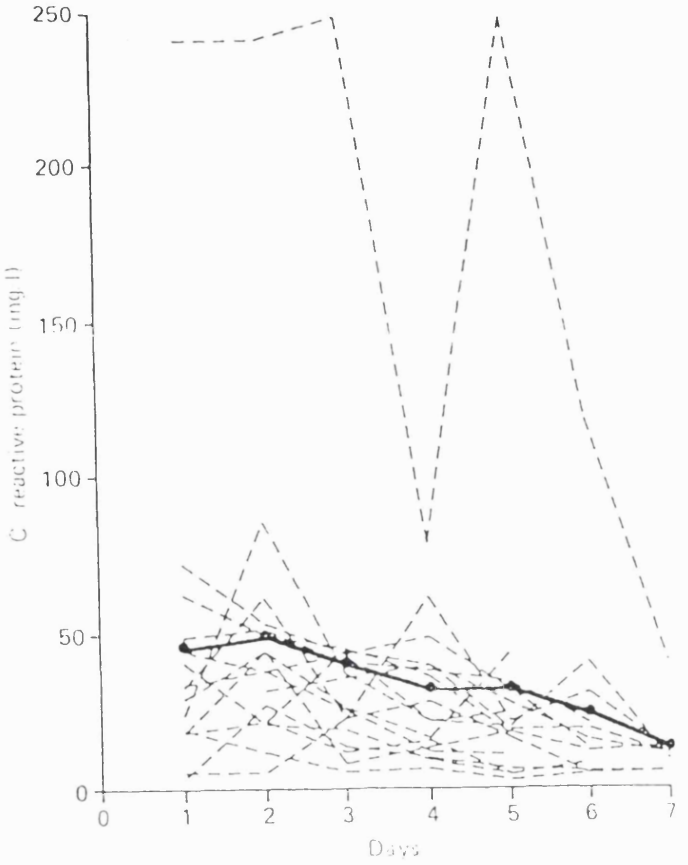
Recent research has confirmed the importance of cytokines e.g. tumor necrosis factor (TNF), Interferon (IFN-γ), Interleukin-1 (IL-1) and Interleukin-6 (IL-6) in the pathogenesis of malaria (Grau *et al.* 1989; Harpaz *et al.* 1992; Jakobsen *et al.* 1995).

Cytokines induce the synthesis of acute phase proteins e.g. C-reactive protein and haptoglobin. While cytokines have extremely short serum half-lives (Harpaz *et al.* 1992) and their determination is sophisticated, measurement of serum concentration of acute phase proteins, is easier (Graninger *et al.* 1992). Sequential measurements of acute phase proteins concentration have shown to be valuable in the assessment of the clinical (Gillespie *et al.* 1991; Hurt *et al.* 1994) and epidemiological *Plasmodium falciparum* malaria (Hurt *et al.* 1994; McGuire *et al.* 1996).

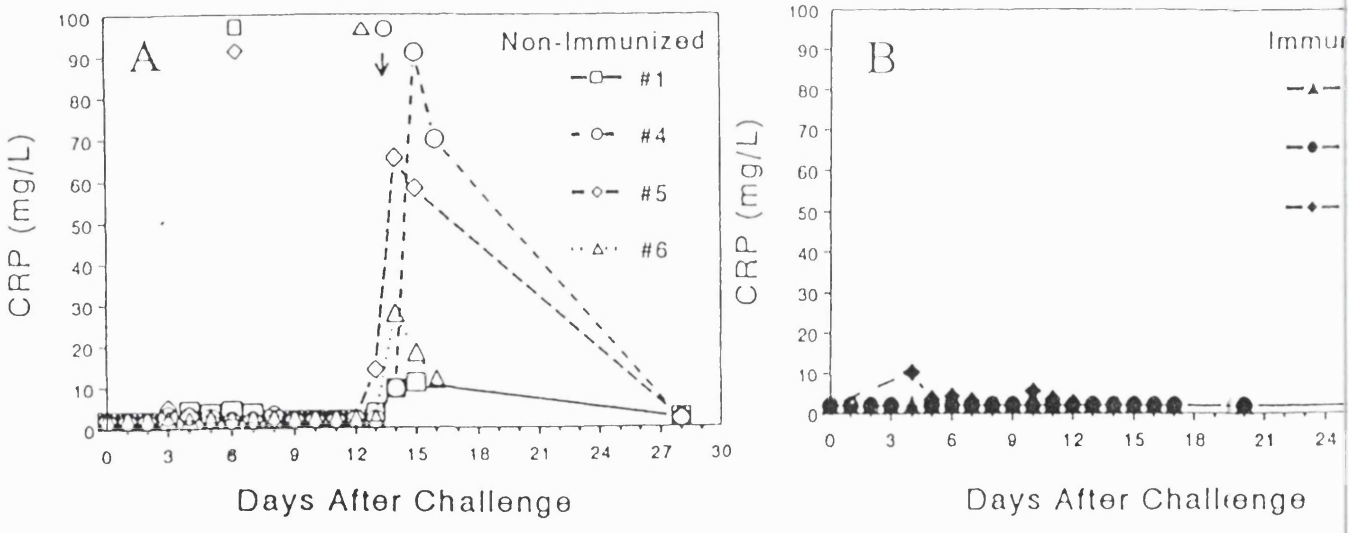
C-Reactive protein (CRP) is an acute phase protein which is elevated following many infections or acute tissue damage. In malaria, CRP is generated in response to pyrogenic cytokines (e.g. IFN γ, IL-1, IL-6) which host monocytes and macrophages secrete when malaria schizonts rupture (Kwiatkowski *et al.* 1989). Increased CRP concentration have been noted *in vivo* after combined administration of recombinant IFNγ and TNF (Demetri *et al.*1989). CRP appears to play a specific protective role in

malaria by preventing penetration of the sporozoite into the hepatocyte and by blocking the parasite division through an antibody-like effect (Pied *et al.* 1989; Nussler *et al.* 1991). It was found that African children with asymptomatic parasitaemia had higher CRP concentrations than others without parasitaemia (Naik, Voller, 1984). It was also found that CRP concentrations were higher in parasitaemic than in non-parasitaemic children and positively correlated with parasite density (Hurt *et al.* 1994; McGuire *et al.* 1996). Recently, Jakobsen and colleagues (1998) have shown that the concentration of CRP was significantly higher in children with clinical malaria than in children with asymptomatic infection or healthy non-parasitaemic children ($P<0.001$) and that the children with asymptomatic infection had significantly higher concentrations of CRP than healthy non-parasitaemic children ($P<0.001$). Also, children with asymptomatic parasitaemia had higher concentrations of soluble TNF receptors (sTNF-RI) than healthy non-parasitaemic children ($P<0.01$). Both CRP and sTNF-R1 concentrations were correlated with parasite density.

Limited information about the time course of changes in CRP concentrations in relation to malaria infection and cytokines is available. In malaria patients who received treatment, it was found that initial high CRP concentrations returned to the normal range by the seventh day of treatment (Gillespie *et al.* 1991). Harpaz and colleagues (1992) found that among the parasitaemic subjects CRP increased abruptly at the onset of fever and it returned to normal concentration within two weeks (fig. 1). In a study that investigated the effect of 1L-6 in eight patients with cancer it was found that CRP, together with many other acute phase proteins, increased following 1L-6 injection

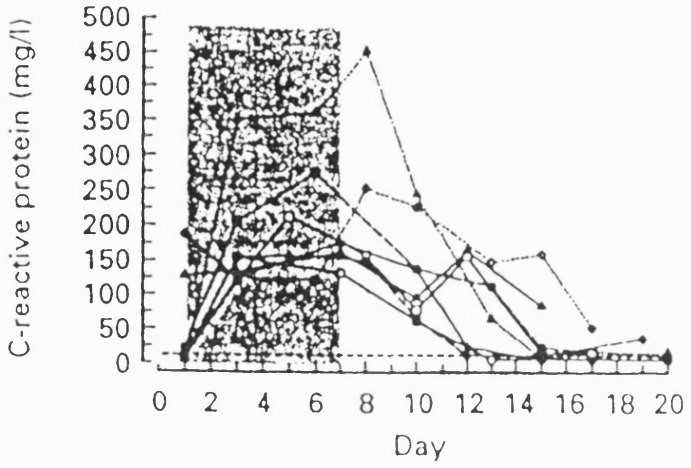


Serum concentrations of CRP in 17 patients with acute *P.falciparum* malaria. Initial CRP concentrations were increased falling towards the normal range by the seventh day of treatment. Mean value indicated by solid line (Gillespie *et al.* 1990).

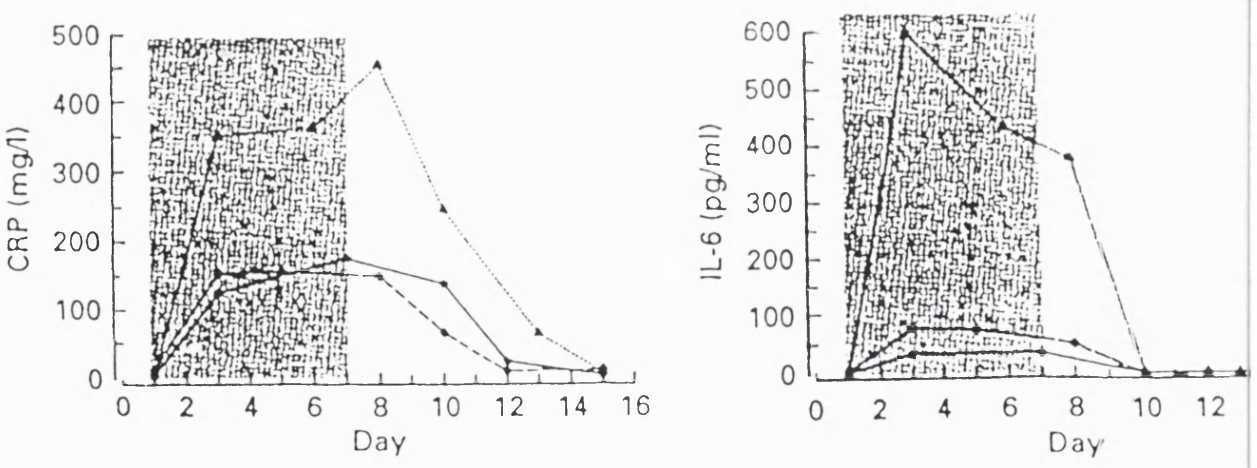


Serum CRP levels after challenge of four non-immune volunteers and three immunized volunteers with virulent *P.falciparum*. Increase in the non-immunized volunteers were significant ($P < 0.01$), as were the differences between the two volunteers groups ($P<0.001$). The abrupt increase coincided with the onset of fever indicated by arrow (Harpez *et al.* 1992).

fig. 1 . C-reactive protein (CRP) in clinical malaria



Serum CRP in patients with advanced cancer during (shaded area) and after IL-6 therapy. IL-6 was capable of causing increase in CRP (Banks *et al.* 1995).



Concentrations of IL-6 and CRP in three patients with different IL-6 doses (1,3, 10 µg/kg/d) during (shaded area) and after IL-6 therapy (Banks *et al.* 1995).

fig. 2. C-reactive protein (CRP) after Interleukin-6 (IL-6) therapy

(Banks *et al.* 1995). They also found that CRP fell to initial values by 5-10 days after the end of therapy (fig. 2). All these findings are limited by the small number of subjects that were studied and no similar data are available about the time course of CRP in asymptomatic parasitaemia.

Another acute phase protein that is closely related to malaria is haptoglobin. It binds rapidly to free haemoglobin whenever intravascular haemolysis occurs. The resulting complex is then rapidly cleared by the mononuclear-phagocyte system. Under these circumstances, haptoglobin disappears rapidly from the plasma, leading to state of ahaptoglobinaemia. Approximately 10-40% of people in some tropical areas have very low serum haptoglobin concentrations. It has been suggested that the absence of haptoglobin may be linked to haemolysis which occurs in malaria and about 95% of the observed low haptoglobin concentrations returned to normal after malaria treatment (Rougemont *et al.* 1988).

Haptoglobin concentrations differ significantly between parasitaemic and non-parasitaemic groups (McGuire *et al.* 1996) and are negatively correlated with parasite density (Hurt *et al.* 1994). Among schoolchildren it was found that there is a correlation between the frequency of ahaptoglobinaemia and parasitaemia (Trape, Fribourg-Blanc, 1988). More recently, Jakobsen and colleagues (1998) found that the haptoglobin concentration in serum was significantly lower in children with asymptomatic parasitaemic than in children with clinical malaria and healthy non-parasitaemic children ($P < 0.05$; < 0.01 respectively).

2.7. Malaria and schooling:

2.7.1 Malaria and school attendance:

Although the saving in days studying and attending classes lost due to illness is important, very few studies have looked at the effect of malaria on school attendance (Halloran, Bundy, Pollitt, 1989).

In an early investigation, Colbourne (1955) observed that annually in Ghana, children seven years of age suffered on average five to six days of sickness per year due to malaria severe enough to lead to absence from school. Freedom from malaria, after chemoprophylaxis lead to a 50% reduction in school absenteeism due to sickness among the treated group.

In another study in a boarding school in the Nairobi, about 152 of 190 students were expected to suffer an episode of malaria over 12 weeks (one term), which represented a considerable period of compromised learning capacity (Nevill *et al.* 1988).

In the Congo, Trape and colleagues (1987) investigated 179 schoolchildren age five to 13 years by weekly surveillance for 17 weeks. They found that out of 53 absences for medical reasons, 22 (42%) were due to malaria. In another daily survey that continued for 10 days, out of 34 absences due to medical reasons 7 (17%) were due to malaria. In another study in the Congo , it was found that malaria was responsible for about

36% of school absences for medical reasons in the high transmission season but for only 3% in the low transmission season (Trape *et al.* 1993).

Recently, local teachers reported that school attendance showed a marked improvement after a successful bed net trial had been implemented in Tanzania (Shiff *et al.* 1996).

In conclusion, although the available data are limited, it appears that the days missed due to malaria represents a considerable period of compromised learning capacity especially over time.

2.7.2. Malaria and cognitive function:

2.7.2.1. Clinical malaria and cognition:

Cerebral malaria is a major manifestation of severe malaria. The disease affects over a million children each year in Africa alone (Warrell, Molyneux, Beales, 1990). Cerebral malaria is fatal in 15-20% and a further 5-10% of patients suffer from various neurological and psychiatric disorders (Nguyen *et al.* 1996). These may take the form of hemiparesis and spasticity (Molyneux *et al.* 1989); hemiplegia, aphasia, and blindness (Brewster, Kwiatkowski, White, 1990); cerebral ataxia (Senanayake, de-Silva, 1994); and psychosis, generalized convulsions, and tremors (Nguyen *et al.* 1996).

In recent years there has been great interest in investigating the neurological sequelae of cerebral malaria (Brewster *et al.* 1990; Bondi, 1992; Nguyen *et al.* 1996). However, neuropsychological aspects have received very little attention. It has been emphasized that gross neurological sequelae such as seizures are likely to represent one end of a spectrum of much wider range problems. There is a possibility that many 'normal' survivors of cerebral malaria have significant cognitive problems, with serious implications for their subsequent educational potential (Crawley *et al.* 1996).

Exciting but conflicting evidence is beginning to emerge regarding the neuro-cognitive effects of malaria. In a recent case-control study, Muntendam and collagues (1996) assessed 36 survivors of cerebral malaria and matched controls with an extensive neuropsychological test battery. No significant intellectual or sensory-motor deficits were found.

In contrast, Dugbartey and Spellacy (1997) found a significant impairment in information processing speed in survivors of cerebral malaria compared with controls matched for age, sex, and education. There was a significant slowing of right-ear modality auditory reaction time and on complex visuomotor sequencing in the cerebral malaria group compared with the control. The authors suggested that these results indicate the presence of localized subacute patterns of cognitive processing deficits that may have serious implications for the cognitive development and functioning of children in malaria-endemic areas.

More recently, a matched case control study of 20 Ghanaian children with recent history of cerebral malaria showed somatosensory discrimination deficits in the cerebral malaria group compared with age, sex and education matched controls (Dugbartey, Spellacy, Dugbartey, 1998).

The long-term emotional and cognitive effects of uncomplicated malaria have been recently investigated among a group 142 individuals with a documented history of clinical falciparum malaria and 30 controls without a lifetime medical diagnosis of malaria. The results indicated the presence of a subclinical mixed anxiety-depressive syndrome after medical recovery from falciparum malaria (Dugbartey, Dugbartey, Apedo, 1998).

2.7.2.2. Asymptomatic malaria and cognition:

It is surprising that in spite of great emphasis on the impact of parasitic infections on cognitive function, very little has been done to investigate the effect of asymptomatic parasitaemia on the cognitive function of schoolchildren (Halloran, Bundy, Pollitt, 1989).

In an early attempt, Colbourne (1955), investigated the impact of malaria control through chemoprophylaxis by a weekly dose of pyrimethamine or placebo on school performance in a group of seven year old schoolchildren in Accra, Ghana. All the children were classified by the teachers before the trial started as 'bright', 'average', and 'dull'. At the end of the investigation these results were scrutinised and the child was

classified as 'improved', 'unchanged', or 'deteriorated'. No difference was observed between the two groups. However, the author admitted that there is a possibility that the tests were not sensitive enough to reveal small differences. Nevertheless, he pointed out that the teachers, who did not know which tablets were the placebo, had the general impression that, the treated group had improved more than the control.

Recently, Boivin and colleagues have investigated the cognitive function of 79 Zairian schoolchildren. They found that children successfully treated for chronic intestinal parasites demonstrated significant improvement in cognitive functions. However, among 35 children with asymptomatic parasitaemia, similar improvement was not obtained after successful treatment (Boivin *et al.* 1993). It is possible that the small sample size, the high chloroquine resistance (40%), and the battery of tests they used confounded their results.

3. Other nutritional and health factors associated with cognition, school achievement and attendance

I am going to review the relevant literature on the effect of nutrition and infection on children's cognition and school achievement. Befor that a short introduction to the cognitive tests will be given.

3. 1. Introduction to the cognitive tests:

Cognition means how knowledge is acquired, organized and used by people (Connolly, Kvalsvig, 1993). It includes perception, memory, attention, problem solving, language, reasoning and imagery. Perception is defined as the detection, recognition, and interpretation of sensory stimuli. Attention commonly used to refer to selectivity of processing or in other words withdrawal from some things in order to deal effectively with others (Eysenck, 1988). A memory which can be auditory or visual is the most important component of cognition. It has two types the short-term memory (sometimes called working memory) that holds information only for about 30 seconds when it can be manipulated and the long-term memory (sometimes called permanent memory) which holds information for a long time. Problem solving is the use of information to achieve a goal. Language is involved in various psychological process such reading and speaking. Imagery is defined as employment of some cognitive process to produce a perception while the stimulus input that would normally give rise to such perception is absent (Neisser, 1972). Because there are many different cognitive skills, a global score such as the intelligence quotient (IQ)

does not provide any information about each specific skills. Therefore, use of different measures for different skills is recommended.

Over the past 40 years there has been a shift in the studies of human cognition from psychometric approach which tries to understand abilities in terms of mental structure or what it is called a "factor" responsible for individual differences in observed performance, to the information- processing approach which tries to find out more about processes that underlie intelligent behaviour. Information processing means how rapidly and accurately specific cognitive processes are performed. A basic assumption in the information-processing approach is that differences in cognition between individuals are based on the differences of small numbers of information-processing components and such differences are reflected in the composite score of intelligence tests such as WISC and Stanford-Binet tests (Sternberg, 1985).

There are different types of cognitive tests that have been used for assessing cognitive skills at different ages. The Bayley Scales of Infant Development (Bayley, 1969) are most commonly used among those under 2 years of age and have two components: the Mental Development Index (MDI) which measures cognitive and perceptual development and the Psychomotor Development Index (PDI) which measures motor development. This test has proven to be sensitive for identifying infants and toddlers with serious development delays (Lewis, Sullivan, 1985). Above the age of three years tests that assess verbal skills, perceptual performance, knowledge of numbers, and short-term memory are more useful. An example of such tests is Stanford-Binet test

which consists of a series of individual tests for children of each age and can be used up to the adulthood. Between the age of six and 12 years, Wechsler Intelligence Scale for Children (WISC) (Kaufman, 1979) has been used frequently. It has two components: one concentrating on verbal skills (e.g. vocabulary, describing similarities between objects, and general information) and the other component focused on performance tests and involves less-verbal types of thinking such as arranging pictures in sequence in order to tell a story, shape matching, pattern with blocks, and test of visual-spatial skills such as completing a maze on paper. WISC gives only a composite score of cognitive function and does not give desegregated scores of the specific skills which have led to the global score (Ulijaszek, 1998).

In this study we decided to measure specific cognitive functions and the speed of conducting these functions rather than a global IQ test. The rationale for the choice of these particular tests used together with their full description is given in the chapter of methods (6.3).

3.2. Protein Energy Malnutrition (PEM)

3.2.1. Introduction:

The aetiology of PEM is complex but both poor nutrition and repeated infections play an important role in its development (Tomkins, Watson, 1989). Different classifications have been designed to define PEM such as the Wellcome and the Gomez classifications, which uses weight-for-age, and Waterlow's classification which used both weight-for-height and height-for-age.

In the Wellcome classification, severe PEM includes different forms e.g. marasmus, kwashiorkor, or marasmus-kwashiorkor. Marasmus is characterised by severe weight deficit (< 60% weight-for- age), kwashiorkor is characterised by oedema, liver enlargement, and hair and skin changes. Children with oedema and body weight less than 60% of the standard are diagnosed as having marasmic kwashiorkor.

The prevalence of malnutrition in children is staggering. Severe malnutrition is common in most developing countries. According to UNICEF (1998) 11% of the world population under five are severely malnourished, but the proportion is as high as 19% in South Asia and 13% in least developed countries.

Mild to moderate PEM affects a majority of children in many developing countries and is characterised by growth retardation. It is important to distinguish between deficits in height-for-age (stunting), weight-for-age (underweight) and weight-for-height (wasting).

3.2.1.1. Stunting:

Stunting or low height-for-age is divided into severe and moderate types. Severe stunting is height-for-age below minus three standard deviations from the median height of the reference population of the same sex and age. Moderate stunting is height-for-age below minus two standard deviations from the median height of the reference population of the same sex and age.

Stunting signifies past or chronic malnutrition. It indicates slowing in skeletal growth which represents an accumulation of retarded growth which usually occurs in the first three years of life. It was found that stunting is usually a reflection of poverty, repeated infections, as well as inadequate food intake.

Stunting is the most common form of malnutrition. The global prevalence of moderate and severe stunting among children under five years is 37% but it is as high as 52% in South Asia and 42% in Sub-Saharan Africa. Approximately 226 million children under five in developing countries are suffering from moderate or severe stunting (UNICEF, 1998).

3.2.1.2. Underweight:

Underweight or low weight-for-age is divided into severe and moderate. Severe underweight is weight-for-age below minus three standard deviations from the median weight of the reference population of the same sex and age. Moderate underweight is

weight-for-age below minus two standard deviations from the median weight of the reference population of the same sex and age.

Underweight is influenced by both height-for-age and weight-for-height and this composite nature makes its interpretation complex. In the absence of significant wasting in the community, both weight-for-age and height-for-age provide similar information that reflects the long-term health and nutritional experience of the individual or population. Also, short-term changes and reduction in weight-for-age reveal changes in weight-for-height (WHO, 1995b).

The global prevalence of moderate and severe underweight among children under five years is 30% but it is as high as 51% in South Asia and 30% in Sub-Saharan Africa (UNICEF, 1998).

3.2.1.3. Wasting:

Wasting or low weight-for-height is divided into severe and moderate forms. Severe wasting is weight-for-height below minus three standard deviations from the median weight of the reference population of the same sex and age. Moderate wasting is weight-for-height below minus two standard deviations from the median weight of the reference population of the same sex and age.

Wasting is serious condition that can endanger life. It is a sign of current malnutrition and it signifies a deficit in tissue and fat mass compared with the amount expected in a

child of the same height or length. It results either from failure to gain weight or from actual weight loss. It may be precipitated by infection or situations which affect family food supply and limit food intake like famine. It differs from stunting in that it can develop very rapidly and under favourable conditions can also be restored rapidly. Wasting is found to be less sensitive as a nutritional indicator in adolescence where there is a marked increase in growth velocity, and body mass index (BMI) for-age is recommended as the best indicator for adolescence (WHO, 1995b).

Wasting is less common than stunting and underweight. The global prevalence of moderate and severe wasting among children under five years is 11% but it is as high as 17% in South Asia and 8% in Sub-Saharan Africa (UNICEF, 1998).

3.2.2. Malnutrition among schoolchildren:

While severe PEM is generally restricted to infants and very young children except in famine condition; mild-to-moderate malnutrition is also found among schoolchildren. Stunting is the more common form of malnutrition among schoolchildren in developing countries. It is believed to reflect a process that almost always happened in early childhood (Martorell, Khan, Schroeder, 1994).

Few studies have looked at the prevalence of malnutrition among schoolchildren. Among Indonesian schoolchildren it was found that 51% of children were stunted

(Pegelow *et al.* 1997). In another study it has been shown that the prevalence of stunting among Zanzibari schoolchildren varies from 14% in 7-y-old children to 83% in 13 y-old children. On the other hand, the prevalence of wasting was only 10% (Stoltzfus *et al.* 1997c). Surveys of schoolchildren in Ghana, Tanzania, India, Vietnam, and Indonesia found that 51% were stunted, 19% severely stunted, 48% were underweight, and 6% severely underweight (Partnership for Child development, 1998).

Stunting is usually associated with poor mental development and many studies have shown that height-for-age was a significant predictor of IQ or school achievement levels when socioeconomic status (SES) variables were controlled (Grantham-McGregor *et al.* 1996). In contrast, weight-for-height was less often found to be a predictor, although it was sometimes so (e.g. Popkin, Lim-Ybanez, 1982; Sigman *et al.* 1989).

3.2.3. Protein energy malnutrition and child development:

Over the past thirty years there has been a steadily growing concern that malnutrition may detrimentally affect intellectual capacities and children's development. To investigate this hypothesis, researchers have used different approaches. Some have examined the association between early severe malnutrition and later development. Other researchers have used different supplementation strategies to look for causal relationship between mild-to-moderate malnutrition and child's development. Others have examined the association between current nutritional status and different school outcomes measures. The state of the art has been reviewed extensively and currently by Grantham-McGregor (1995), Wachs (1995), and Gorman (1995).

3.2.3.1. Severe malnutrition in early childhood and its long-term effects at school age on cognitive function and school achievement:

There has been a long-standing interest in whether under-nutrition at a sensitive or critical period of brain growth or maturation could have a long lasting or permanent influence on later cognitive performance. Many studies have been conducted on school age children who suffered from severe malnutrition during their early childhood to explore such a possibility. Investigators have used unrelated controls, siblings, or both.

Studies with matched controls:

While in some of these studies (e.g. Bartel *et al.* 1978; Pereira, Sundararaj, Begum, 1979) there was little attempt to control for confounding factors (e.g. social background, effect of hospitalisation etc..), others (e.g. Champakam, Srikantia, Gopalan, 1968; Hoorweg, Stanfield, 1976; Grantham-McGregor *et al.* 1987) tried to match carefully for many possible confounders. However, in such research, it is well known that it may not be possible to control for all confounders (Grantham-McGregor, 1993).

Out of eight studies that used matched control, the index child performed worse than the control in seven studies (Champakam, Srikantia, Gopalan, 1968; Hertzig *et al.* 1972; Hoorweg, Stanfield, 1976; Nwuga, 1977; Pereira, Sundararaj, Begum, 1979; Galler *et al.* 1983; Grantham-McGregor *et al.* 1987). Only in one study was no difference found (Bartel *et al.* 1978).

The areas found to be affected were: intellectual development and cognitive performance (Hertzig *et al.* 1972; Stanfield, 1976; Nwuga, 1977; Galler *et al.* 1983; Grantham-McGregor *et al.* 1987), motor abilities (Stanfield, 1976, Galler *et al.* 1984), and neurosensory-integration (Champakam, Srikantia, Gopalan, 1968; Pereira, Sundararaj, Begum, 1979).

Studies that used siblings as control:

To overcome the difficulty in matching for all social background variables that may affect cognition and school performance, some researchers have used siblings as controls. Siblings are probably the best control for these variables but not for the birth order and age. However, the main problem with the use of siblings as controls is the possibility that because they share the same environments with their severely malnourished siblings they may have suffered mild to moderate malnutrition that was not reported, although it may have affected their development. If this is the case, comparison with siblings may minimise the differences between the groups.
Four studies have used siblings only as controls. In another four studies both siblings and unrelated matched control group have been used (Hertzig *et al.* 1972; Nwuga, 1977; Bartel *et al.* 1978; Pereira, Sundararaj, Begum, 1979).

Out of these eight studies, four studies found that the survivors of severe malnutrition performed worse than their siblings (Birch *et al.* 1971; Hertzig *et al.* 1972; Nwuga, 1977; Pereira, Sundararaj, Begum, 1979). The skills affected were verbal, performance, and full-scale of Wechsler's Intelligence Scale for Children (WISC) (Birch *et al.* 1971,

Hertzig *et al.* 1972; Nwuga, 1977) and scholastic performance (Pereira, Sundararaj, Begum, 1979).

In the other four studies, no difference was found between the previously malnourished children and their siblings (Evans, Moodie, Hansen, 1971, Bartel *et al.* 1978; Moodie *et al.* 1980; Graham, Adrianzen, 1979). In these studies no significant differences were found in full scale intelligence score, verbal score, or non-verbal score (Evans, Moodie, Hansen, 1971), motor development (Bartel *et al.* 1978), school achievement (Moodie *et al.* 1980), and school grade (Graham, Adrianzen, 1979).

Conclusions of studies on severe malnutrition in early childhood and its long-term effects on cognitive function and school achievement at school age:

As retrospective studies, none of the above studies can provide conclusive evidence for a causal relation between the malnutrition and impaired child development. Nevertheless, these studies strongly suggested that severe malnutrition in early childhood could lead to long-standing deficits in child development.

3.2.3.2. Mild-to-moderate malnutrition in early childhood and its effects on cognitive function:

Observational studies:

Few studies have used the observational design to investigate the effects of mild to moderate malnutrition in early childhood on later child development (e.g. Lasky *et al.* 1981; Powell, Grantham-McGregor, 1985).

In Guatemala, Lasky and colleagues (1981) have shown that length and weight were the indices most strongly correlated with behavioral development in Guatemalan children.

In Jamaica, Powell and Grantham-McGregor (1985), have found that height-for-age and weight-for-age had significant effects on Development Quotient (DQ), whereas wasting (weight-for-height) did not.

Supplementation trials:

The most powerful approach to investigate how mild to moderate malnutrition can affect child development is through the randomised controlled trials. Several supplementation trials have been conducted to investigate this causal relationship.

Preventive supplementation (i.e. before malnutrition occurred):

Supplementation from pregnancy:

Four supplementation trials have been conducted to prevent malnutrition. In three studies the supplement was given to pregnant mothers and children (Freeman, *et al.* 1980; Waber *et al.* 1981; Chavez, Martinez, 1982; Joos *et al.* 1983). In the fourth study, the supplement was directed towards the pregnant mothers while their infants received no direct supplementation (Joos *et al.* 1983).

In Guatemala, a supplement was given to mothers, infants and young children in four villages: two of them received a vitamin and mineral fortified high protein energy supplement (*Atole*), and the other two villages received a vitamin fortified supplement containing no protein and a relatively small amount of energy (*Fresco*). It has been shown that children three to seven years old who received the high protein energy supplement and whose mothers received it during pregnancy and lactation, were more likely to score higher in cognitive performance (Freeman *et al.* 1980). A recent study has looked at the long-term benefits from early supplementation and found that high protein energy supplement was associated with faster reaction time in information processing and higher scores in tests of knowledge, numeracy, reading, and vocabulary (Pollitt *et al.* 1995).

In Bogota, Colombia, a supplement was given from the beginning of the 3rd trimester of pregnancy until the child reached three years. It has been shown that children who received food supplementation performed better than those who did not, especially in tests of motor functions (Waber *et al.* 1981). Benefits were still observed at the age of seven when the supplemented children performed better than controls in a test of reading readiness, a difference which was approximately equivalent to eight months of cognitive development (Super, Herrera, Mora 1991).

In Mexico the supplement which was given to the pregnant mothers and children up to seven years of age resulted in substantial benefits in all areas of development on infants'tests, and in first grade at school. The supplemented children were more active

and explored more than the non-supplemented children (Chavez, Martinez, 1982). Follow-up at adolescence showed that the supplemented boys scored higher on the Raven's Progressive Matrices than the non-supplemented boys. However, such a difference was not found in the supplemented girls (Chavez *et al.* 1994).

Maternal nutritional supplementation during pregnancy and lactation did not have any effect on the mental score of the infants of the supplemented group. However, the motor scores of the supplemented infants were higher than those of the controls (Joos *et al.* 1983).

Supplementation in early childhood:

Fourteen children (supplemented group) in whom under-nutrition was prevented for the first two years of life by supplementary feeding were compared with 3 groups of their siblings: kwashiorkor group, medical attention and management group, and neither extra feeding nor medical management group. At mean age of 8.9 years, the mean full scale IQ of the supplemented group was significantly higher than that of any of other three groups (Evans *et al.* 1980).

Therapeutic supplementation (supplementation of children who were already malnourished):

Some studies have investigated the effect of supplementation on the cognitive function and development of moderately malnourished children (e.g. McKay *et al.* 1978; Husaini *et al.* 1991; Grantham-McGregor *et al.* 1991).

A group of under-nourished Colombian children participated in a treatment program of nutrition, health, and education but no group received no treatment. Supplementation alone had no benefits on the performance of three-year olds whereas supplementation with stimulation produced a marked improvement that was greater in the groups which began treatment first. The data also showed that the gains were still evident at the end of the first grade in primary school, a year after the experiment had ended (McKay *et al.* 1978). Benefits were found after treatment stopped up to age ten where the grade level achieved was slightly higher and school failure was lower (McKay, McKay, 1983).

In Bogota, Colombia, both undernourished and adequately nourished were supplemented. After one year the supplemented malnourished children showed a small improvement on the Griffith Test (Mora *et al.* 1974).

In Indonesia short-term (14 weeks) supplementary feeding of malnourished infants aged 6-20 months showed that motor development responded favourably to dietary supplementation (Husaini *et al.* 1991). In a recent follow-up study it has been found that the supplemented children who had received the supplementation before the age of 18 months performed better than control children on the Sternberg Tests of the working memory (Pollitt, Watkins, Husaini, 1997).

Grantham-McGregor and colleagues, conducted a two year-intervention study of nutritional supplementation, with or without psycho-social stimulation, of growth retarded (stunted) children. Stimulation and supplementation had significant

independent beneficial effects on the children's development which were additive. Only the children who received both treatments caught up to the non-stunted control group in developmental levels (Grantham-McGregor *et al.* 1991). A recent follow-up study looked for the long-term benefits and found that supplementation only benefited the children whose mothers had higher verbal intelligence quotient. On the other hand, the children's perceptual motor functions showed significant benefit from stimulation (Grantham-McGregor *et al.* 1997).

Conclusions of studies of mild to moderate malnutrition in early childhood and its effects on cognitive function:

Few observational studies have been conducted but the main evidence comes from the supplementation trials. There are some design problems with these trials e.g. lack of random assignment, lack of proper controls, possibility of supplement sharing, and no control for extra attention. Nevertheless the findings are reasonably consistent. Preventive supplementation appears to have benefited development, especially when it was given in the first three years of life and where undernutrition is prevalent. There is some evidence from the few follow-up studies (Chavez *et al.* 1994; Super, Herrera, Mora 1991; Pollitt *et al.* 1995) that there are long-term benefits.

There is evidence that therapeutic supplementation can also have some benefits (Husaini *et al.* 1991; Grantham-McGregor *et al.* 1991). However, the long-term benefits are doubtful (Grantham-McGregor *et al.* 1997, Pollitt, Watkins, Husaini, 1997). Both stimulation and supplementation may be necessary to achieve better and long-lasting effects (Grantham-McGregor *et al.* 1997).

3.2.3.3. Mild-to-moderate malnutrition in school-age children and its association with cognitive function and school achievement:

Many studies have examined the association of children's school performance, cognition and IQ with their current nutritional status. Some studies found that current nutritional status was associated with school achievement. Popkin and Lim-Ybanez (1982) found that weight-for-height, haemoglobin concentration, and feeling hungry all predicted school achievement. In India it was found that scholastic performance was influenced by height-for-age and haemoglobin (Agarwal *et al.* 1987). In the Philippines, Florencio (1988) found that low weight-for-age or height-for-age and skipping breakfast were related to teacher's assessment of the children ability to concentrate. Once family and school factors were controlled for, height-for-age, visual ability, and haemoglobin were related to school achievement levels, whereas weight-for-height was not. In a Jamaican study that investigated a comprehensive number of determinants of failure in primary schoolchildren found that the failing children had significantly lower heights-for-age, weights-for-heights, and they ate worse breakfasts. They also, had lower haemoglobin concentration, higher blood lead concentrations, had visited a clinic with illness more often, and were more likely to have been admitted to hospital with injuries. They were also poorer in many socioeconomic measures (Clark, Grantham-McGregor, Powell, 1991). More recently, Hutchinson and colleagues (1997) showed that school achievement and attendance were correlated with height-for-age.

In addition others workers have shown that there is an association between current nutritional status and enrollment and grades. In Guatemala, Wilson (1970) has shown that weight-for-height was associated with enrollment and grades while in Nepal, Moock and Lesile (1986) found that both height-for age and weight-for-age were associated with enrolment and grades.

Finally, some workers found that nutritional status is associated with cognitive and motor development. Johnston and colleagues (1987) found that height-for-age is significantly related to cognitive development. In India Agarwal and colleagues (1987) found that the relative risk of having an IQ below 90 was 4.5, 2.4, 1.7 times higher than the normal in the 'severe', 'moderate' and 'mild' forms of malnutrition as indicated by low height-for-age. In Kenya Sigman and colleagues (1989) found that the children who were better nourished , as judged by height-for-age and weight-for-height, had higher composite scores on a test of verbal comprehension and Raven's matrices. In Mexico, Cravioto and colleagues (1966) found that height-for-age is associated with neuro-sensory integration especially among those who were nutritionally at-risk.

Conclusions of studies of mild to moderate malnutrition in school-age children and its association with cognitive function and school achievement:

There is strong evidence that current nutritional status is associated with different school outcome measures. Height-for-age was the nutritional indicator most frequently associated with school grades and achievement levels. It predicted enrolment (Moock, Leslie, 1986), intelligence or cognitive function (Agarwal *et al.* 1987; Florencio, 1988;

Sigman *et al.* 1989), neurosensory integration (Cravioto, DeLicardie, Birch, 1966), attendance (Hutchinson *et al.* 1997) and school failure (Clark, Grantham-McGregor, Powell, 1991). On the other hand, weight-for-height (Wilson, 1970; Popkin, Lim-Ybanez, 1982; Sigman *et al.* 1989; Clark, Grantham-McGregor, Powell, 1991) and weight-for-age (Moock, Lesile, 1986) were less consistently associated with school outcome measures although sometimes they were.

3.2.4. Impact of short-term hunger and missing breakfast on cognitive function, school achievement, and attendance:

Although the question of whether having breakfast influences cognition and school performance is important and has a public health implication, the findings of studies on this issue have been inconsistent. The literature about the relation between the breakfast and cognition has recently been extensively reviewed (Pollitt, 1995; Pollitt, Mathews, 1998). In investigating the possible effects of missing breakfast on cognitive function some researchers conducted experiments in a laboratory setting, others conducted studies at schools, and some have investigated school breakfast programmes.

In the studies that used a laboratory design (e.g. Pollitt, Leibel, Greenfield, 1981; Simeon, Grantham-McGregor, 1989) children were admitted for a night under controlled condition and the subjects were their own controls i.e. a cross-over design was used in which each child was tested twice, once having received breakfast and once have received placebo. In an early study it was found that fasting had an adverse effect on the accuracy of response in problem solving and that breakfast improved the children's performance on Matching Familiar Figures Test (MFFT) (Pollitt, Leibel, Greenfield, 1981). In a Jamaican study, it was found when undernourished children missed breakfast their performance on tests of verbal fluency, auditory short-term memory, and perceptual speed was detrimentally affected and breakfast improved their performance (Simeon, Grantham-McGregor, 1989).

However, Upadhyay and colleagues (1988) did not find a detrimental effect of missing breakfast on cognitive performance of Indian schoolchildren.

Some investigators used the natural setting (schools) to find out the effect of giving breakfast for varying period of time on cognition and school outcome measures. In Jamaica, Powell and colleagues (1983) investigated children in three classes in grade seven where one class was given breakfast, one a low energy syrup, and the third received nothing. After controlling for children's progress in the first term, those who received breakfast improved significantly more than controls in arithmetic and attendance. A second Jamaican study, showed that undernourished children's performance improved significantly on a test of verbal fluency when they received breakfast whereas that of the adequately nourished group did not change (Chandler *et al.* 1995).

In Peru, Pollitt and colleagues (1996) found that children who where nutritionally at-risk, were slower in the short-term memory scanning with the placebo than with the breakfast. However, among the non-risk group, children showed more rapid discrimination between visual stimuli under the placebo than under post-breakfast conditions. In contrast, Lopez and colleagues (1993) studied 279 Chilean schoolchildren categorised nutritionally as normal, wasted or stunted. Irrespective of having received breakfast or not, neither nutritionally normal, wasted, or stunted children showed obvious differences in short-term memory, problem solving, or attention.

Other researchers used school breakfast programmes to study their effect on cognitive function and school outcome measures. In a field evaluation of one-month feeding program in 10 rural schools, mean total cognitive scores did not differ between School Breakfast Program (SBP) subjects and non-SBP subjects. However, the children who were nutritionally at-risk improved in their vocabulary test scores after the SBP participation (Pollitt, Jacopy, Greenfield, 1996). More recently it has been shown that higher rates of participation in school breakfast program were associated with improved student function in a broad range of psychosocial and academic measures (Murphy *et al.* 1998).

In addition, some observational studies that discussed before found that feeling hungry at school predicted school achievement (Popkin, Lim-Ybanez, 1982) and missing breakfast and feeling hungry were related to teacher's assessment of the children ability to concentrate (Florencio, 1988). A Jamaican study that investigated the determinant of failure in primary schoolchildren, found that failing children ate significantly worse breakfasts (Clark, Grantham-McGregor, Powell, 1991).

Conclusions of studies of the impact of short-term hunger and missing breakfast on cognitive function, school achievement, and attendance:

In spite of the different study designs applied, it is still possible to conclude that short-term hunger can detrimentally affect cognitive function. Findings from some studies at schools and some school breakfast programs showed improvement in cognitive function and school achievement and attendance. Those children who are nutritionally at risk are

more likely to benefit. The association that was found between feeling hungry at school or missing breakfast and school achievement or failure suggests that in areas where many children are hungry in schools, school feeding programs may improve some school outcome measures.

3.3. Iron deficiency anaemia (IDA)

3.3.1. Introduction:

Anaemia is defined as a reduction in red cell mass or blood haemoglobin concentration (Oski, 1993). It has many different causes but iron deficiency (ID) is the most common cause, especially in developing countries. ID is lack of iron that is sufficient to cause an impairment in haemoglobin production. IDA represents a severe form of iron deficiency that leads to a drop in haemoglobin concentration below the 95 per cent reference range for age (Dallman, Yip, Oski, 1993). The aetiology of IDA is multifactorial but increased iron requirements, a poor diet, and blood loss are the main causes. In areas where malaria and parasitic infestations are common, the prevalence of IDA is usually high.

IDA is the most prevalent nutritional deficiency in the world, affecting about 1.3 billion people (Stoltzfus, 1997a). Because of their higher physiological requirements, children together with pregnant women are the two groups most likely to be affected. Globally, 40 to 50% of children under five and over 50% of pregnant women are affected (UNICEF, 1998). Leaders from 159 countries at the 1990 World Summit for Children, agreed to reduce the prevalence of IDA by a third by the year 2000. However, it seems that only little progress has been made to achieve this goal. The United Nations, based on surveillance data from 1975 and 1990, reported that the global prevalence of anaemia did not decline (Stoltzfus, 1997a).

3.3.2. Iron deficiency anaemia among schoolchildren:

Among schoolchildren, IDA is highly prevalent especially in developing countries where 46% of primary school-age children are affected (DeMaeyer, 1989). The highest prevalences were reported from South Asia (50%) and Africa (49%). A recent study conducted among Zanzibari schoolchildren found that 62% were anaemic and 83% of anaemia was associated with iron deficiency (Stoltzfus *et al.* 1997b). Biomedical surveys of 8-12 years old schoolchildren in Ghana, Tanzania, India, Vietnam, and Indonesia found that 47-81% were anaemic (Hb < 120 g/L) (Partnership for Child Development, 1997).

3.3.3. Iron deficiency anaemia and child development:

The physiological and developmental consequences of IDA have been emphasised recently and an association with less than optimal behaviour has been reported (Haas, Fairchild, 1989). Several mechanisms have been postulated. Hypomyelination, delayed neurotransmission, and impaired neurotransmitter function, together with the functional isolation and disadvantaged environments, have all been implicated (Lozoff, 1998).

There is a great concern that IDA in infancy may interfere with brain development at a critical time and may lead to long-term deficits that cannot be reversed even when anaemia is corrected (Scrimshaw, 1998). On the other hand, IDA in school-age children can detrimentally affect their mental functions and scholastic achievement in several ways. Schoolchildren may have lower IQ scores, decreased attention, restricted

perception, and poor efficiency in problem solving. Therefore prevention of IDA may contribute to the promotion of child development and better school achievement.

Different approaches have been used in investigating the association between IDA and cognitive functions and school achievement. Some workers have investigated early anaemia in infancy and poor cognitive performance and scholastic achievement later in life. Others have examined the impact of current anaemia either in infancy and early childhood or at school age and its concurrent effects on cognitive functions and school achievement. Some researchers have explored the association between iron deficiency without anaemia and child development.

3.3.3.1. Anaemia in infancy and later development:

The hypothesis that IDA in infancy in an otherwise healthy child may have a long standing effect on development has been investigated in five studies.

In four studies it was found that anaemia in early childhood was associated later in life with poor intelligence (Palti, Pevsner, Adler, 1983; Lozoff, Jimenez, Wolf, 1991), motor function (de-Andraca *et al.* 1990; Lozoff, Jimenez, Wolf, 1991) and school achievement (Palti, Meijer, Adler, 1985). More recently, Hurtado and colleagues (1999) showed that those who had hemoglobin (Hb) below 9 g/dL in infancy were more likely to be in special educational classes. In most of these studies, the effect remained significant after controlling for many background variables e.g. birth weight, sex, and mother's education and IQ.

In conclusion, the findings of these studies, although there are not many, are consistent and suggest that there are long-term developmental consequences of anaemia in early childhood. The fact that these children were properly treated in infancy and at the time of the follow-up they are no longer anaemic raises great concerns about the irreversibility of the developmental consequences of anaemia in early childhood.

3.3.3.2. Anaemia in early childhood and current development:

Anaemia in infancy:

The hypothesis that anaemia during the latter part of the brain growth spurt i.e. six to 29 months of age, may have a detrimental impact on infants' mental and motor development has been tested in many studies, mainly through experimental trials. The age of the participants in these trials varied from six to 26 months. The outcome measure used most frequently was the Bayley Scales of Infant Development, which is a standardised test for infants with three main components: a mental scale, a motor scale, and a behaviour record.

Some of these trials evaluated children's performance at the base-line, i.e. pre-intervention, and found an association between current anaemia and children's development. Many studies have shown that anaemic children had lower mental scores than non-anaemic controls (Lozoff *et al.* 1982; Walter, Kovalskys, Stekel, 1983; Lozoff *et al.* 1987; Walter *et al.* 1989) and/or lower motor scores (Lozoff *et al.* 1982; Lozoff *et al.* 1987; Walter *et al.* 1989).

Post-intervention, those given a short period of treatment e.g. one week, usually did not show a difference (Oski, Hong, 1978; Lozoff *et al.* 1982; Lozoff *et al.* 1987; Walter *et al.* 1989). Some of those given a longer period of treatment e.g. two to three months, found that the children treated with iron showed an improvement compared with the placebo group, especially among those who showed an improvement in their haematological indices (Walter, Kovalskys, Stekel, 1983; Aukett *et al.* 1986; Idjradinata, Pollitt, 1993). However, some did not find a difference even after prolonged iron therapy (Walter *et al.* 1989; Lozoff , Wolf, Jimenez, 1996).

Anaemia at pre-school age:

Two trials (Pollitt *et al.* 1986; Soewondo, Husaini, Pollitt, 1989) were conducted among pre-schoolers three to five years of age. Pre-intervention both trials showed that there were differences between anaemic and iron-replete children. Post-intervention these differences were successfully reversed and were no longer significant. The tasks found to be impaired were discrimination and oddity learning in the first study, and visual attention and concept acquisition in the second.

Out of four studies conducted by Seshadri and Gopaldas (1989), two included pre-schoolers and also showed an improvement on treatment.

Conclusions of studies of anaemia in early childhood and later development:

The main problems with many of these trials are lack of randomisation and absence of a placebo group. The small sample sizes and the short duration of iron therapy in some of these trials were also important limitations. Although some of the trials tried to control during the analysis for different confounders (e.g. home environment), it may be difficult to be sure that differences were not due to the effect of some of these confounders. All these problems may explain some of the inconsistency in the findings. The only strong evidence comes from the Indonesian study (Idjradinata, Pollitt, 1993) but its small sample size is a problem. The question of whether iron therapy can correct performance remains open question.

Among the few studies that looked at the pre-schoolers the findings are more consistent, and all studies showed a difference in performance at the pre-intervention between anaemic and non-anaemic groups. The fact that the Indonesian trial, with a robust design (Soewondo, Husaini, Pollitt, 1989), was able to show that IDA had a detrimental effect on the aptitude of children before school enrolment and that the best improvement in the learning process was noticed among anaemic children who received iron and the worst was among anaemic who received placebo, has important policy implications.

3.3.3.3. Anaemia in school-age children and cognition and school achievement:

Several observational and experimental studies have examined the performance of anaemic schoolchildren in developmental tests and scholastic achievement.

Observational studies:

Effects on cognitive function:

In an early study, Waite and Neilson (1919) investigated 196 children infected with hookworm of varying intensity. They reported that infected children were far behind uninfected peers in the Binet and Proteus mazes IQ tests. More recently, Watkins (1995) reanalysed the data and found that Hb concentration was positively correlated with IQ. Pre-intervention measurements in some trials have shown a significantly lower IQ among anaemic children than non-anaemic children (Pollitt *et al.* 1985; Pollitt *et al.* 1989, Seshadri, Gopaldas, 1989 -first study-).

Effects on School achievement:

Webb and Oski (1973) compared a group of anaemic students aged 12 to 14 years (n=92) with a non-anaemic group (n= 101). School performance was measured by Iowa Tests of Basic skills, which measured performance in subsets of vocabulary, reading, comprehension, spelling, arithmetic's etc.. Performance was found to be significantly poorer in anaemic than in non-anaemic students.

Many studies that reviewed previously in the section on malnutrition (3.1.3.3) also found that Hb, among other nutritional indicators, was associated with school achievement (e.g. Popkin, Lim-Ybanez, 1982; Agarwal *et al.* 1987; Florencio, 1988) and school failure (Clark, Grantham-McGregor, Powell, 1991).

In addition two intervention trials have found significantly low school achievement at the baseline among anaemic schoolchildren (Soemantri, Pollitt, Kim, 1985; Pollitt *et al.* 1989).

Intervention studies:

The most important evidence for a causal relationship between IDA and poor achievement or performance comes from intervention studies. Many of them were conducted among schoolchildren.

Soemantri and colleagues investigated the effect of iron supplement for a three month period on school performance in Indonesia in a randomised controlled trial. The results showed that an improvement in the Hb concentration was associated with significant improvement in the school achievement and concentration tasks but not in IQ (Soemantri, Pollitt, Kim, 1985). In another trial, supplementation also resulted in an apparent improvement in anaemic subject's haematological status and learning achievement scores, but no statistical evidence was provided (Soemantri, 1989).

In a four-month, double blind trial carried out on iron anaemic (n= 28) and non-anaemic (n= 40) primary school Egyptian children, anaemic subjects treated with iron reduced their errors and improved their efficiency scores in the Matching Familiar Figures Test (MFFT) compared with the pre-intervention scores and the placebo group (Pollitt, *et al.* 1985).

Seshadri and Gopaldas (1989) investigated the effect of iron supplementation on cognitive function in four trials. In the first study (n=94) five to eight year old children who received iron and folic acid supplementation for 60 days showed a significant rise in their haemoglobin concentration as well as in scores on (WISC). In the second study, 14 pairs of five to six year old anaemic boys matched for known factors that affect cognition showed among the treated group a significant improvement in the verbal and performance IQ scores of WISC. In the third study, 48 boys aged 8-15 years were randomly assigned to one of three groups: 30 mg ferrous iron, 40 mg ferrous iron , or placebo. The treatment was given daily for 60 days. Both iron supplemented groups significantly improved in their individual and overall cognitive scores compared with the placebo group . In the fourth study, 65 pairs of 8-15 years girls were randomly assigned to receive either iron 60 mg a day or a placebo for one school year. The treated girls performed significantly better in mazes, clerical task, and visual memory compared with the placebo.

In contrast, two other trials failed to show any significant improvement. In India iron given to schoolchildren for one academic year failed to show an improvement in school achievement tests (Agarwal *et al.* 1989). In Thailand a double blind clinical trial found an association between anaemia and IQ and school achievement at the basline but no improvement has been found after 14 weeks of iron therapy (Pollitt *et al.* 1989).

Conclusions of studies of anaemia in school-age children and cognition and school achievement:

There is evidence that current IDA can detrimentally affect cognitive performance. The studies which allowed for pre-treatment comparison showed that anaemic children's performance was significantly poorer than non-anaemic controls. More importantly those randomised controlled iron supplementation trials led to improvement in performance on follow-up which supports a causal relationship. The only study which failed to show treatment effect (Pollitt *et al.* 1989) has no clear explanation.

Regarding the effect on school achievement, many observation studies have identified anaemia as a predictor for school achievement and school failure. In the pre-intervention phase of the four trials that investigated school achievement, an association was found between anaemia and school achievement at the base-line. Two of these trials found an improvement in school achievement after treatment (Soemantri, Pollitt, Kim, 1985; Soemantri, 1989) but the other two did not find a difference (Agarwal *et al.* 1989; Pollitt *et al.* 1989).

3.3.3.4. Iron deficiency without anaemia and child development:

The question as to whether iron deficiency without anaemia may produce alteration in development was examined by some workers. Three studies investigated only iron deficient non-anaemic subjects, two were randomised controlled trials, and one was an observational study.

Oski and colleagues (1983) examined four groups of non-anaemic infants with varying degree of iron deficiency. Iron therapy produced a significant increase in the score of Bayley Mental Development Index (21.6 points) in infants with iron deficiency without anaemia (Hb ≥ 11 g/dL, ferritin < 12 ng/ml, erythrocyte protoporphyrin > 30 μg/dL, mean corpuscular volume (MCV) ≥ 70 fL) compared with iron sufficient infants (Hb ≥ 11 g/dL, ferritin > 12 ng/ml, erythrocyte protoporphyrin < 30 μg/dL, MCV ≥ 70 fL) or iron depleted infants (Hb ≥ 11 g/dL, ferritin < 12 ng/ml, erythrocyte protoporphyrin < 30 μg/dL, MCV ≥ 70 fL) (Oski *et al.* 1983).

Recently a randomised clinical trial found that in non-anaemic iron-deficient adolescent girls iron supplementation led to improvement of some aspects of cognitive functioning even in the absence of anaemia (Bruner *et al.* 1996).

However Pollitt (1997) has raised scepticism as to whether a modest degree of ID affects cognition. In his recent review of an unpublished correlational Guatemalan study that investigated the association between ID without anaemia and cognitive test

performance, he found no evidence to support an association between iron deficiency without anaemia and poor performance.

Many other studies that examined the relation between IDA and development included both IDA subjects and non-anaemic iron deficient children. Pre-intervention, some found that iron deficiency without anaemia detrimentally affected the development (e.g. Pollitt *et al.* 1989) but others did not find an effect (e.g. Soewondo, Husaini, Pollitt, 1989; Walter, *et al.* 1989). Post-intervention, some have shown an improvement with iron therapy (Walter *et al.* 1983; Lozoff, *et al.* 1987) while others did not find an improvement (Soewondo, Husaini, Pollitt, 1989; Pollitt *et al.* 1989, Lozoff, Jimenez, Wolf, 1991).

In conclusion, the results of studies of the relationship between iron deficiency without anaemia and children's development are inconsistent. There is little evidence to suggest that in absence of anaemia, iron deficiency impair behaviour in infants. However, this merits further research.

3.4. Intestinal parasitic infections

3.4.1. Geohelminthic infection:

3.4.1.1. Introduction:

Intestinal parasitic infections are among the commonest infections world-wide. It is estimated that one third of the world's population is infected with one or more species of geohelminths and that polyparasitism is the rule rather than the exception (WHO, 1987; Bundy, 1994).

3.4.1.2 Prevalence among schoolchildren:

Among school age children geohelminthic infections are very common (table 6). Not only do schoolchildren have the highest prevalence of *Trichuris trichiuria* and *Ascaris lumbricoides*, but they also have the highest worm load (Halloran, Bundy, Pollitt, 1989).

3.4.1.3 Geohelminthic infection and cognitive function, school achievement and absenteeism:

Until recently the public health impact of intestinal helminthic infections has been consistently and considerably underestimated. Since the peak of infection with many helminths occurs at an age when children are receiving what may be the only education they will ever receive, concerns about the possible impact on their cognitive function and educational progress have recently been increasing (Pollitt, 1990; Nokes, Bundy, 1994). Studies have shown that the extra-intestinal effects of geohelminthic infection

Table 6. Prevalence of geohelminthic infection among schoolchildren in some developing countries

Country	*Trichuris trichiuria*	*Ascaris lumbricoides*	*Hookworm*	*Reference*
South Africa	47 %	30 %	5%	Kvalsvig, Cooppan, Connolly, 1991
Jamaica	67 %	15-37 %	6%	Nokes *et al.* 1991
	42-47 %	36 %	-	Wong *et al.* 1994
Guatemala	82 %.	91 %	-	Watkins, Cruz, Pollitt, 1996
Tanzania	94%	72%	96%	Albonico *et al.* 1997

include malnutrition, growth retardation and anaemia (Bundy, Cooper, 1989; Callender *et al.* 1992; Bundy, 1994), all of which have been shown to be associated with impaired mental development (3.1 and 3.2). It has been hypothesised that parasites may also have specific effects through their excreted toxins that could directly influence cognitive function (Baddeley, 1992). However the problem is probably more complex than it is thought to be. Different helminths have different impacts on the host and are likely to affect host performance in different ways. Both the intensity of infection and the underlying nutritional status of the host may play a critical role in determining the magnitude of the effect (Callender *et al.* 1994, Simeon *et al.* 1995a, Simeon *et al.* 1995b).

Different investigators have used different approaches to investigate the relationship between geohelminthic infections and cognitive functions and school achievement. Both observational studies and randomised controlled trials have been conducted.

Observational studies:

The hypothesis that geohelminths can detrimentally affect the cognitive function and school achievement has been investigated through many correlational studies.

Cognitive functions:

The association between parasitic infection and mental development was identified as early as the beginning of this century. Waite and Neilson (1919) showed that hookworm infection is associated with arrested mental development. Heavily infected children were 19.5 months behind and lightly infected children were 4.5 behind uninfected

children on the Binet-Simon Intelligence Scale. Similarly, on Proteus mazes, heavily and lightly infected children were 13.3 and 2 months respectively behind uninfected ones. Gordon (1925), found that hookworm was associated with a lower IQ. Smillie and Spencer (1926) also found that the heavily infected children with hookworm had a lower IQ.

There is evidence of an association between worms and cognitive function from the baseline comparisons in treatment trials conducted recently. In South Africa, two trials have shown an association between parasitic infection and information processing and sustained attention (Kvalsvig, Cooppan, Connolly, 1991). Children with Trichuris Dysentery Syndrome (TDS) were found to have a serious deficit in developmental levels measured with Griffith mental developmental scales (Callender *et al.* 1994). Finally, Simeon and colleagues (1995a) found that infected children with *Trichuris* had lower scores than uninfected ones in fluency (generation of ideas), visual search (sustained attention), and French vocabulary test (paired associate learning).

School achievement and absenteeism:

In another early study, Stiles (1915) found that the children infected with hookworms and to lesser extent *A. lumbricoides,* advanced through school more slowly compared with uninfected children. Strong (1916) has also shown that children infected with hookworms had lower scores in arithmetic tests. However in these studies little was done to control for possible confounders (e.g. socioeconomic status). Better-designed correlational studies with proper matching of cases and control are also suggestive. Nokes and colleagues found that those who were ranked by their teachers to be

academically poorest were not only more likely to be infected, but were also more likely to be heavily infected (Nokes *et al.* 1991). Simeon and colleagues (1994) after controlling for socioeconomic status, gender, age, school and presence of *A. lumbricoides*, found that uninfected children had higher reading and arithmetic scores than children heavily infected with *Trichuris*. More recently, it has been demonstrated that children who were infected with *Trichuris* and *Ascaris* scored significantly lower on the reading and spelling tests (Hutchinson *et al.* 1997).

In contrast pre-intervention results of a trial conducted in South Africa found no association between infection and educational attainment (Kvalsvig, Cooppan, Connolly, 1991).

A relationship between infection and absenteeism has been also found. Nokes and Bundy (1993) found that the children infected with moderate and heavy *Trichuris* infection were absent from school more often than their uninfected counterparts.

Conclusions of observational studies:

The main problem with this type of research is that even when an association is found, this is not necessarily causal relationship. Possible underlying factors (e.g. low socio-economic status) could be responsible for both poorer development and educational attainment and infection. These factors were not taken into account in some studies. Although recent studies tried carefully to match for possible confounders, it may be not possible to match for all of them. In conclusion, there is evidence of an association between parasitic infection and cognition or school outcome variables. However a

causal relationship between geohelminthic infection and developmental measures can never be confirmed on the basis of cross-sectional and retrospective studies. Therefore randomised controlled trials are needed as the only satisfactory way of isolating the effect of infection.

Intervention studies:

Many investigators have used randomised controlled trials to determine whether there is a casual relationship between parasitic infection and developmental outcomes. Strong (1916) conducted one of the earliest experiments in this field. He reported that after the treatment of hookworms, improvements were observed in mental and motor tests. Recently 159 schoolchildren aged nine to 12 years old infected with *Trichuris*, were randomly assigned to treatment or placebo. It was demonstrated that removal of worms led to a significant improvement in tests of auditory short-term memory (digit span) and in the scanning and retrieval of long-term memory (verbal fluency). The treated children were no longer significantly different from an uninfected control group nine weeks after treatment (Nokes *et al.* 1992) .

In Zaire Boivin and colleagues (1993) showed that the children who were successfully treated for intestinal parasites demonstrated significant improvement in spatial memory. However iron was also given and this made it difficult to determine whether the effect was due to treating the parasites or treating the anaemia.

In another study, after a year of anthelminthic treatment of a group (n= 19) of children with TDS, an improvement in locomotor development compared with controls was noticed (Callender *et al.* 1994).

In contrast, some recent trials did not find a treatment effect. Simeon and colleagues (1995a) compared *Trichuris* infected children (n= 189) with uninfected group (n= 100). Although there was a difference at pre-intervention, there was no significant treatment effect. However, the children with low weight-for-age showed an improvement in fluency with treatment (Simeon *et al.* 1995a). In another study, only heavily infected and malnourished children showed improvement with treatment in spelling and school attendance respectively (Simeon *et al.* 1995b). Gardner and colleagues (1996) found no significant improvement in cognitive function on treatment among those who were lightly to moderately infected. Finally, in Guatemala a comparison of the treated and placebo groups showed no effect of removing *Ascaris* on indicators of school performance (Watkins, Cruz, Pollitt, 1996).

Conclusion from intervention trials:

Although some treatment trials have shown an effect on cognitive function (e.g. Nokes *et al.* 1992; Boivin *et al.* 1993), findings from recent robust trials did not support a casual relationship in children with light to moderate infection (Simeon *et al.* 1995a, Simeon *et al.* 1995b). However, there is little doubt that heavy infections in nutritionally at risk children have a detrimental affect (Callender *et al.* 1994, Simeon *et al.* 1995a, Simeon *et al.* 1995b). The question of whether mild or moderate infections in adequately nourished children have any detrimental effects is not resolved and needs to be further investigated.

3.4.2. Schistosomiasis:

3.4.2.1 Introduction:

Schistosomiasis is a parasitic infection which affects over 200 million people in Africa and Asia. A large percentage of those who are infected are schoolchildren. It is not only the school age children who usually have the highest prevalence, but they also have the highest infection load (Halloran, Bundy, Pollitt, 1989).

3.4.2.2 Schistosomiasis and cognitive function, school achievement and absenteeism:

Research on the effects of schistosomiasis on mental functioning and scholastic ability has yielded confusing and often contradictory results.

Observational studies:

Some studies found that there was an association between schistosomiasis and lower school performance. Clarke and Blair (1966) observed that infected children had lower class and stream positioning. In another study it was found that uninfected children performed significantly better than infected children (Castle, Clarke, Hendrikz, 1974). Kvalsvig (1981) found that low level infections of *S. haematobium* and *S. mansoni* show little evidence of decrease in energetic activity under normal conditions. However, children with high egg counts had lower activity levels.

In contrast, other studies did not find detrimental effects on school attendance and academic performance, and even found that infected children performed better. Abdulla and colleagues (1964) found that a comparison of the mental power and scholastic

achievement of a control group with a group of bilharzial cases showed no significant difference. Recently, a cross-sectional study of urinary schistosomiasis among 510 primary schoolchildren in Nigeria found no association between infection and school attendance or academic performance (Ekanem *et al.* 1994). On the other hand, Walker and colleagues (1970) studied 500 schoolchildren in South Africa and reported that intelligence, as reflected by examination results, appeared to be higher among infected children than uninfected group. The same finding was reported earlier by Loveridge and colleagues (1948).

Conclusions of the Observational studies:

There is a marked inconsistency among the above results. A possible explanation for this is that most of these studies have serious design problems. Many of them neglected the importance of the intensity of the infection which may play an important role. Indeed, when this issue was considered, it showed an effect (e.g. Kvalsvig, 1981). On the other hand, little has been done to control for some of the confounding variables that are known to affect performance e.g. SES or nutritional status. Therefore, in many instances, it is not possible to differentiate the effect of infection from that of the confounders. Also the mental tests used were relatively crude, unreliable, and subject to gross errors (Connolly, Kvalsvig, 1993). The school examination results in particular that many studies relied upon are a very complex measure of intellectual development and may be affected by many factors other than health, such as the availability of schooling or the quality of teaching.

A possible explanation for the higher intelligence scores among infected children may be the fact that the more intelligent children, being more active, come into contact with infected water more frequently than the less active and less intelligent classmates.
All these issue, and maybe many more, have not been taken in account and this may explain the great inconsistency among the results, making it difficult to make any scientifically based conclusion.

Intervention studies:

The fact that the best method to prove a causal relationship is through experimental studies, has made many researchers use this design to find out if schistosomiasis has an effect on children's development and school achievement.

Jordan and Randall (1962) in an early trial, found that the scholastic ability of the treated children improved compared with the untreated group. Also, Bell and colleagues found that treating schoolchildren produced a significant increase in the Raven's score and school work, but only the first was significant (Bell *et al.* 1973). Recently a treatment trial showed an improvement in the scores of attentiveness and concentration tests but only among children who got high scores in the first tests (Kimura *et al.* 1992).

Conclusion from the intervention studies:

While intervention designs can provide better proof of a causal relation, unfortunately, with many experimental studies of schistosomiasis this was not the case and in many instances the design employed was not adequate. Some had no control group, some did

not have random assignment to treatment (e.g. Jordan, Randall, 1962), and some did not have a control group (e.g. Jordan, Randall, 1962, Bell *et al.* 1973). As with the correlational studies, the performance tests used were often relatively crude. Sometimes it was not possible to differentiate the effect of treatment on infection from that of the confounding variables that may have differed between the groups at the base-line such as SES. There is an obvious need for well-designed randomised controlled trials to determine the effect of schistosomiasis on cognitive development and school achievement.

3.5. Acute infection and cognition

3.5.1. Introduction:

Most of the work conducted to investigate the effect of infection on cognition was done by Smith and colleagues at Medical Research Centre (MRC), Common Cold Unit in Salisbury, UK. It was mainly on viral infections, especially the common cold and influenza. These infections are common . It is estimated that each individual has between one and three attacks per year and it is common for people to continue with their normal routine and work. Therefore, there has been a growing concern that if these infections detrimentally affect performance it may reduce people's efficiency and safety at work.

Both naturally occurring and experimentally induced respiratory viral infections were investigated and studies involved both cross-sectional and prospective designs. In these studies, the performance of infected subjects was compared with their performance when they were not infected or with performance of healthy controls. In most of these studies, computerized tests have been used to measure reaction time and fine motor functions.

3.5.2. Evidence from studies on cold:

Studies of both naturally occurring and experimentally induced colds have shown that a cold detrimentally affects performance. It was found that a cold impaired hand-eye coordination in a motor task (Smith *et al.* 1987a; Smith *et al.* 1988; Hall, Smith, 1996a), slowed the speed of response in a serial reaction task (Smith *et al.* 1987b;

Smith *et al.* 1989b; Hall, Smith, 1996a) and impaired learning and recall from stories (Smith 1990, Smith *et al.* 1990). Subjects with a cold also reported an increase in negative mood and they were significantly more feeble, clumsy, lethargic, mentally-slow and incompetent than those who were non-infected (Hall, Smith, 1996a). These effects were observed during the incubation period (Smith *et al.* 1987b; Smith *et al.* 1990), with sub-clinical infections (Smith *et al.* 1990) and after the clinical symptoms disappeared (Smith *et al.* 1989b). On the other hand, it was found that a cold did not impair some other functions such as working memory and semantic memory (Smith 1990; Smith *et al.* 1990; Hall, Smith, 1996a).

3.5.3. Evidence from studies on influenza:

Naturally occurring or experimentally induced influenza can also impair performance, but in different tests. Those who were infected with influenza were found to be slower in both speed and accuracy in a reaction-time task and a detection task than uninfected controls (Smith *et al.* 1988; Smith *et al.* 1993). Infected subjects were also slower in a search task and they made significantly more errors (Smith *et al.* 1989b, Smith *et al.* 1993). These effect were observed during the incubation period (Smith *et al.* 1988; Smith, 1990, Smith *et al.* 1989b) and after the symptoms had gone (Smith, 1990). Other functions such as memory, logical reasoning, hand-eye coordination and mood were not affected by influenza (Smith *et al.* 1987a; Smith *et al.* 1993).

3.5.4. Evidence from Other infections:

A study on infectious mononucleosis (IM) showed an impairment of performance similar to that observed with influenza in acute IM. In chronic IM, similar effects to those described in chronic fatigue syndrome (CFS) were found. Patients with acute IM had significantly slower reaction and impaired attention while those with a chronic infection showed an impairment in free recall and logical reasoning (Hall, Smith, 1996b).

In another study, the performance of patients suffering from depression, CFS, or acute infective illness such as acute Epstein-Bar virus or influenza-like illness was compared with the performance of healthy controls. Results showed that the three groups of patients made significantly more errors and were slower in attention and concentration tasks (e.g. auditory discrimination, short-term memory etc.). However, patients with CFS and depression showed greater impairment than those with an acute infection (Vollmer-Conna *et al.* 1997).

3.5.5. How infection affects cognition?

It is hypothesized that cytokines' effects on the central nervous system (CNS) may provide a mechanism through which infections influence cognition in a direct but transient way (Smith *et al.* 1991). Evidence from different research areas is emerging.

In viral infections such as common cold and influenza it has been shown that even in the absence of clinical symptoms infection may alter performance, and that this occurs before the appearance of symptoms, during the incubation period in subclinical infection, and after the symptoms have disappeared (3.4.2 and 3.4.3). It is postulated that performance is sensitive to immunonolgical changes that occur before, during and after the symptoms and these effects may occur through cytokine production which is increased in upper respiratory tract infections (Smith *et al.* 1991). In a double-blind controlled trial it was demonstrated that injecting volunteers with different doses of interferon produced influenza-like symptoms and impairments in mood and performance similar to those described in acute viral infections. Challenged subjects were slower in a simple reaction task and a peg board task, the two tasks that were found to be impaired in respiratory viral infections. Although the effects produced by IFN-α were less marked by the second day after the challenge, the authors mentioned that there was some evidence to suggest that after-effects of IFN-α may occur (Smith *et al.* 1991). Some other tasks found to be impaired in viral infections such as search and memory tasks were not found to be affected by IFN-α challenge and other cytokines such as interleukin-1 may be responsible for the impairment of these tasks (Smith *et al.* 1991).

Preble and Torrey (1985) found that 25% of psychotic patients had high titters of INF-α. There is suggestive evidence from research conducted among cancer and hepatitis B patients who have been treated with INF-α that it has detrimental effects on cognition and mood. These effects were dose related, reversible with discontinuation

of therapy, and the purification of interferon did not prevent symptoms (McDonald, Mann, Thomas, 1987).

In ten patients who received INF-α therapy for cancer it was found that it produced slight to moderate cognitive changes and moderate to severe changes in behaviour. The fact that such changes appeared or increased after initiation of treatment and their reversibility on stopping the treatment, support the view that the effect was drug-related and not disease-related. The authors hypothesized that these changes are probably caused by a diffuse toxic encephalopathy that interferes with frontal lobe function (Adams, Quesada, Gutterman, 1984).

In another study reversible central nervous system toxicity manifested by somnolence, confusion and parasthesia was noticed during INF-α therapy. Patients returned to normal within seven to 10 days of stopping of treatment. Some changes were noticed in their electroencephalograms (EEG), such as an increase in slow wave activity during receiving INF-α in comparison to a normal pre-treatment EEG. A repeated EEG after withdrawal of treatment showed a return to pre-treatment normal activity (Smedley *et al.* 1983).

In a randomized controlled trial, 43 patients were allocated into one of four groups: three groups were treated with different doses of INF-α and one acted as a control group. The analysis showed a rise in psychiatric morbidity in the treatment groups compared with the controls (McDonald, Mann, Thomas, 1987).

The problems with these studies are their small sample sizes and the high therapeutic dose of INF-α used. However even small doses were found to have an effect. Honigsberger and colleagues showed that small dose of INF-α produced gross changes in the EEG but no neurological symptoms (Honigsberger, Fielding, Priestman, 1983). Smith and colleagues (1991) have shown that injecting subjects with small doses of INF-α produced influenza-like symptoms and that impairments in mood and performance mimic those described in acute viral infections.

More recently, two review articles have been published. In a review of mood and the cognitive side effects of INF-α, Valentine and colleagues (1998) described common cognitive dysfunctions that were associated with INF-α therapy, including memory impairment, apathy and cognitive slowing. They pointed out that even after a single small dose of INF-α, normal healthy volunteers can suffer from reduced alertness. In the other review, Licinio and collagues (1998) explored possible mechanisms for the INF-α effect on CNS. They suggested that cognitive and mood effects that were seen during IFN- α therapy could be caused by a its direct effect on CNS or through other cytokines that are induced by IFN- α such as interleukin-1, interleukin-2, interleukin-10 and tumour necrosis factor (TNF). They also, stressed that different CNS cell types such as microglial cells and astrocytes, are sensitive to cytokines.

4. The Yemen Republic

4.1. Introduction:

The Republic of Yemen was recently formed on May 22, 1990, by combining the Yemen Arab Republic (North Yemen) and the People's Democratic Republic of Yemen (South Yemen). It is bordered by Saudi Arabia to the north, the Red Sea to the west, the Sultanate of Oman to the east and the Gulf of Aden to the south (fig. 3). The total population is 15.8 million, of whom 52% are under 15 years and 70% live in rural areas. The annual growth rate is 3.7 % and the average total fertility rate is 7.7 (Central Statistical Organization, 1994).

In Yemen, Arabic is the language of the nation and Islam is the religion. Topographically, the country could be divided into five regions, mountainous, plateaus, coastal, Ar Rub-Al Khali and islands. There are 17 governorates in the unified Yemen: Sana'a: the political capital of the country, Aden: the economic and commercial capital of the country, Al-Hudaydah: the main port of the country, and Taiz: The third biggest city in the country.

The climate in the coastal area (the Tihamah region), is primarily dry and hot with an annual rainfall of about 229mm (9in). This region is prone to severe sandstorms and maximum daytime temperatures range from 32°C in winter to over 40°C in summer. The highland areas boast a mild, temperate climate with pleasantly cool winters. Temperatures range from 21.7°C in June to 13.9°C in January. Highland rainfall

fig. 3. Map of the Yemen Republic

varies from about 406mm (16in) to 762mm (30in). Two periods of increased rainfall are linked with the eastern and western monsoons; the first of these arrives in April/May and the second in July to September. Most of the rain falls on the western mountains. Rainfall gradually diminishes towards the east and in the Rub' al-Khali desert (Empty Quarter) no rain ever falls.

The natural resources are petroleum, fish, rock salt, marble, small deposits of coal, gold, lead, nickel, and copper, fertile soil in west. Agriculture: accounts for 26% of the gross domestic products (GDP). Topographical variations in the country give rise to a wide range of climatic conditions, and its fertile highland plateaux are ideal for growing a wide variety of both tropical and temperate zone crops. These highland regions are interspersed with wadi - river valleys- which are dry in the summer months. Crops include coffee, cotton, sorghum, corn, oats, barley, dates, almonds grapes and qat (a leaf which is widely chewed by Yemeni people). Farming activities also include the breeding of livestock, such as cattle, sheep and goats.

Yemen has got some horrific health indicators. Maternal mortality is 1400 per 100 000 live births. The infant mortality rate is 78 per 1000 live births and under five mortality is 105 per 1000 live births. Only 61% of the total population have access to safe water and only 24% have access to adequate sanitation. Live expectancy is 57 years. Malnutrition is widespread and the percentage of under five suffering from moderate and severe underweight and from moderate and severe stunting are 39% for each. The percent of one-year children who were fully immunized is 59%. The literacy rates are 53% and 26% for male and females respectively (UNICEF, 1998).

4.2. Malaria in the Yemen Republic

4.2.1. Introduction:

Malaria is one of the priority health problems in the Yemen Republic. Sixty per cent of the total population lives in areas at risk of malaria. Reports indicate that malaria has increased in recent years. In 1992 it was estimated that the true incidence was around 500 000 cases (National Malaria Control Programme, 1992). Outbreaks with malaria deaths were reported in 1992 from several governorates including the mountainous areas (WHO, 1996b). In 1998, it was estimated that 1.5-2 million people are suffered from malaria annually (National Malaria Control Programme, 1999).

4.2.2. Epidemiology of malaria in the Yemen Republic:

Malaria endemicity varies with the topographical features of the country. In the coastal plain area (Tihama region) malaria is hypoendemic to mesoendemic and the spleen rate which is the prevelance of splenomegaly in children aged between two and nine years (Granham, 1966) is up to 25%. Hot seasons are not generally favorable for malaria transmission while the less hot seasons (October through April) are more favorable for transmission in this area. The endemicity is generally higher in the foothills where the plain coastal areas meet mountains. Pockets of mesoendemicity and hyperendemicity may be found here with the spleen rate up to 67% and malaria transmission seems to be relatively prolonged in this type of area. Malaria endemicity is considered to be hypoendemic in the highlands with the spleen rate up to 9%. The warmer season (May through September) is more suitable for malaria transmission in

this region. Malaria has been considered to be sporadic in the desert area sloping to the Empty quarter (Rub Al Khaly). Malaria is hyeperndemic in Sokotra island where the spleen rate of 60% has been reported (Thuriaux, 1971; National Malaria Control Programme ,1993a).

Ninety to 97% of malarial infections are due to *P. falciparum* (WHO, 1996b). *Anopheles arabiensis* is the principal vector and *A. culicifacies* is suspected to be a secondary vector in Sokotra island (National Malaria Control Programme, 1993b). Other *Anopheline* species such as *A. sergentii*, *A. cinereus*, *A. rhodesiensis* and *A. d'thali* have been reported also.

The vector mosquitoes are present in rural as well as in urban areas. Water courses along wadi (river valleys) are natural breeding places of *A. arabiensis*. However, the mosquito has adapted to breed in man-made water collections, such as irrigation canals, household water containers (e.g. cement tanks, clay pots, barrels etc.) and waste water collections near water-pumps (National Malaria Control Programme, 1991).

The country is prone to periodic malaria outbreaks with considerable morbidity and some mortality. Outbreaks following heavy or prolonged rainfall and flooding in otherwise dry areas are common, particularly in southern governorates and mountainous areas (National Malaria Control Programme, 1993a).

4.2.3. Present malaria situation in the Yemen Republic:

In the Yemen the prevalence of malaria have increased in last 10 years and the prevalence was the highest ever recorded. In 1990 110,000 cases were confirmed and the slide positivity rate (SPR) was 14% compared with 4.6% in 1985 (National Malaria Control Programme, 1993b). In 1994 malaria came only after gastroenteritis in the incidence of reported infectious diseases when 158,935 cases were reported and the SPR went up to 23% (MOPH, 1994). However, it should be noticed that data given above were collected from a selected number of health facilities, mainly in cities, and reports were often incomplete. The data do not represent the whole country nor a part of the country all the year round. At best the data come from approximately 50% of the country. However, they do indicate a trend in malaria incidence.

The increase of malaria cases in the recent years has been attributed to several factors: an increase in rainfall and man-made breeding sites, a decrease in anti-malaria activities due to financial constraints, an increase in the movement of the population, and an increase in people seeking diagnosis and treatment are the main contributors.

4.2.4. Malaria control in the Yemen Republic:

The National Malaria Control Programme (NMCP) was established in the northern governorates (previously known as the North Yemen) in 1978, and much earlier in the South Yemen. The main goals are to support and strengthen malaria control throughout the country. The main antimalarial activities are DDT spraying, larviciding, and passive and active case detection and treatment.

DDT spraying in houses was carried out in the seventies and early eighties with fairly good results but it was stopped in late eighties as it could not be sustained for financial and logistical reasons. Larviciding using organophosphorus insecticide(Temphos) was initiated in the eighties in high malaria risk areas where more than 300,000 people benefited, but was stopped in 1992 due to financial constraints. Diagnosis and treatment of malaria have been carried out by all levels of health services. However diagnosis is mainly on clinical criteria and microscopical examination of blood smears is only carried out by hospitals or main health centres (National Malaria Control Programme, 1992).

Chloroquine continues to be the first line antimalarial drug although resistance is an increasing problem. The evidence obtained to date indicates that the problem of chloroquine resistance is a real one. The proportion of resistant cases among subjects tested varies from 5% to 19% and is higher in the southern part of Tihama (National Malaria Control Programme, 1993b). WHO (1996b) reported that simplified *in vivo* tests carried out in 1992 showed chloroquine resistance (R-I and R-II) in 12% of cases. Chloroquine resistance has not been recorded in the southern governorates so far (National Malaria Control Programme, 1993b).

The main limitation of the NMCP in the battle against malaria, besides the financial constraints, is a shortage of trained technical personnel such as epidemiologists, entomologists, parasitologists and operational specialists) who can evaluate, monitor, train and coordinate antimalarial activities in the country.

4.3. Education in the Yemen Republic:

In the Yemen there was a marked improvement in the provision of education services in the last thirty years, and the number of schools has enormously increased. The total student population grew from less than 250,000 in 1970 to almost 2.75 millions in 1994 (World Bank, 1998). However educational services are still inadequate and the system suffers from many shortages and limitations. Teacher unavailability, especially in rural areas, low qualification level of teachers, and out of date curriculum together with poor physical school environment are major obstacles.

The illiteracy rate in the Yemen is very high. The 1994 census revealed that 56% of the whole population was illiterate (34% in urban and 64% in rural areas), a figure which is higher than the average illiteracy rate in the least developed countries, which is 47%. Illiteracy is highly concentrated among women as compared with men. In the urban areas the females' illiteracy is 48% and in rural areas it jumps to 85%. The figures for male illiteracy in urban and rural areas are 23% and 42% respectively (Central Statistical Organization, 1994). The Yemen Demographic Maternal and Child health Survey showed that nine out of ten women had not received any formal education and less than five per cent have received formal schooling above the primary level (Central Statistical Organization, 1992).

Total school enrollment has risen 11-fold since 1970 when schooling first became available in large scale (World Bank, 1998). However this increase in the total

enrollment is not been equally distributed across the population. Enrollment rates in basic education for age six to 15 years in the academic year 1994-1995 was 55%, 80% and 49% for the whole population, the urban and the rural areas respectively. The female enrollment rates were only 37%, 75% and 27% respectively. The data also revealed great differences in school enrollment among different governorates with the highest enrollment rates (86%) in the capital, Sana'a (Central Statistical Organization, 1992).

High dropout rates are also a major problem. It has been found that by the end of the primary school over half of both girls and boys have dropped out and that by the end of the preparatory school three quarters of children who started school no longer attend (World Bank, 1998).

Learning achievement is another big concern. The poor quality of education together with poor health of schoolchildren has resulted in low levels of student performance in the Yemen Republic. A recent learning achievement assessment administered to a sample of 983 fifth-grade schoolchildren in seven governorates found that students performed poorly in all three of the subject areas covered (mathematics, science, and Arabic). Eighty-five students failed the mathematics' component of the test, 65% failed the science's component, and 80% failed the Arabic component. Only 3% of mathematics students, 14% of science students, and 5% of Arabic students received satisfactory passes (World Bank, 1998).

4.4. Schoolchildren's health in the Yemen Republic:

There is extremely little information on the health of schoolchildren. In a preliminary limited survey conducted among rural and semirural areas in Sana'a governorate 641 schoolchildren were investigated to estimate the prevalence of intestinal and blood parasites. The results showed that the prevalences of *A. lumbricoides* and schistosomiasis among the rural areas were 49% and 32% compared with 17% and 5% among semirural areas. However, no concentration method was used in stool analysis which may explain the low prevalence of other intestinal parasites (8% of *Trichuris* and 3% for *E. histolytica*). No malaria parasites were detected, which is also not unexpected at 5000 feet above sea level (el-Qirbi, Sadek, Ghaly, 1987).

In another study, schoolchildren from lowland and highland southern areas were screened for blood and intestinal parasites. Malaria was detected by microscopy only in 7% and 4% respectively compared with 67% and 12% by an immunofluorescent antibodies test. *S. haematobium* was detected in 29% and 13% from lowland and highland areas respectively while *S. mansoni* was detected in 19% of those from the highland area only. However the small sample size (approximately 26 and 30 for malaria and 26 and 57 for intestinal parasites in lowland and highland areas respectively) was a major limitation of this study (Kopecky *et al.* 1992).

Three studies investigated schistosomiasis among schoolchildren in the Yemen Republic. Hazza and colleagues (1983) found a patchy distribution of schistosomiasis in Taiz province with an overall prevalence of 37%. In another study, conducted in

Amran, Sana'a governorate, it was found that the prevalence of *S. haematobium* was 29% (Janitschke *et al.* 1989). In a third study, a prevalence of 23% of *S. mansoni* and 17% of *S. haematobium* was found in Dhamar governorate (Schaap, Den-Dulk, Polderman, 1992).

Recently, I conducted a survey among schoolchildren in Aslam district (Al Serouri, 1995). 30% of students attending grade four to six (n = 330) were selected randomly from 34 primary schools in Aslam. Demographic data and medical history were recorded, a clinical examination was carried out and blood, urine and stool samples were collected and examined microscopically. The results pointed out the low standard of health among the schoolchildren. Anemia defined as less than 11 g/dL was present in 85% of the children. Malnutrition is highly prevalent and 45% were stunted and 54% were underweight. Overall asymptomatic malaria parasitaemia was 35% and more than 50% in the rainy season. Schistosomiasis, mainly *S.haematobium*, was common (36%). The study also showed a marked lack of basic health education among schoolchildren regarding the method of transmission of common diseases e.g. malaria and schistosomiasis.

5. The study

5.1. Rationale for the present study:

Asymptomatic malaria parasitaemia is highly prevalent among schoolchildren, especially in hyperendemic and holoenedemic areas where up to 90% of schoolchildren are affected (2.3). The impact of asymptomatic parasitaemia on cognitive function and school achievement of schoolchildren has not been studied and there is an urgent need for this to be addressed.

In the present study the effects of asymptomatic parasitaemia on cognitive functions and school achievement are examined taking into account other possible confounding factors which are not uncommon in endemic areas, and which could affect cognitive function and school achievement e.g. socioeconomic background and nutritional status.

5.2. Hypotheses:

Malaria might effect cognition either through its long-term or its short-term effects.

5.2.1 Long-term effects:

There is some evidence that geohelminthic infection can detrimentally affect cognition (3.3.1.3). Although the mechanisms through which this occurs is not well understood, indirect mechanisms such as malnutrition, growth retardation, and anaemia (Bundy and Cooper, 1989; Callender *et al.* 1994) have been postulated.

By extrapolation malaria may affect children's cognitive function in a similar way. Children with parasitaemia are usually more anaemic than those without parasitaemia (2.4.1). Improvement in the prevalence of anaemia has been found in children following chemoprophylaxis and bed net trials (2.4.2). Although the anaemia of falciparum malaria has an extremely complex pathogenesis, iron deficiency is also likely to be present (2.4.4).

Children with parasitaemia also have a poorer nutritional status than children without parasitaemia (2.5.1). Following malaria control using chemoprophylaxis, combined chemoprophylaxis and residual spraying or, more recently, with impregnated bed nets, children have shown improvement in their nutritional status (2.5.2).

Both iron deficiency anaemia (3.2) and protein energy malnutrition (3.1) may affect cognition, thus malaria acting through these indirect mechanisms might also affect cognition.

5.2.2. Transient effects:

Parasitaemia may also have a direct but a transient effect on cognition mediated through cytokines. Recent research has confirmed the importance of changes in cytokine concentrations (e.g. IFN γ, TNF, IL-1, IL-6) and their acute phase response (e.g. CRP) in asymptomatic malaria (2.6). It has been also shown that infection e.g. cold and influenza can detrimentally affect cognition (3.4). In a double blind controlled trial, interferon produced changes in performance similar to those described

during colds (Smith *et al.*1991). Thus cytokines acting on the CNS provide a possible mechanism through which infections influence cognition in a direct but transient way (3.4.5). Therefore it is possible that children's cognition may be affected through cytokines in asymptomatic malaria. Children tend to have repeated episodes of asymptomatic malaria parasitaemia (Bruce *et al.* 1996, Farnert *et al.* 1997). If their cognition is detrimentally affected over substantial periods of time then this is likely to have long term effects on their development and school achievement.

The long-term effects i.e. anaemia and malnutrition, are most likely to be manifested over fairly long periods of time and need a long follow up. For this study we are more interested on the transient effects that can occur through cytokines and acute phase response as has been shown in other acute infections such as common cold and influenza (3.4).

5.3. Aim and specific objectives of the present study:

5.3.1. Aim:

To determine whether asymptomatic malaria parasitaemia affects schoolchildren's cognitive functions.

5.3.2. Specific objectives:

1. To compare the cognitive functions of schoolchildren with and without asymptomatic malaria parasitaemia.
2. To determine the effect of treatment or natural cure on the cognitive functions of children with asymptomatic malaria parasitaemia.

5.4. Summary of chapter one: Background information:

In this chapter:

- the literature about the asymptomatic malaria among schoolchildren and the relationship between malaria , anaemia and malnutrition has been reviewed. The importance of cytokines and acute phase proteins in pathogenesis of symptomatic malaria and possible effects on cognition has been discussed. Available literature about malaria and schooling and cognition has been also reviewed.
- the literature about nutritional and health factors (e.g. parasitic infections) associated with cognition, school achievement, and attendance has been reviewed.
- the available literature about impact of acute infection -mainly common cold and influenza- on cognition together with the possible mechanisms for such impact also reviewed.
- the health status, malaria situation and education in the Yemen Republic has been discussed.
- finally the rationale of the present study and possible long-term and short-term mechanisms by which malaria may affect cognition together with the aim and specific objectives of this study has been discussed.

Chapter Two

Methods

6. Methods

Two separate studies with different designs were conducted. I first conducted a randomized controlled trial of the effect of treatment of asymptomatic malaria parasitaemia on cognitive functions of schoolchildren. Due to the unforeseen rapid clearance of parasitaemia among the placebo group I abandoned the trial after half the children had been enrolled and I ran a second study designed as a natural trial.

Due to the differences in the study designs, the method of each study will be described separately. However, since the measurements are applicable to both studies, with only few additional measurements for the second study, I discuss them at the end of the second study underlining any differences from those used in the first.

6.1. The clinical trial:

In this study I planned first to compare cross-sectionally the cognitive function of schoolchildren with asymptomatic parasitaemia with those of non-parasitaemic group matched for school and grade. Then we planned to conduct a double blind, randomized controlled trial to assess the effect of treating schoolchildren with asymptomatic parasitaemia on their cognitive function.

6.1.1. Study Area (See fig. 4):

The study was conducted in a rural community in the Aslam district of the Hajjah governorate, the Yemen Republic. It is six to seven hours drive from the capital Sana'a.

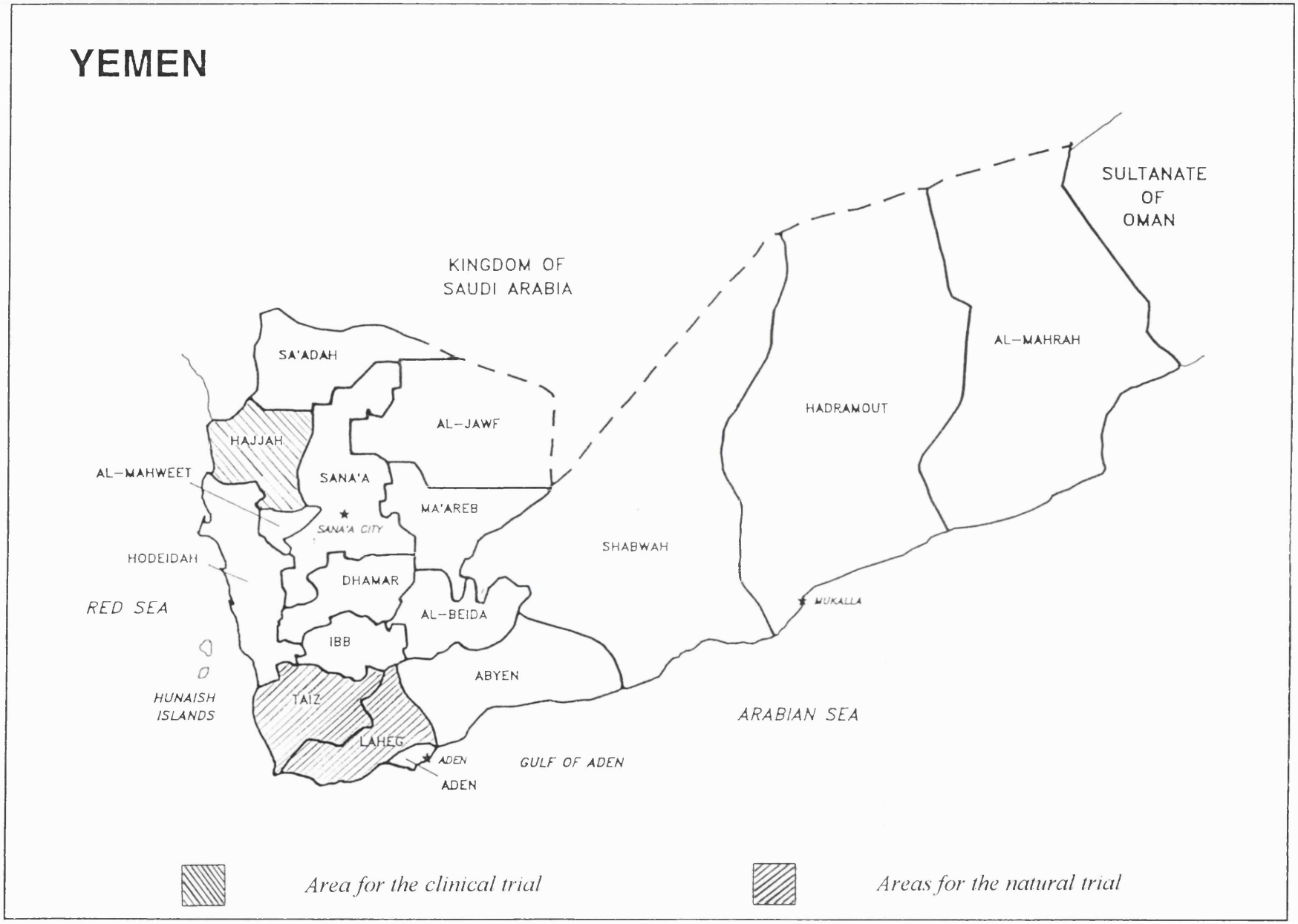


fig. 4 . Map of the study areas

Hajjah is one of the poorest governorates in the Yemen Republic and Aslam contains some of the poorest families in Hajjah. The community is partially agricultural but also engaged in tending animal herds or in daily paid labour. The community is linked together by inadequate dirt roads and the main methods of transportation are walking, riding animals and a few four-wheel drive cars. The principal food crops are maize, sorghum, rice and potatoes.

There is only limited cash crop production e.g. qat (*Catha edulis*). There are two rainy seasons: a long one from February to May and a short one from September to November.

Most of the population lives in huts or in thatched houses built of mud with dirt walls and floors. Houses usually have an adjacent open area for animals (cows, goats and cattle). Drinking water comes from wells and most of the population use open fields for defecation. Electricity is usually unavailable except for very few private generators. Education (mainly primary) is available through scattered public schools that are in poor condition, some in the open air or under trees. The children have to come to school from the surrounding villages on foot, which may take up to one hour a day. Hajjah governorate has one of the lowest rates of female primary school enrollment and most schools are attended by boys. In a limited number of schools a few girls are enrolled, but they only attend the first four grades.

The area is endemic for malaria and a previous pilot study conducted in 1995 showed that among schoolchildren the overall asymptomatic parasitaemia rate was 35% and more than 50% in the rainy season. This made the area an ideal setting for this study.

6.1.2. Study Design (See fig. 5):

A randomized controlled trial of Fansidar (sulphadoxine and pyrimethamine) on the cognitive function of schoolchildren with asymptomatic parasitaemia was conducted. The trial was scheduled to run in two phases in order to accommodate the testing schedule. Approximately half the children were to be enrolled in the first phase and, following completion of their pre-treatment tests, the remaining children were to be enrolled in the second phase. After completion of the pre-treatment tests of the second phase the post-treatment tests of the first phase were to be started, and after that the post-treatment tests of the children in the second phase completed.

6.1.3. Time-line of the clinical trial:

1996:

November - December	- Piloting the cognitive and school achievement tests by the principal investigator
	- Recruiting different team members
	- Obtaining the necessary permission from the Ministry of Education and Ministry of Public Health

1997:

1st January - 31st January	- Training of the cognitive testers
1st February - 14th March	- Getting reliability for the cognitive testers
15 th February -14th March	- Screening schoolchildren for parasitaemia
	- Choice of schools
15th March - 15th April	- The clinical trial
15th April - 15 th May	- Data Entry
July	- Principal investigator back to the institute in London
	- Data analysis and reporting

6.1.4. Sample:

6.1.4.1. Rationale for sample size:

The sample size was calculated using different formulae (Kirkwood, 1994). The outcome variables are the cognitive function test scores. As there are no local data on these variables, we used data from studies of children in other developing countries (Jamaica and Bangladesh) in similar age groups to estimate the sample size required. For the case control study, the sample size required to show a difference of one third SD of the mean between parasitaemic and non-parasitaemic groups, taking 5% level of significance and 80% power, was approximately 100. For the clinical trial the sample size calculated to be able to detect a difference of 10-15% in cognitive function tests scores, a sample size of 120 was required to achieve this at 90% power and 5% level of significance. We increased the sample size to allow for unplanned changes in parasitaemia and for drop-outs.

6.1.4.2. Selection of schools:

A list of primary schools within possible access by the research team was produced and the number of children in each of these schools was obtained from the Education Officer in Aslam. We had originally intended to work in 10 schools. However during screening (see 6.1.3. 3) it became obvious that the prevalence of parasitaemia was much lower than we previously estimated. Therefore, we screened children in thirty-two out of thirty-four public primary schools in the district of which 14 schools were chosen to participate in this study. In 9 schools there were no girls and in the other 5 schools there were only 45 girls in grade three to six, so we decided to enroll only boys. The main selection criteria for these schools were the availability of sufficient parasitaemic children and the willingness of the headmasters and teachers to participate. The prevalence of asymptomatic malaria among the selected schools was 30 to 50% but most of the infected children had a very low parasite density.

6.1.4.3. Screening for malaria parasitaemia and measurement of parasite density:

A thick blood film was prepared on a microscope slide from blood obtained by pricking the child's finger. The dry film was fixed and stained with Giemsa and examined for *Plasmodium spp.* by an experienced malaria technician, who is teaching at the National Malaria Control Program (NMCP).

For positive films the number of the parasites per μl was determined. One hundred high power fields (HPF) of the Giemsa-stained thick blood film were examined under the microscope using × 10 eyepieces and a × 100 objective. The blood film was

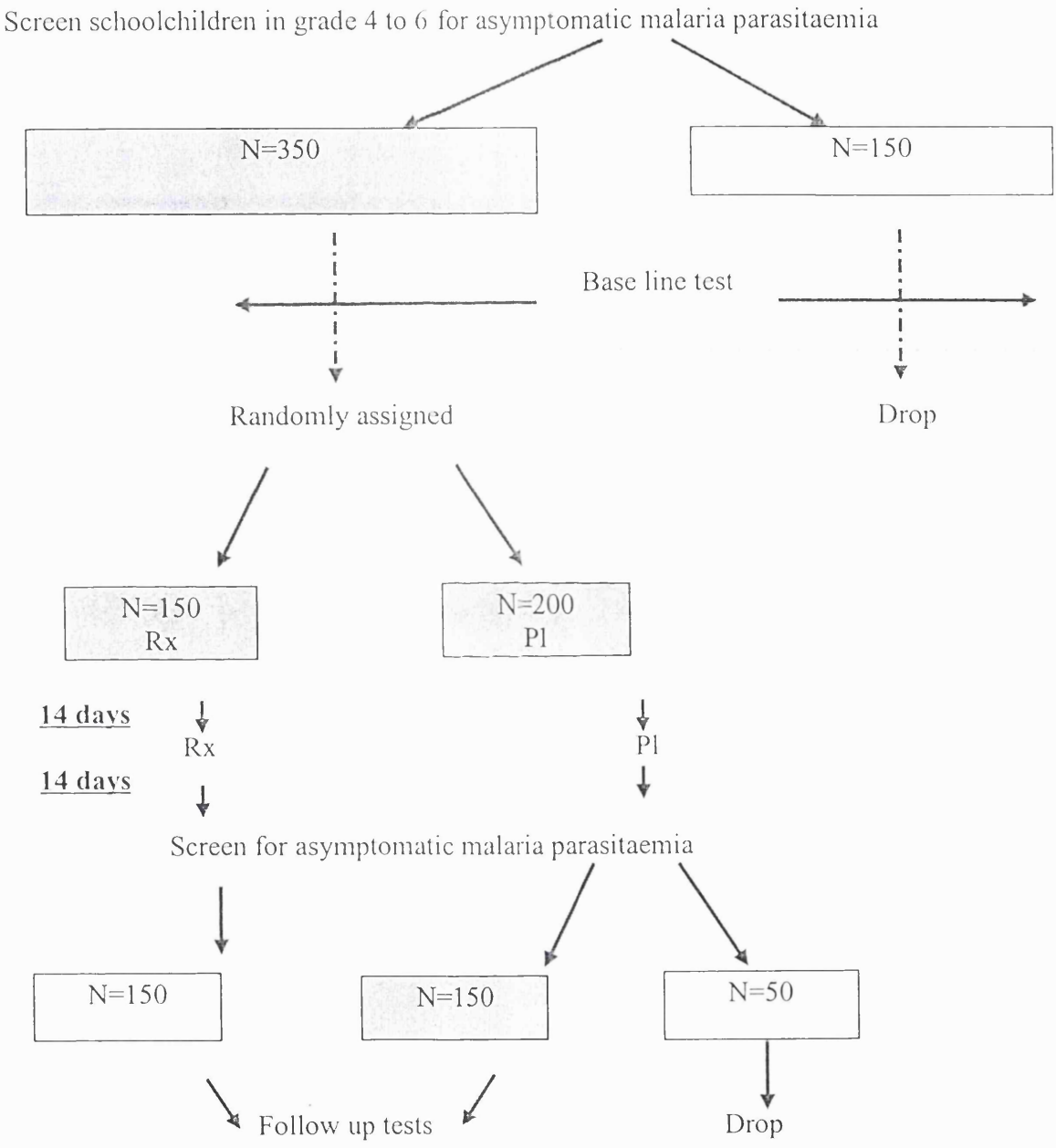
examined systematically to ensure that each section of the film is covered by screening the film from side to side and then moving downwards until 100 HPF had been viewed. The numbers of HPF containing one or more parasite were determined. When each field contained one or more parasite, 10 to 50 HPF were examined to determine the number of parasites per HPF. The number of the fields counted was determined by the parasite density and only 10 were counted when it was very high. The average number of parasites per HPF was multiplied by 500 to give an estimated parasite density per μl.

Previous research have shown that this method proved to be more accurate than that based on determination of the parasite/white blood cell ratio, probably because the variability in the volume of blood used in preparation of thick blood films is less than the variability of the white blood cell count (Greenwood, Armstrong, 1991).

6.1.4.4. Selection of children:

All study children were boys, aged between nine and 14 years, in grades three to six from the 14 selected schools in the first phase of the trial. The following children were excluded: those who have learning difficulties or serious chronic illness, those with a history of chills or fever in the previous three days, those with a an axillary temperature more than 37.5 °C and those whose parents refused to give their consent.
Since a previous study conducted in the same area in 1995 (Al Serouri, 1995) showed that among schoolchildren the overall prevalence of urinary schistosomiasis was 36% and there are some reports that schisostosmiasis may have a detrimental effect on

fig 5. Proposed study design of the clinical trial



Key:

Parasitaemic

non-parasitaemic

Rx treatment

Pl placebo

cognition (Clarke, Blair, 1966; Castle, Clarke, Hendrikz, 1974), we added another exclusion criterion by excluding those with a positive strip test for haematuria.

Two groups of children were then chosen:

Parasitaemic group: in whom *Plasmodium falciparum* seen in a stained thick blood smear. Two exclusion criteria were added to the previous criteria. The parasitaemic children with parasite density less than 500 parasites per μl were excluded as this level of infection was less likely to have an effect. I had originally intended to use 1000 parasites per μl of blood as the cut-off point but since the parasitaemic children had a very low parasite density, the first 165 infected children who had a minimum parasite density of 500 parasites per μl and fulfilled the selection criteria were enrolled into the study. The other exclusion criteria was those with a parasite density more than 5000 parasites per μl were also excluded because their health may been in jeopardy and they were treated.

Four children were subsequently excluded; two refused to attend the cognitive function tests and two devloped fever on the day of cognitive testing. The final sample comprised 161 parasitaemic children.

Non-parasitaemic group: this comprised 60 non-parasitaemic children who had no *Plasmodium spp.* seen in 100 high power field (HPF) in a stained thick blood smear. This group was matched with the parasitaemic group for grade

and school in ratio of one non-parasitaemic child to three parasitaemic children. When the number of parasitaemic children was not dividable by three and there was just one extra child, this child was not matched with a non-parasitaemic one but if there were two extra children then the second parasitaemic child was matched with a non-parasitaemic one.

6.1.5. Procedure:

All parasitaemic and non-parasitaemic children were given a set of baseline measurements on enrollment which included cognitive function tests. They were interviewed to determine details of their socioeconomic background and had a general and abdominal examination. The children's weights and heights were recorded. Haemoglobin was measured using the HemoCue apparatus (HemoCue Inc., Mission, Vieja, California) (Hudson-Thomas, Bingham, Simmons, 1994). Blood samples collected on filters paper for measuring the C-reactive protein (CRP). Venous blood samples were also collected, centrifuged, and sera were kept frozen. However, due to repeated power cut and unsatisfactory storing conditions these samples were not used. All tests were repeated at the end of the treatment. Details of measurements are given later (see 6.3).

The non-parasitaemic group was not studied further. The parasitaemic group was randomly assigned to treatment or placebo.

6.1.6. Treatment:

Since chloroquine resistance has been reported from the Yemen Republic (WHO, 1996b), I decided to use fansidar tablets in a single dose of 25 mg /kg of sulpha-doxine and 1.25 mg /kg of pyrimethamine (Hagos *et al.* 1993). Both fansidar and placebo tablets were offered as a gift from the Yemen Drug Company (YDCO) and of similar colour and size. Since I expected that some of the children in the placebo group will lose their infection due to immunity I increase the size of the placebo group to be 200 children compared with 150 in the treatment group . Therfore, the fansidar and placebo divided into seven groups: 4 for placebo and 3 for fansidar.The groups were coded alphabetically from A to G by Prof. Sally McGregor during her visit to the Yemen and the code was taken with her and kept at the Institute of Child Health, in London, till the end of the study. Prof. Sally McGregor did not participate at any stage of study implementation and she left the Yemen before the study began. The research team was unaware of the treatment allocation. Throughout the study treatment received was not known until all subjects had completed post-intervention testing and the principal investigator was back in the UK. The tablets were kept in similarly labeled drug bottles. After a child completed initial screening he was assigned in order to one of these bottles and was given tablets according to his weight. The tablets were given by the principal investigator and were swallowed in front of him.

6.1.7. Follow-up (See fig. 6):

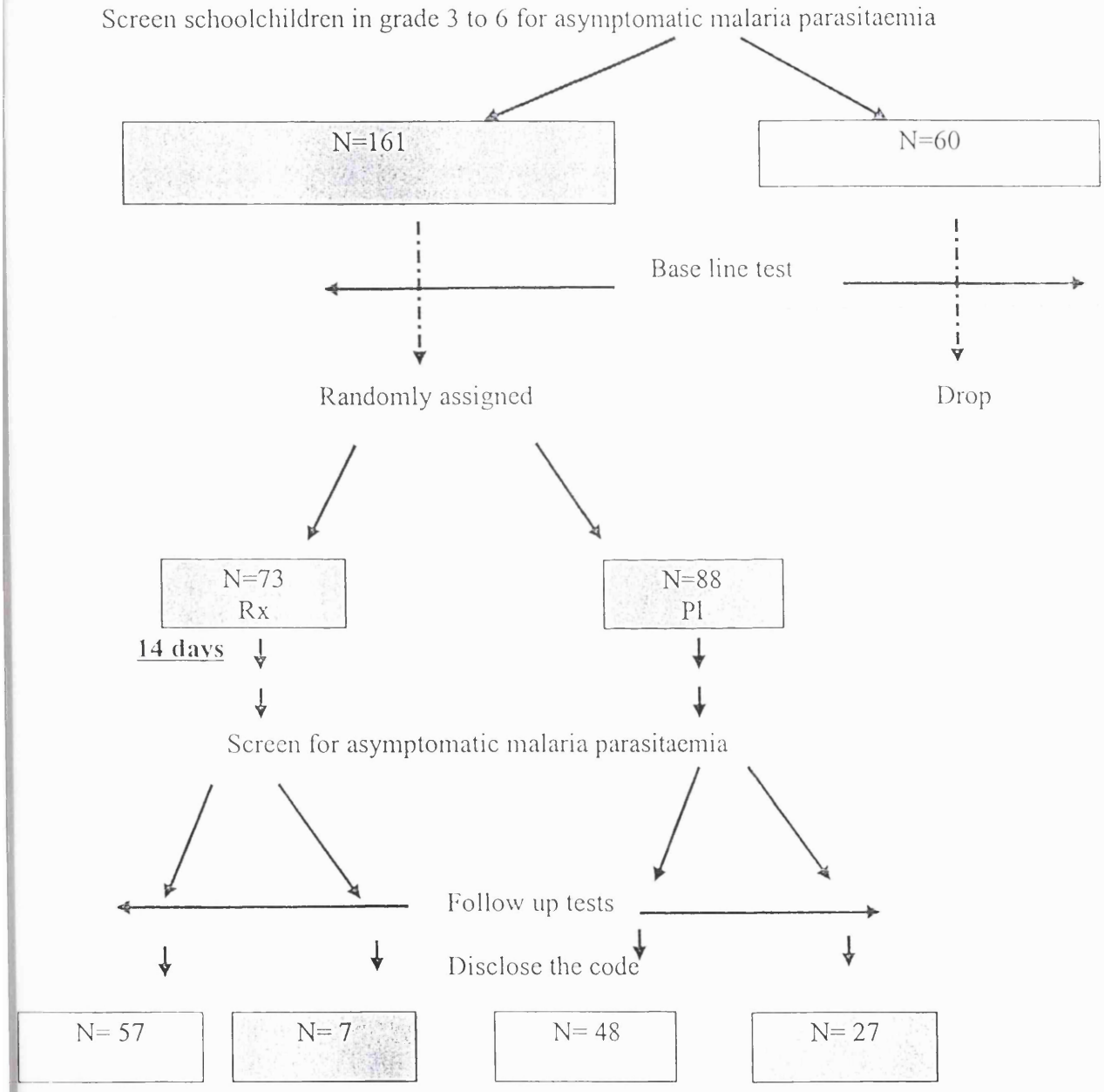
To prevent re-infection in the treated group, which is unlikely to occur in treated children within 14 days of treatment (Salako *et al.* 1995), it was planned that the treatment should be repeated 14 days later. At that time we repeated the malaria smear

for 50 of the parasitaemic group to be sure that the necessary numbers of infected children would be available. To our surprise, only 11 of those who were screened remained infected. After discussion with Prof. Sally McGregor we decided not to wait for four weeks to do the follow-up tests as planned. Instead we started the follow-up tests two weeks after initial treatment before further loss of infection from the infected placebo group occurred.

I planned to enroll into the second phase of the clinical trial immediately following the follow-up but there was an unexpected drought that led to low prevalence of malaria in that season. I felt that I could not take the risk of conducting the study as planned because it would be too difficult to identify a sufficient number of infected children. Further the rapid loss of infection in the placebo group made the number required much larger. I therefore abandoned this study and planned the second one.

The principal investigator traveled to the UK where the treatment code was disclosed and a preliminary analysis of data collected was done. It was shown that the cognitive function testing was good and the scores of different tests correlated with age and with each other reliably . Fig 6 summarises the design and the sample sizes. Out of the 161 parasitaemic children who were enrolled at the base-line, 73 were assigned randomly to the treatment group and 88 assigned to the placebo group. Out of the 161 parasitaemic children enrolled at the base-line, 139 (86%) were enrolled at the follow-up study of whom 64 received the treatment and 75 received placebo. After two weeks, 48 of the placebo group (64%) were no longer found to be infected *versus* 27 (36%) who

fig 6. Performed study design of the clinical trial



Key:

Parasitaemic

non-parasitaemic

Rx treatment

Pl placebo

were still infected. Among those who received the Fansidar 57 (89%) were no longer infected *versus* 7 (11%) who were still infected.

In the view of this rapid clearance of the parasitaemia irrespective of treatment we felt that the controlled treatment trial design was no longer tenable and we decided to conduct a natural trial that I shall discuss next.

6.2. The natural trial:

This study compared the cognitive function of schoolchildren with asymptomatic parasitaemia with a uninfected group matched for grade and school. Two weeks later, the infected group was screened for parasitaemia and those who remained parasitaemic were compared with children matched for grade and school who were no longer parasitaemic.

6.2.1. Study area (See fig. 4:)

Due to unforeseen rapid clearance of asymptomatic parasitaemia among the placebo group in the clinical trial we felt that it might be important to change the study area to an area with higher prevalence. Therefore this study was conducted in rural communities in four districts belonging to two adjacent governorates: Taiz and Laheg, 300- 400 km to the south of the capital Sana'a. The study area was known to be endemic area for malaria. After the last rainy season there was a marked increase in the number of reported cases and the community contacted the Health Offices asking for help. A malaria survey conducted by the National Malaria Control Program (NMCP) team showed that prevelance of asymptomatic malaria among the schoolchildren was 50%. This made the area an ideal setting for the study.

These communities are predominantly agricultural but some are engaged in paid labour. The communities are linked by inadequate dirt roads and the main ways of transportation are by foot or riding animals, and there are few four-wheel drive cars. The principal food crops are maize, sorghum, bananas and potatoes. The main

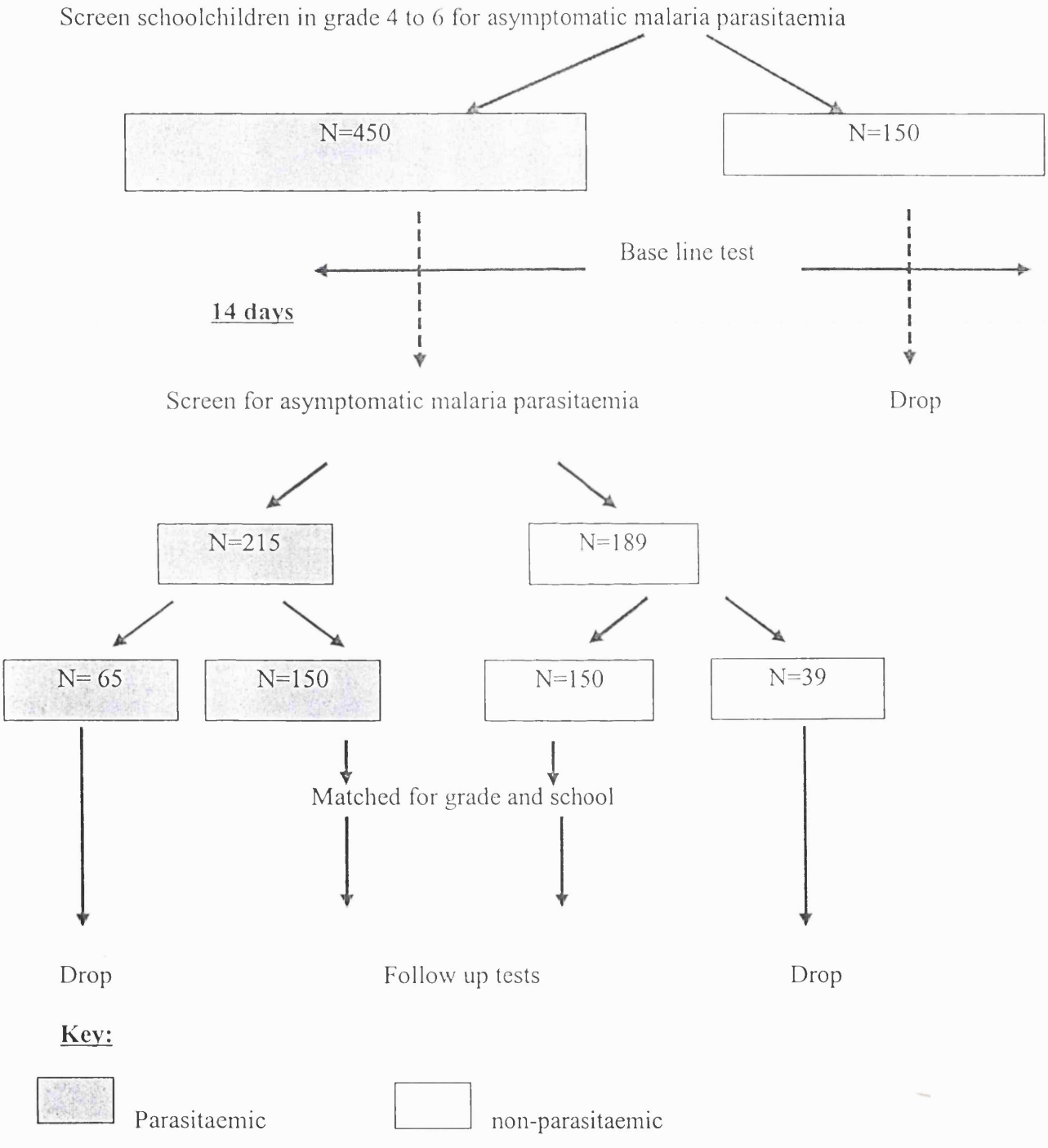
cash crop is qat (*Catha edulis*). There are two rainy seasons: a long one that lasts from March to May and a short one from September to October.

A typical house is built of rock or mud and has one to two rooms with dirt walls and a floor with a separate area for animals (i.e. cows, goats and cattle). Drinking water comes from wells and many families have to use open fields for defecation. There is no electricity except for few private generators and only few families own TV. Education (mainly primary) is available through scattered public schools that are in a poor condition and overcrowded. The children have to come to these schools from the surrounding villages by foot. This may take up to one to one and half hours walking a day.

6.2.2. Study Design (See fig. 7):

This study comprised a natural trial of the effect of asymptomatic parasitaemia on cognitive function and school achievement tests scores of schoolchildren. A group of asymptomatic parasitaemic and non-parasitaemic children were first compared on their performance on cognitive and school achievement tests. Two weeks later the parasitaemic children were re-screened and those who remained parasitaemic were matched for grade and school with children who were previously parasitaemic but no longer parasitaemic. These children were then re-tested.

fig 7. Study design of the natural trial



6.2.3. Time-line of the natural trial:

1997:

September	- Getting reliability for the cognitive testers - Screening schoolchildren for parasitaemia - Choice of schools
1st - 15th October	- Screening of schoolchildren and base line testing for Group I-1 (142 infected) and Group I-2 (48 non-infected)
15th -31st October	- Re-screening of GI-1 and follow up testing for 50 children who remain infected and 50 who were no longer infected
1st -15th November	- Screening of schoolchildren and base line testing for Group II-1 (150 infected) and Group II-2 (52 non-infected)
15th-30th November	- Re-screening of GII-1 and follow up testing for 50 children who remain infected and 50 who were no longer infected
1st -15th December	- Screening of schoolchildren and base line testing for Group III-1 (150 infected) and Group III-2 (45 non-infected)
15th -31st December	- Re-screening of GIII-1 and follow up testing for 50 children who remain infected and 50 who were no longer infected

1998-1999:

January - May	- Data entry, checking, screening etc..
May - June	- Preliminary data analysis
July- September	- Principal investigator back to the Institute in London
	- Data analysis
October- April 1999	- Writing

6.2.4. Sample:

6.2.4.1. Rationale for sample size:

The sample size was calculated using different formulae (Kirkwood, 1994). Data collected in the clinical trial were used to calculate the sample size required. The outcome variables are the cognitive function tests. For the case control study, the sample size required to show a difference of one third SD of the mean between parasitaemic and non-parasitaemic groups - taking 5% level of significance and 80% power- was approximately 140 to 150. For the natural trial the sample size calculated to detect a difference of 10-15% in cognitive function tests scores, a sample size of 120 was required to achieve this at 90% power and 5% level of significance. The clinical trial showed that about one third from the infected group would still be infected after 2 weeks. Therefore, 450 infected were recruited at the baseline to end up with 150 infected at the follow-up. We increased the sample size to allow for changes in parasitaemia and for drop-outs.

6.2.4.2 Selection of schools:

A list of primary schools within possible access by the research team was obtained from the Education Officers in the four districts. Twenty-seven public primary schools were screened and the prevalence of parasitaemia in these schools was 20-60 % and the parasite density was moderate. The main selection criteria for these schools were the availability of sufficient parasitaemic children and willingness of the headmasters and teachers to participate. Out of these schools, 16 were chosen to participate in this study.

6.2.4.3. Selection of children:

One thousand and twenty-nine children in the eligible schools were screened for malaria. Many headmasters warned us that enrolling girls in a study in a conservative rural community might create problems for the research team and for the school administrators. This, together with low female enrollment, led us to enroll only boys. All boys selected were aged between nine and 14 years in grades four to six from the 16 selected schools.

The following children were excluded: those with unknown age, those reported to have learning difficulties or serious chronic illness, those with a history of chills or fever in the previous three days, those with an axillary temperature more than 37.5 ^{o}C or those whose parents refused to give their consent.

Two groups were then chosen:

Parasitaemic group: in whom *Plasmodium falciparum* seen in a stained thick blood smear. Two exclusion criteria were added to the previous criteria The parasitaemic children with parasite density less than 750 parasites per μl were excluded as they were less likely to have an effect on cognition and those with parasite density more than 5,000 parasites per μl were also excluded because their health might be in jeopardy and they were treated. 445 children who had 750 to 5000 parasites per μl of blood in two consecutive malaria smears 24 hours apart were identified first.

Non-parasitaemic group: this comprised 142 non-parasitaemic children who had no *Plasmodium spp.* seen in 100 high power field (HPF) in a stained thick blood smear in two consecutive malaria smears 24 hours apart. This group was matched with the parasitaemic group for grade and school in ratio of one non-parasitaemic child for three parasitaemic children. When the number of parasitaemic children was not dividable by three and there was just one extra child, this child was not matched with a non parasitaemic one but if there were two extra children then the second parasitaemic child was matched with a non-parasitaemic one.

6.2.5. Procedure:

All parasitaemic and non-parasitaemic children were given a set of baseline measurements on enrollment. The measurements included the cognitive function and school achievement tests. They were interviewed and had a general and abdominal examination. Weight and height were recorded. Haemoglobin was measured by the HemoCue apparatus - HemoCue Inc., Mission, Vieja, California - (Hudson-Thomas, Bingham, Simmons, 1994) and a blood sample collected on a filter paper for measuring the C-reactive protein (CRP). For details of these measurements see 6.3. The uninfected group was not studied further.

Two weeks later, all parasitaemic children were screened for parasitaemia by finger prick and a thick blood film examined. 48% from the parasitaemic group were still parasitaemic after two weeks. The first 150 children who were parasitaemic at the baseline and still parasitaemic at follow-up were matched for grade and school with 150 of those who were parasitaemic at the baseline but no longer parasitaemic at follow-up. If two or more of the non-parasitaemic children were available, the nearest in age were chosen.

6.3. Measurements

For all measurements, test re-test and inter-observer reliability were assessed at the beginning and throughout the study. An $r \geq 0.70$ was set as the minimum level of test re-test reliability to be considered acceptable for inclusion in the study.

6.3.1. Clinical examination:

6.3.1.1. Temperature:

Axillary temperature was measured by an electronic thermometer. Although it may be less sensitive than the core body temperature, it represents a less invasive and less hazardous procedure (Smith *et al.* 1995).

6.3.1.2. Spleen size:

The spleen size was assessed in the standing position using Hackett's grading system on scale 0-5 (Hackett, 1944):

0 = non -palpable spleen (normal).

1= spleen palpable only when the subject draws a deep breath.

2= spleen ranging from those palpable at the costal margin without assistance from the subject to those whose lower border reaches a point halfway to a horizontal line through the umbilicus.

3= spleen projecting more than halfway to the umbilical level but not below it.

4= spleen below the umbilical level but not more than halfway to the line of the symphysis pubis.

5= Spleen is larger than those mentioned above.

6.3.2. Age determination:

The age of the child was taken from the school registry. The government has started a campaign to encourage the use of the birth certificates. It is becoming compulsory for the father of each new student at the time of registration to present his child's birth certificate. However, this system is not working well, especially in rural areas. For those born more than 10 years ago, we found that many of them did not have birth certificates. At the time of registration the father usually gave the date of the birth using local events calendar and the schoolteachers, usually from the same area and who know about the calendar, translated it to the proper age in years. In the Aslam district, where the clinical trial was conducted the school registry was not well developed and we could not be certain about children's ages but in the natural trial areas the school registry was better and we obtained the age for all children.

6.3.3. The questionnaire:

The children were given a questionnaire by the principal investigator concerning socioeconomic conditions at home and whether they ate breakfast or experienced hunger. At the beginning of the study, the validity of children's responses was checked by comparing the children's response to these given by 20 mothers at home. The agreement between children's answers and their mothers ranged from 70%-90%.

6.3.3.1. Socioeconomic status (SES):

The children were asked about the house quality (type of the house and floor), house facilities (toilet, electricity, and water supply), possessions (TV and radio), and the animals they had (cows, goats, and sheep).

6.3.3.2. Breakfast and hunger history:

This was taken only in the natural trial. Each child was asked if he ever felt hungry before mid-morning break, how many days he came to the school without breakfast during the last week, the type of the breakfast he had had on that day (nothing, drink, drink with milk, full breakfasts), and finally how many days he went to bed hungry during the previous week.

6.3.3.3. Observations:

The quality of children's shoes (no shoes, flip-flops, or proper shoes), clothes (poor: both the shirt and trouser torn, faint colour or no button; fair: mixture or half was good and half was bad; good: both trouser and shirt in good condition) and school bag (none or plastic, material bag, proper school bag) were assessed by the principal investigator whilst interviewing the child.

6.3.4. The anthropometric measurements:

Height and weight were measured by standard procedures (Lohman, Roche, Martorell, 1989). Before data collection, the principal investigator was trained by an anthropometrist. The inter-measurer reliabilities between the two were high (r=0.99, n=30).

6.3.4.1. Height:

Standing height was measured and recorded to the nearest 0.1 cm by using a portable stadiometer. The child was requested to remove his shoes and anything that could interfere with measurement such as a hat. The base of the

stadiometer was placed on a hard flat surface. The child was asked to put his feet flat and together in the centre against the back of the base. To be certain that the child's legs were kept straight and against the board, the health worker placed his left hand just above the child ankles and the right hand on the child's knees. The principal investigator placed his left hand on the child's chin and made sure that the child looking straight forward, the shoulders were leveled, the hands were at the sides and the head, the shoulder blade, and the buttocks were against the board. Then the principal investigator lowered the head piece with his right hand and pushed it gently through the child's hair and read the measurement to the nearest 0.1 cm.

6.3.4.2. Weight:

The child's weight was measured to the nearest 0.1 kg using a portable digital measuring scale (Soehnle 7504, 0-150 kg, 0.1 kg). The child was asked to remove his shoes, socks, school bag, and any thing that could interfere with measurement e.g. heavy object in his pockets. The scale was placed on a flat hard service. The child weight's was recorded twice and the mean was taken. The scale was checked daily with known weights before starting the field work.

6.3.5. Cognitive function tests:

A wide range of functions was measured using tests with which we were familiar. Speed of information processing and some other functions such as memory and attention, previously found to be sensitive in other parasitic infections, were included.

6.3.5.1. Choice of tests:

Several considerations influenced our choice of tests. There were no previous studies on asymptomatic malaria and cognition, so there was no empirical evidence to guide us. In addition, we had to use simple tests that required little equipment as the testing conditions were difficult and we had to test in different schools.

We hypothesized that children with parasitaemia would be less efficient in several cognitive functions, but speed of processing, attention and working memory were more likely to be affected than more complicated functions such as problem solving.

Probably the most similar conditions that had been studied were the viral infection of influenza and cold that were studied by Smith and colleagues (e.g. Smith *et al.* 1988; Smith, 1992). They found that influenza affected performance on the Stroop and visual search test, while the common cold affected performance on a peg board task, effects which were present even before symptoms appeared. Other tests affected were reaction time tests but these required a computer. Therefore, we selected the Stroop test which has been modified for children in a study conducted in Bangladesh (Huda, 1998), a visual search task and a peg board task. We also used a verbal fluency task because it has been shown to be sensitive to several subtle biological insults in studies in Jamaica (Simeon, Grantham-McGregor, 1989; Simeon *et al.* 1995a). There were also, some suggestions that speed of processing may be affected more than other cognitive functions by biological insults in general (Connolly, Kvalsvig, 1993). Therefore, we added speed of processing task (symbol/symbol verbal test). Two memory tests were added, an auditory working memory task (digit span) and a visual memory (pictorial memory) task.

6.3.5.2. Piloting:

Extensive piloting was carried out to ensure that the tests were culturally appropriate. To assess the difficulty of the tests in the Yemen in these age groups, the cognitive tests were piloted in ten very clever children and ten slow children as ranked by their teachers and from different grades. This was important to ensure that all children, even the slow ones, could score on these tests and also to ensure that even the superior ones had not reached a ceiling and had some space to show improvement at re-testing.

The sequence of the tests was arranged in the best possible way to keep the child interested and cooperative till the end of the testing session which usually lasted about 35-45 minutes.

6.3.5.3. Training:

Extensive training was carried out to ensure that testers had a good rapport with the children and that the reliability of testing was good. We know that testing children especially in developing countries has many problems because children tend to be very shy with strange adults and necessary steps were considered during training to put each child at his ease as far as possible before beginning the tests. Frequent positive feedbacks were given throughout the test.

In November 1996, 12 qualified psychologists recently graduated from Sana'a University were interviewed by Head of Psychology Department, Sana'a University and the principal investigator and the best three, who had previous experience with

testing of schoolchildren, were recruited. In January 1997, Susan Chang, a psychologist from The Tropical Metabolism Research Unit in Jamaica, arrived in the Yemen for the purpose of training the principal investigator and the three psychologists. Intensive training was conducted and the necessary modifications of the battery of cognitive tests were made to be more acceptable in the Yemeni context. After indoor training we chose two schools for the training and piloting. Each tester conducted around 20 tests while he was observed by the trainer, the principal investigator and the other two testers. After each testing session the trainer commented on the testing and gave advice to improve the testing. Only when the trainer and the testers were confident about the testing, did the testers and the principal investigator move to the study area. Extensive and repeated testing was done in order to reach the accepted reliability of test-re-test. Each tester tested 20 children on two occasions seven days apart. This was repeated until a test- re-test reliability of ≥ 0.70 was reached for each test for every tester (table 7& 8). Throughout the study, the testers were observed by the principal investigator in about 10% of all tests. The observation indicated a good level of standardization throughout the study ($r \geq 0.90$).

Professor Sally McGregor, the supervisor, visited the team in the Yemen in February 1997. She was briefed by the principal investigator about all the activities going on. She observed each tester during several testing sessions and made the necessary and final corrections.

6.3.5.4. Testing procedure:

The battery of cognitive tests was administered by one of three testers who were unaware of the children's group assignment. For each child, the same tester administered the tests at baseline and at follow-up in order to remove any effect of having different testers.

All tests were given at the school when possible, but if no room was available, a nearby house or mosque was used. All visual distractions were removed from the test room. Since the time of the day may be critical in determining a child's cognitive performance, re-testing was standardised to be within one hour of the time the baseline test was given. A small snack was given to each child prior to testing to remove the effect of short-term hunger that has been shown to affect test performance (Simeon, Grantham-McGregor, 1989).

Testers were especially trained to create good rapport with the children. The child was welcomed by the tester and he introduced himself and asked the child his name, grade etc. The tester then told the child that they were going to play some interesting games and he would get a gift at the end of the session. The session started with both tester and the child drawing anything the child liked. Before scoring began, the child was given practice items until it was clear that he understood the task. The child, irrespective of how he conducted in the tests, was praised and encouraged. Marking was done under the table out of sight of the child so the child was not put-off if he did not perform well. At the end of the session the child was given a small gift such as a ruler, eraser or other school material.

Table 7. Test re-test reliability (Pearson's correlation) for cognitive function tests before the clinical trial (n=20).

Test	Tester one r	Tester two r	Tester three r
Digit span	0.85	0.79	0.74
Peg board dominant	0.90	0.82	0.82
Peg board non-dominant	0.81	0.76	0.79
Picture learning	0.84	0.75	0.70
Symbol - symbol	0.89	0.74	0.83
Stroop direct	0.81	0.73	0.75
Stroop indirect	0.78	0.70	0.73
Verbal fluency	0.91	0.83	0.75
Visual search	0.84	0.89	0.79

Table 8. Test re-test reliability (Pearson's correlation) for cognitive function tests before the natural trial (n=20).

Test	Tester one r	Tester two r	Tester three r
Digit span	0. 88	0.79	0.81
Peg board dominant	0. 87	0.80	0.80
Peg board non-dominant	0.84	0.79	0.75
Picture learning	0.80	0.85	0.75
Symbol - symbol	0.90	0.79	0.83
Stroop direct	0.88	0.87	0.75
Stroop indirect	0.75	0.77	0.70
Verbal fluency	0.85	0.80	0.75
Visual search	0.85	0.84	0.80

6.3.5.5. Details of cognitive tests:

Verbal fluency:

This test measures the children's ability to generate items from two semantic categories at speed. Children are asked to name as many animals and then food items as possible, and are given one minute for each category. The repeated or the non relevant items are excluded. The score is the total number of correct items. this test is considered to measure speed of semantic processing and to be an indicator of the central executive component of working memory (Baddeley, *et al.* 1995). The test has been used in Jamaica, where it was found to be sensitive to missing breakfast (Simeon, Grantham-McGregor, 1989) and parasitic infection (Nokes *et al.* 1992).

Digit span:

This test measures the phonological loop of working memory (Baddeley *et al.* 1995). Children are asked to repeat a series of string of spoken numbers which are increased by one digit, every third string. The digits are spoken at the rate of one per second in a monotone voice. The tests are stopped after two consecutive failures. The score is the number of correctly repeated strings. The test was also sensitive to missing breakfast and parasitic infection in previous Jamaican studies (Simeon, Grantham-McGregor, 1989; Nokes *et al.* 1992).

Visual search (see appendix):

This test is usually considered to measure speed of visual information processing and sustained attention (Kvalsvig, Cooppan, Connolly, 1991). The test was modified by Baddeley and colleagues (1995) to consist of rows of pictures instead of letters. To the right of each line there is a target picture and on the left, separeated by space, a row of 16 pictures, one or more of which is the same as the target picture. The child has to scan each line and to mark all the target pictures in each line as fast as possible. The time taken to complete two pages of 12 lines is recorded. The score is the mean time for each correctly identified target. The test has been shown to be affected by influenza (Smith *et al.* 1988; Smith *et al.* 1989b).

Modified Stroop test (see appendix):

This test measures the ability to inhibit concurrent perceptual cues. It was modified to consist of pages of rows of circles of black and white colours. The child has to say the colour of each circle as fast as he can (the direct Stroop). Then he has to reverse, saying black for white and white for black (the indirect Stroop). The child has to do two pages in a correct way and two in an opposite way. The total time required to complete two pages of the direct and two of the indirect and the number of mistakes are recorded. The score is the mean time per correctly named circle. The test was found to be sensitive for influenza (Smith *et al.* 1988; Smith, 1992).

Symbol-symbol verbal test (see appendix):

This test measures simple paired associate learning and speed of information processing. The children are presented with a key consisting of two shapes that are each paired with picture. They are given two pages of the shapes and asked to name the pictures paired with them as fast as possible. The time require to complete the two pages is recorded as well as the mistakes. The score is the time per correctly identified shape.

Peg-board (see appendix):

This is a measure of fine motor coordination. The Lafayette grooved peg board is used to measure fine motor function (Grooved pegboard test, 1989). The peg, which resembles a key, has hooks on one side and has to be rotated to fit into a hole. Only one hand is used each time. The dominant hand is tested first then the non-dominant. Only one hand is used and before the test starts the child is asked to keep the other hand on his lap. Frequently, it is necessary to point the first hole of the new row, especially in the non-dominant hand trials. Two trials are given for each hand and the tester encourages the child to perform the task as fast as possible. There are some extra pegs kept in the tray and if any peg is dropped to the floor the child is asked not to pick it up and to take another one instead. The score is the total time required to do the two trials of the dominant then the non-dominant hand. The test was found to be affected by cold (e.g. Smith *et al.* 1988; Smith *et al.* 1989b).

Pictorial memory:

This test measures visual recognition memory. The test is a modified version of the pictorial learning used by Tiwari and colleagues (1996). Two different sets of photographs one for the baseline and one for the follow-up were used. Each set consists of 39 black and white photographs (55 mm × 45 mm) of boys all approximately the same age with more or less similar dress and neutral facial expressions with moderate identifiability. These photographs are divided into three equal groups, 13 in each. The first group is called the "targets" and the other two groups are called "non-targets 1" for the first trial and "non-targets 2" for the second trial. The choice of the group and the order they were presented to the child is randomly selected. Two trials are given for each child. In each trial, the "targets" are presented to the child first, one after another, each for duration of 3 seconds. The child is asked each time to concentrate upon the photograph and to say if he looks handsome or not. Then these "targets" which already mixed and shuffled with 13 photographs of the "non-targets" group are presented to the child and he is asked to identify those "targets" that are shown to him previously from the "non- targets" by just saying "yes" for the one he saw before and "no" for the one he did not. The number of correctly recognized from both the "target": yes he saw, and the "non-target": no he did not see in each trial is recorded.

6.3.6. The school achievement Tests:

In the natural trial only, the Wide Range School Achievement Test (WRAT) (Jastak, Bijou, 1946) was used to measure children's school achievement in mathematics and the NFER-Nelson Group Reading Test to measure school achievement in reading. These tests have been used in other developing countries such as Jamaica and have found to be reliable (Powell, Grantham-McGregor, 1985; Clark, Grantham-McGregor, Powell, 1991; Simeon *et al.* 1994). Modified translated versions of the tests were used after extensive piloting and after discussion with schoolteachers and headmasters. To assess for the difficulty of the tests in the Yemen in these age groups the tests were piloted in ten clever children and ten slow children as ranked by their teachers and from different grades. This was important to ensure that all children - even the slow ones- could score in these tests. During piloting the children had a difficulty in understanding some items of the WRAT reading subset therefore we used instead NFER-Nelson Group Reading Test that seemed to be more understandable by this group of children. Each test took about 20- 30 minutes.

The tests were administered to small groups of four to six children under continuous supervision from one of the research team. To avoid the children becoming tired the school achievement tests were not given on the same day as cognitive tests.

6.3.6.1. Mathematics test:

This section consisted of problems in addition, subtraction, multiplication and division, arranged in order of difficulty. Before starting the testing session, the tester explained to the children that the test had different types of problems for different grades and there would be some above their grade level.

6.3.6.2. Reading test:

The reading test had two parts. The first part consisted of five pictures and five different names. The child had to choose the appropriate name for the picture and to encircle it. The second part of the test consisted of incomplete sentences and the child had to choose from a list of words the most appropriate one to complete the sentence.

6.3.7. Absenteeism:

The number of days the child was absent from the school during the previous three months in the clinical trial and the previous two months in the natural trial was determined from the school records. In some schools the records were not reliable so, we recorded it as missing.

6.3.8. Blood Measurements:

6.3.8.1. Screening for malaria parasitaemia and measurement of parasite density:

The method of screening for malaria parasitaemia and measurement of parasite density has been described before (6.1.4).

6.3.8.2. Haemoglobin:

Haemoglobin was measured in the field by using a B-haemoglobin photometry, HemoCue apparatus, HemoCue Inc., Mission, Vieja, California (Hudson-Thomas, Bingham, Simmons, 1994).

The finger of the child was pricked using a disposable stylet under aseptic conditions by a trained laboratory technician. The first drop of the blood was wiped off with sterile cotton and formation of subsequent drops of blood was encouraged by gentle pressure on the child's finger. The microcuvette was allowed to fill completely without air bubbles. The outside of the cuvette was wiped with tissue paper and then the cuvette was placed in the photometer. The haemoglobin concentration was read directly from the HemoCue in g/L . The calibration of the HemoCue was checked daily by using the control cuvette supplied by the manufacturer of the apparatus.

6.3.8.3. C-reactive protein (CRP):

A Guthrie card (Her Majesty's Stationery Office) was used to collect three blood spots for C-reactive protein analysis. Each card was labelled with the child's name and study number.

After a finger of the child was pricked (see 6.3.8.2) the filter paper was made to touch the three blood drops and the technician ensured that the drop was enough by observing that the blood appeared from the opposite side. The spots were allowed to dry in the air for two hours then were kept in an envelope and stored at room temperature.

Whole blood CRP was determined by enzyme-linked immunosorbant assay (ELISA) based on the method of Voller and colleagues (1980). Bloodspots were punched out using a standard 5 mm hole punch and placed into a

microtitre plate. These spots were eluted using 100 µl of elution buffer (0.05% Tween-80 in phosphate buffered saline, PBS, with sodium azide preservative) overnight at 4°C. A second microtitre plate was coated with 50 µl of rabbit anti-human CRP polyclonal antibody (Dako) diluted 1 in 1000 in buffer (0.1 mollar (M) sodium carbonate and 0.1 M sodium hydrogencarbonate in distilled water) overnight at 4°C. The following morning, the coated plate was washed with 0.05% Tween-20 in PBS (PBST) and blotted dry, and then non-specific binding was minimised using 100 µl per well of a blocking buffer (1% bovine albumin, in PBS).

After a one hour incubation at 37°C, the plate was washed and dried before the eluted samples were added, diluted 1:1 in 0.2% bovine albumin in PBST (diluting buffer), to the plate in duplicate and CRP standards (Behring) in the range 1.23 to 300 µg/L were added. Two quality control samples were also added and the plates incubated for one hour at 37°C. After washing with PBST, horse-radish peroxidase (HRP) conjugated anti-CRP antibody (Dako) was added, diluted 1:2000 in diluting buffer, at 50 µl/well. After a further 1 hour, 37°C incubation, the plates were washed again before 3,3',5,5'-tetra-methyl -benzidine dihydrochloride (TMB) (Sigma) dissolved in 0.11 M sodium acetate, pH 5.5, was added at 50 µl/well. After 10 minutes, the reaction was stopped by adding 25 µl/well 2 M sulphuric acid and the optical density at 450 nm was measured.

A factor to convert CRP values from blood spot to serum was developed based on comparisons of the 2 quality control sera (CRP= 0.14 mg/L and CRP= 22.96 mg/L). These were analysed both as serum and spotted on to, and subsequently eluted from, Guthrie cards. The comparison of these two media gave a liquid/spot value of 157 (SD= 15, n=17). Assuming that blood spots are actually only 60% serum, the above value was multiplied by 0.6 to give a blood spot to serum conversion factor of 94.

6.4. Ethical approval:

The study protocol was approved by the Review Board of the Institute of Child Health, London. In Yemen, permissions were obtained from the Committees of the Faculty of Medicine and Post Graduate Studies at Sana'a University and from the Research Department in the Ministry of Public Health. A permission letter was also obtained from the Ministry of Education. In the schools the headmasters, teachers and community were briefed about the purpose of the study and asked to give their permission and support. Only ten children were excluded because they or their parents refused to give blood samples. During screening those with a parasite density above 5,000 parasites per μl were given chloroquine tablets according to their weight. At the end of the study those who had haemoglobin between 90 and 110 g/L were given ferrous gluconate and asked to have their haemoglobin checked one month later at the nearby health centers. Also, those who had haemoglobin concentrations below 90 g/L or had serious medical problems were referred to the nearest health center for proper management.

6.5. Data management:

6.5.1. Data collection:

Data collected in the field were checked daily. Each tester carefully checked and scored the tests and recorded the score on a pre-coded form and in a record book. After he finished scoring he passed the sheet to another tester for rechecking. When there was any doubt about scoring procedures, testers checked with the principal investigator. After that the sheets and forms were given to the principal investigator, who also re-checked them again before keeping them in a safe place.

6.5.2. Data Entry:

At the end of each study, data entry was done in Yemen. Data were entered into an IBM PC computer using Epi Info program (CDC, Atlanta, 1994). Print-outs were made and were checked with the original forms.

6.5.3. Statistical analysis:

On arriving in England, data were converted into the Statistical Package for Social Science program (SPSS Inc., Chicago, IL, 1994). Further cleaning and cross checking for consistency was done. The children's heights and weights were expressed as Z-scores of the National Centre for Health Statistics (1997) references and the body mass index (BMI) was calculated (weight in kg/ height in meter squared). The SPSS program was used for statistical analysis. The differences were considered statistically significant at $P < 0.05$.

6.5.3.1. Clinical trial:

Data were first normalized. Peg board dominant, Stroop direct, days absent from school, parasite density, and CRP were mildly positively skewed and needed log-transformation, while symbol-symbol, Stroop indirect, and visual search were more positively skewed and needed reciprocal transformation (Armitage, Berry, 1994)

Arithmetic means, geometric means, and harmonic were presented when appropriate. I also presented geometric SD of geometric means however, its use is controversial between statisticians (Kirkwood, 1979). Correlations between the initial and final cognitive scores, cognitive scores with each other, cognitive scores with other variables (e.g. SES, anthropometry, etc..) , and correlations between other variables (e.g. haemoglobin and parasite density, and SES and anthropometry) were examined. Arithmetic means, or geometric means, and harmonic of transformed data, of continuous variables were compared by using Student's t-test and proportions were compared using the Chi square test. Analysis of covariance (ANCOVA) was used to determine differences between parasitaemic and non- parasitaemic groups in cognitive scores. The effect of potentially confounding variables was examined in multiple regression analyses. For analysis of data on enrollment, we used the model described in table 9. We predicted each cognitive score in turn, controlling for age which was entered, then offering stepwise socioeconomic status, father's literacy, anthropometric indices (WAZ, HAZ, BMI), and tester then finally, parasitaemia group was forced to enter in the equation (0= No / 1= Yes). As anaemia could be the mechanism through which parasitaemia may affect cognition we repeated all the regression analyses offering anaemia group instead of parasitaemia.

Table 9. Multiple regression model used to examine the difference between parasitaemic and non-parasitaemic groups in cognitive function and school achievement tests controlling for age, SES, father's literacy, anthropometry and tester on enrollment to the clinical and natural trials

Variables	Method
Dependent variable:	
- Cognitive tests scores	
- The school achievement Test scores	
- Days absent from the school	
Independent variables:	
Block 1:	Enter
- Age	
Block 2:	Offered stepwise
- SES:	
- wealth factor	
- animal factor	
- school items	
- house factor	
- father literacy	
- Anthropometry	
- HAZ	
- WAZ	
- BMI	
- Tester	
Block 3:	Enter
- Parasitaemia (0 = no/ 1 = yes)	

To assess the effect of the clinical trial, we used a multiple regression analysis (table 10) where we predicted the final cognitive scores controlling for initial scores, and offering age and tester, and finally forcing the treatment group (Placebo/ treatment) to enter the equation.

6.5.3.2. Natural trial:

We first normalized the data. Peg board dominant, peg board non-dominant, symbol-symbol, Stroop direct and indirect, visual search, days absent from school, BMI and CRP were mildly positively skewed and needed log-transformation. Parasite density was normalized through square root transformation (Armitage, Berry, 1994).

To reduce the data and to identify the underlying constructs within the set of socioeconomic variables and cognitive tests, a principal component factor analysis with varimax rotation was conducted (Kendall, Stuart, Ord, 1979).

The approach to the analysis was similar to that used in the clinical trial. The school achievement data, which were not previously assessed, were analysed in the same way as cognitive scores.

Table 10. Multiple regression model used to examine the effect of treatment in the clinical trial or natural cure in the natural trial in cognitive function tests controlling for the initial cognitive test score, age and tester.

Variables	Method
Dependent variable:	
- Final cognitive tests score	
Independent variables:	
Block 1:	Enter
- Initial cognitive test score	
Block 2:	Offered stepwise
- Age	
- Tester	
Block 3:	Enter
- Treatment or natural cure group	

6.5.4. Summary of chapter two: Methods:

In this chapter:

- the study area, design, sampling method, and procedures for the clinical trial and the reasons why this trial has been aborted has been discussed.
- a detailed description of the design of the second study (the natural trial) and how the sample has been chosen was given.
- details of the measurements used, rationale behind the choice of such measurements, piloting, training, and how such tests were standardized has been discussed.
- finally data management techniques and how the analysis was done has been also mentioned.

Chapter Three

Results

7. Results

I will briefly describe the results of the clinical trial but not give details because the study was abandoned. Then I will give detailed results of the natural trial.

7.1. Results from the clinical trial:

It was planned to enroll the sample in two waves, in the first wave a total of 221 children (161 parasitaemic and 60 non-parasitaemic) were enrolled at the baseline. Out of those who were parasitaemic, 73 were assigned randomly to treatment and 88 assigned to the placebo.

7.1.1. Results from the enrollment data:

Table 11 gives the general characteristics of the study population by parasitaemia group on enrollment.

The mean age of the combined samples was 11.6±1.7 years. There were no differences between the groups in anthropometry. In general, the children were stunted and 42% had height-for-age Z-score (HAZ) below -2 s.d. and 13% had HAZ below -3 s.d. Also, they had a low weight-for-age (WAZ); 55% were below -2 s.d. and 9% had a WAZ below -3 s.d. The mean haemoglobin concentration was 109 ± 13 g/L and the range was 50 to 140 g/L. A large of number of children was anaemic and 55% had a haemoglobin below 110 g/L and 4% were below 90 g/L. The parasitaemic group had a significantly lower haemoglobin concentrations than the non- parasitaemic: 107 ± 13 compared with 113 ± 12 g/L ($P< 0.01$). There were no other significant differences

between the two groups. 39% had splenomegaly. Among the parasitaemic group, the median parasite density was 5,00/μl and the 25 th and 75 th percentiles were 500 and 800/μl respectively.

The children's homes had few facilities, five per cent of children had piped water, 17% had a toilet and 1% had electricity. 62% of children reported having a radio and 15% had TV.

The initial cognitive test scores by parasitaemia group on enrollment to the clinical trial are shown in table 12. For the timed-scores tests (i.e. peg board tests, symbol – symbol, Stroop tests, and visual search), the lower the score the better the performance. For other tests (i.e. digit span, picture memory, and verbal fluency), the higher the score the better the performance. The parasitaemic children were significantly slower on the peg board non-dominant test than non-parasitaemic group ($P < 0.05$). There were no other significant differences.

7.1.2. Results from the follow-up:

At the follow-up, out of the 161 parasitaemic children who were assigned to treatment or placebo, 139 (64 received treatment and 75 received placebo) had completed the trial. Table 13 shows the initial and final cognitive tests scores for those who completed the trial by the treatment group. No significant differences were detected between the two groups at either test sessions.

Although the numbers were extremely small we assessed the effect of the treatment versus the placebo. We conducted multiple regression analysis where we predicted the final cognitive scores controlling for initial scores and offering stepwise age and tester, and finally forcing the treatment group (Placebo =0/ treatment =1) to enter the equation. Offering SES and anthropometry made no difference for the results. There was no significant difference between the treatment and the placebo groups in their performance in cognitive tests.

Table 11. Age, nutritional status, socioeconomic status, days absent from school and splenomegaly by parasitaemia group on enrollment to the clinical trial

Variable	n	Non-parasitaemic Mean ±SD	n	Parasitaemic Mean ±SD
Age (years)	51	11.9 ± 1.8	144	11.5 ±1.7
Weight-for-age (Z score)	51	- 1.9 ± 0.9	140	- 2.0 ± 0.9
Height-for-age (Z score)	50	- 1.6 ± 1.3	140	- 1.8 ± 1.1
Body Mass Index	56	14.5 ± 1.6	155	14.4 ± 1.4
Hemoglobin (g/L)	55	113 ± 12	151	107± 13*
Days absent from school [a]	41	4.9 ± 2.2	130	4.8 ± 2.2
Father illiterate [b]	56	45 (80%)	147	115 (78%)
Father Job: Farmer [b]	51	19 (37%)	142	42 (30%)
Splenomegaly [b]	60	23 (38%)	153	63 (41%)

[a] Geometric mean± geometric SD

[b] Number (%)

* P <0.01 (t-test).

Table 12. Initial cognitive tests scores by parasitaemia group on enrollment to the clinical trial

Test	Non-parasitaemic n= 60 Mean ± SD	Parasitaemic n= 161 Mean ± SD
Digit span	5.8 ± 1.6	5.8 ± 1.4
Peg board dominant (seconds) [a]	146.4 ± 1.2	151.9 ± 1.2
Peg board non-dominant (seconds)	164.9 ± 27.7	179.2 ± 39.2*
Picture memory	40.9 ± 5.2	40.6 ± 5.3
Symbol - Symbol (seconds) [b]	1.4 ± 5.8	1.5 ± 5.4
Stroop direct (seconds) [a]	1.4 ± 1.3	1.5 ± 1.3
Stroop indirect (seconds) [b]	2.2 ± 6.5	2.3 ± 6.7
Verbal fluency	20.1 ± 6.7	19.0 ± 6.1
Visual search (seconds) [b]	2.5 ± 8.4	2.7 ± 8.9

[a] Geometric mean ± geometric SD [b] Harmonic mean ± SD

* $P < 0.05$ (t-test).

Table 13. Initial and final cognitive tests scores for the children who were initially parasitaemic and completed the clinical trial by the treatment group

Test		Treatment n=64 Mean ± SD	Placebo n=75 Mean ± SD
Digit span	initial	6.0 ± 1.5	5.9 ± 1.3
	final	5.9 ± 1.5	6.0 ± 1.5
Peg board dominant (seconds) [a]	initial	148.5 ± 1.2	150.6±1.2
	final	128.8 ± 1.1	128.8±1.2
Peg board non-dominant (seconds)	initial	175.8 ± 36.8	176.6 ±38.8
	final	150.2 ± 27.3	149.8 ±29.8
Picture memory	initial	41.9 ± 5.1	40.4 ± 5.2
	final	41.6 ± 4.2	40.2 ± 4.4
Symbol - Symbol (seconds) [b]	initial	1.4 ± 5.5	1.4 ± 5.5
	final	1.3 ± 5.0	1.3 ± 5.3
Stroop direct (seconds) [a]	initial	1.5 ± 1.3	1.4 ± 1.3
	final	1.2 ± 1.3	1.3 ± 1.3
Stroop indirect (seconds) [b]	initial	2.2 ± 6.1	2.2 ± 7.1
	final	1.7 ± 5.8	1.8 ± 6.8
Verbal fluency	initial	19.0 ± 6.3	19.6 ± 6.1
	final	19.8 ± 6.7	20.6 ± 5.7
Visual search (seconds) [b]	initial	2.8 ± 8.3	2.5 ± 9.6
	final	2.2 ± 8.6	2.1 ± 10.0

[a] Geometric mean ± geometric SD

[b] Harmonic mean ± SD

7.2. Results of the natural trial

I will first report the sample characteristics, then the analysis of enrollment data. I will then consider the effect of the natural cure and finally I will comment on the C-reactive protein concentrations at baseline and at follow-up.

7.2.1. Characteristics of the sample:

A total of 587 children (445 parasitaemic and 142 non-parasitaemic) were enrolled at the base-line. Table 14 gives the general characteristics of the study population by parasitaemia group on enrollment. The mean age of the whole sample was 11.5±1.2 years. There was no significant difference between the groups in anthropometry. As in the clinical trial but a large population of children was stunted and underweight: 51% had HAZ below -2 s.d and 9% had a HAZ below -3 s.d., 55% had a WAZ below -2 s.d. and 2% had a WAZ below -3 s.d. The mean haemoglobin concentration was 113 ± 14 g/L and the range 66 to 150 g/L. A large of number of children was anaemic: 41% had a haemoglobin below 110 g/L and 6% were below 90 g/L. The parasitaemic group had a significantly lower hemoglobin than the non-parasitaemic: 111 ± 14 g/L compared with 120 ± 14 g/L ($P<0.01$). 35% of children had splenomegaly which was significantly more common among parasitaemic children: 41% compared with 16% among the non-parasitaemic group ($P<0.01$). Among the parasitaemic group, the median parasite density was 1,000/µl and the 25th and 75 th percentiles were 750 and 1,700 /µl respectively. Only 10% had parasite density above 2,400/µl.

Eight per cent of children's households had piped water, 51% had toilets and 12% had electricity. 72% of children reported having radio and 34% had a TV.

Table 14. Age, nutritional status, socioeconomic status, days absent from school and splenomegaly on enrollment to the natural trial by parasitaemia group

Variable	n	Non-parasitaemic Mean ±SD	n	Parasitaemic Mean ±SD
Age (years)	142	11.4 ± 1.2	445	11.6 ± 1.3
Weight-for-age (Z score)	140	- 1.9 ± 0.6	437	- 2.0 ± 0.6
Height-for-age (Z score)	141	- 2.0 ± 0.8	442	- 2.0 ± 0.8
Body Mass Index	139	14.8 ± 1.1	437	14.7 ± 1.1
Hemoglobin (g/L)	142	120 ± 14	441	111 ± 14*
Days absent from school [a]	70	3.0 ± 2.3	245	3.4 ± 2.3
Father illiteracy [b]	135	42 (31%)	412	169 (41%)•
Father Job: Farmer [b]	142	27 (19%)	445	123 (28%)•
Splenomegaly [b]	142	23 (16%)	442	183 (41%)••

[a] Geometric mean± geometric SD

[b] Number (%)

* P <0.001 (t-test).

• P < 0.05 (X^2 test)

•• P < 0.001 (X^2 test)

Parasitaemic children were more likely than non-parasitaemic children to have fathers who were farmers and illiterate ($P < 0.05$ for both).

To reduce the data and to identify the underlying constructs within the set of socioeconomic variables, a principal component factor analysis with varimax rotation was conducted. Four factors were identified: wealth which included toilet, electricity, water supply, TV and radio; animals which included cows, goats and sheep; school items which included clothes, school bag and shoes; and housing which included type of the house and floor. All items loaded ≥ 0.40 (Table 15). The parasitaemic group had significantly lower scores in school items factor ($P < 0.05$). There were no differences between the groups in the other factors. Table 16 shows the details of SES by parasitaemic group on enrollment to the natural trial. The parasitaemic group had significantly fewer school bags ($P < 0.01$). There were no significant differences between the groups in the other items.

There were no differences between the groups in the prevalence of hunger reported. In the combined groups 62% of children said that they were hungry before break, 12% said they went to bed hungry during the previous week, and 5% had had no breakfast during the previous week. Of those who went to bed hungry during the last week among 6% this was for one day, in 4% this was for two days, and in 2% this was for three days. Of those who had no breakfast during the previous week among 3% this was for one day and in 1% this was for either two or three days.

Table 15. Results of the factor analysis of SES variables on enrollment to the natural trial

Factor	Variable	Loading	Eigen value
Factor one:			
	Wealth factor		2.5
	- Electricity	0.71	
	- Toilet	0.67	
	- TV	0.71	
	- Radio	0.46	
	- Piped water	0.66	
Factor two:			
	Animal factor		1.4
	- Cows	0.67	
	- Goat	0.62	
	- Sheep	0.61	
Factor three:			
	School items		1.3
	- Clothes	0.73	
	- Shoes	0.63	
	- Bag	0.54	
Factor four:			
	Housing factor		1.2
	- House type	0.78	
	- Floor	0.65	

Table 16. Details of SES by parasitaemic group on enrollment to the natural trial

Factor		Non-parasitaemic	Parasitaemic
Wealth factor:			
		Yes	Yes
- Electricity		16%	11%
- Toilet		50%	52%
- TV		38%	33%
- Radio		74%	71%
- Piped water		6%	9%
Animal factor			
		Geometric mean ± geometric SD	
- Cows		1.7 ±1.6	1.8±1.6
- Goat		3.0±3.0	3.2±3
- Sheep		2.2±2.3	2.5±2.4
School items			
- Clothes*	poor	23%	35%
	fair	76%	65%
	good	4%	4%
- Shoesno	1%		2%
	flip-flop	89%	93%
	proper shoes	10%	5%
- Bag*	no	23%	35%
	material	76%	65%
	school bag	1%	-
Housing factor			
- House type	thatched	1%	1%
	rocks	83%	87%
	blocks	16%	12%
- Floor	mud	40%	40%
	cement	60%	59%
	Garnet	-	1%

* Clothes: poor: both the shirt and trouser were torn, faint color or no button
fair: mixture or half was good and half was bad
good: both trouser and shirt were in good condition

* $P < 0.01$ (X^2 test)

7.2..2. Analysis of the enrollment data:

7.2.2.1. Cognitive tests and school achievement tests:

The initial cognitive and school achievement tests scores of parasitaemic and non-parasitaemic children are shown in table 17. There were no significant differences between the groups.

Table 18 shows the correlation among cognitive function tests and school achievement tests. Both were highly correlated to each other.

In order to identify underlying constructs, a principal component factor analysis was conducted with the initial cognitive scores. The result is shown in table 19. Two factors emerged with all tests loading ≥ 0.40. The two peg boards and visual search loaded on factor one formed the motor factor. Other cognitive tests loaded on factor two formed the general cognitive factor. These factors were used in analyses as dependent variable.

The motor factor correlated with school achievement: mathematics and reading ($r= - 0.14$, $P <0.01$, $r= - 0.15$, $P <0.01$). The general cognitive factor also correlated with reading and mathematics ($r= 0.36$, $P <0.001$ for both).

7.2.2.2. Correlations with cognitive and school achievement tests:

Table 20 shows the significant correlations between initial cognitive tests and school achievement tests on one hand and SES, and nutritional status on the other. SES significantly correlated with cognitive and school achievement tests, the wealth factor was associated with peg board non-dominant, picture memory and Stroop indirect, and the housing factor was correlated with motor and cognitive factor and reading. HAZ and WAZ were significantly associated with visual search and motor factor. The correlation with visual search was strong and highly significant while correlation with motor factor was weak but still significant.

7.2.2.3. Correlations with parasitaemia:

Correlations between parasitaemia and nutritional status, splenomegaly and SES are shown in table 21. Parasitaemia was negatively correlated with WAZ, BMI and haemoglobin but it was positively correlated with splenomegaly. The correlations with haemoglobin and splenomegaly were strong and highly significant. Parasitaemia was also strongly associated with poor socioeconomic status as indicated by negative correlation with school items factor.

Both peg boards tests were correlated with parasitaemia but the correlation was only significant with the dominant test ($r=0.10$, $n=585$; P: <0.05) and approaching significant with the non-dominant ($r=0.08$, $n=585$; P: $= 0.07$).

Table 17. Initial scores of cognitive and school achievement tests by parasitaemia group on enrollment to the natural trial

Test	Non-parasitaemic n=142 Mean ± SD	Parasitaemic n=445 Mean ± SD
Digit span	5.8 ± 1.3	5.7 ± 1.2
Peg board dominant (seconds) [a]	127.4 ± 1.2	130.5 ± 1.2
Peg board non-dominant (seconds) [a]	153.7 ± 1.2	156.6 ± 1.2
Picture memory	38.8 ± 5.3	38.7 ± 4.9
Symbol - Symbol (seconds) [a]	0.7 ± 1.2	0.7 ± 1.3
Stroop direct (seconds) [a]	0.7 ± 1.2	0.7 ± 1.2
Stroop indirect (seconds) [a]	1.0 ± 1.2	1.0 ± 1.2
Verbal fluency	22.2 ± 5.3	22.5 ± 6.3
Visual search (seconds) [a]	2.5 ± 1.3	2.5 ± 1.2
Mathematics score	11.7 ± 4.9	11.9 ± 4.7
Reading score	13.4 ± 5.9	13.7 ± 5.8

[a] Geometric mean ± geometric SD

Table 18. Correlation among cognitive and school achievement tests (n=550-569) controlling for age

Test	Mathematics r	Reading r
Digit span	0.28***	0.27***
Peg board dominant (seconds)	- 0.20***	- 0.23***
Peg board non-dominant (seconds)	- 0.14**	- 0.13**
Picture memory	0.11*	0.20***
Symbol-symbol (seconds)	- 0.28***	- 0.27***
Stroop direct (seconds)	- 0.30***	- 0.30***
Stroop indirect (seconds)	- 0.33***	- 0.28***
Verbal fluency	0.13**	0.15***
Visual search (seconds)	- 0.32***	- 0.28***

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table 19. Results of the factor analysis of cognitive function tests on enrollment into the natural trial

Factor	Test	Loading	Eigen value
Factor one:			
	Motor factor		2.4
	- Peg board dominant	0.91	
	- Peg board non-dominant	0.91	
	- Visual search	0.67	
Factor two:			
	General Cognitive factor		2.0
	Digit span	- 0.60	
	Verbal fluency	- 0.52	
	Picture learning	- 0.52	
	Stroop indirect	0.66	
	Symbol-symbol	0.68	

Table 20.Significant correlations (r) between cognitive and school achievement tests and SES and nutritional status (n=550-584) controlling for age

Test	Wealth	Housing	School items	Animal	HAZ	WAZ
Digit span	_	_	_	_	_	_
Peg board dominant (seconds)	_	_	_	_	_	_
Peg board non-dominant (seconds)	- 0.10*	_	_	_	_	_
Picture memory	0.13**	_	0.09*	_	_	_
Symbol-symbol	_	_	_	_	_	_
Stroop direct (seconds)	_	_	_	_	_	_
Stroop indirect (seconds)	- 0.10*	_	_	_	_	_
Verbal fluency	_	_	_	_	_	_
Visual search (seconds)	_	_	_	_	- 0.15***	- 0.16***
Motor factor	_	0.10*	_	_	- 0.09*	- 0.09*
General cognitive factor	_	0.08*	_	_	_	_
Reading score	_	0.13**	_	_	_	_
Mathematics	_	_	_	_	_	_

* P< 0.05 ** P <0.01 ***P <0.001

Table 21. Correlations between parasitaemia group and cognitive tests, nutritional status, splenomegaly, and SES controlling for age

Test	r	n	P
I- Cognitive tests			
Peg board dominant	10	585	<0.05
II. Nutritional status			
WAZ	- 0.11	573	< 0.01
BMI	- 0.09	573	< 0.05
haemoglobin	- 0.35	580	< 0.001
III. Splenomegaly	0.24	581	< 0.001
IV. SES			
School items factor	- 0.16	578	< 0.001

7.2.2.4. Factors associated with cognitive tests scores:

Multiple regression analysis was conducted to study the association between parasitaemia and cognitive functions taking into account SES and nutritional status. We predicted each cognitive score in turn controlling for age and offering stepwise socioeconomic status, anthropometry (WAZ, HAZ, BMI) and tester, and finally parasitaemia group was entered in the equation (No / Yes). The results are shown in table 22. As anaemia could be the mechanism through which parasitaemia may affect cognition, the first analysis did not include haemoglobin. We then repeated all the regressions entering anaemia group instead of parasitaemia group.

Age:

Age influenced significantly all the cognitive function tests except for the digit span which was not significant.

Socioeconomic status:

Wealth, school items, housing and father's education were significantly related to cognitive function tests. Children who came from wealthy families did better in the peg board non-dominant, the picture learning, the symbol-symbol, the Stroop indirect and the general cognitive factor ($P < 0.05$ for all). Also, children who had more school items tended to do better in the picture memory but this only approached significance ($P = 0.06$). Children whose fathers were literate had better scores in the peg board dominant ($P < 0.05$) and the visual search ($P < 0.01$).

Table 22. Significant regression coefficients and R^2 in multiple regression analysis of the initial cognitive tests scores on parasitaemia (No/Yes) controlling for age, SES, father literacy and nutritional variables *

Test	B	SE	P
Peg board dominant [a] (R^2 = 0.17)			
- age	- 0.05	0.01	< 0.001
- father literacy	- 0.02	0.01	< 0.05
- malaria	0.03	0.01	< 0.05
Peg board non-dominant (R^2=0.16)			
- age	- 0.05	0.01	< 0.001
- wealth factor	- 0.02	0.01	< 0.01
- malaria	0.03	0.02	0.05
Picture memory (R^2=0.10)			
- age	0.5	0.2	< 0.01
- wealth factor	0.8	0.2	< 0.001
- school items factor	0.4	0.2	0.06
Symbol-symbol (R^2 =0.14)			
- age	- 0.06	0.01	< 0.001
- wealth factor	- 0.06	0.01	< 0.05
Stroop direct (R^2= 0.20)			
- age	- 0.06	0.01	< 0.001
Stroop indirect (R^2=0.11)			
- age	- 0.05	0.01	< 0.001
- wealth factor	- 0.02	0.01	< 0.01
Verbal fluency (R^2=0.07)			
- age	1.27	0.20	< 0.001
Visual search (R^2=0.24)			
- age	- 0.08	0.01	< 0.001
- WAZ	- 0.06	0.01	< 0.001
- father literacy	- 0.05	0.02	< 0.01
Motor factor (R^2=0.20)			
- age	- 0.33	0.03	< 0.001
- WAZ	- 0.16	0.07	< 0.05
- father literacy	- 0.16	0.04	< 0.05
General cognitive factor (R^2=0.10)			
- age	- 0.2	0.03	< 0.001
- wealth factor	- 0.09	0.04	< 0.01

* d.f.: 566-569

Nutritional status:

Children with lower WAZ were slower on the visual search test ($P < 0.001$) and had poorer scores on the motor factor (<0.05).

Parasitaemia:

The parasitaemic children were significantly slower in the peg board dominant test than non- parasitaemic group ($P < 0.05$). The model predicted 17% of the variance ($R^2 = 0.17$). This relationship was not mediated through anemia because after controlling for hemoglobin, it remained significant. The parasitaemic group was also, slower in the peg board non-dominant test but this effect only approached significance (P=0.05).

Haemoglobin:

We repeated all the regressions entering haemoglobin group instead of parasitaemia group. Haemoglobin was entered as three dummy variables for four groups (Hb ≥110, Hb 100-100.99, Hb 90-90.99 and Hb <90 g/L). The results are shown in table 23. Haemoglobin was significantly associated with three cognitive tests. Those with haemoglobin below 90 g/L were slower in the peg board non-dominant and both Stroop tests (P <0.05 for all).

Splenomegaly:

To investigate the effect of having splenomegaly - as an indicator of chronic malaria- on cognitive and school achievement tests scores we used splenomegaly as dichotomous variable (0= no / 1= yes) instead of parasitaemia group. No significant association between having splenomegaly and cognition was found.

Table 23. Significant regression coefficients and R^2 in multiple regression analysis of the initial cognitive tests scores on haemoglobin group controlling for age, SES, father's literacy, nutritional variables and parasitaemia group*

Test	B	SE	P
Peg board non-dominant (R^2=0.17)			
- age	- 0.05	0.01	< 0.001
- wealth factor	- 0.02	0.01	< 0.05
- haemoglobin (< 90 g/L)	0.07	0.03	< 0.05
- malaria	0.03	0.02	< 0.05
Stroop direct (R^2= 0.21)			
- age	- 0.06	0.01	< 0.001
- haemoglobin (< 90 g/L)	0.08	0.03	< 0.05
Stroop indirect (R^2=0.11)			
- age	- 0.05	0.01	< 0.001
- wealth factor	- 0.02	0.01	< 0.01
- haemoglobin (< 90 g/L)	0.08	0.04	< 0.05

*d.f.: 566-569

7.2.2.5. Factors associated with school achievement:

The above model was also used to investigate the different variables which were associated with school achievement (dependent variable). School was significantly associated with school achievement tests. Age and SES (animal factor) were associated with the perormance in mathematics test. The model explained 21% of the variance (R^2= 0.21). Older children and those with better SES (higher scores in the animal factor) had better scores in mathematics. Age, haemoglobin, and housing were significantly associated with the score of reading test. The model predicted 13% of the variance (R^2= 0.13). Older children scored better (P <0.001), those who had haemoglobin below 90 g/L scored less (P <0.05) and those who came from better SES background (higher scores in housing factor) had better reading scores (P < 0.01). The results are shown in table 24.

7.2.2.6 Factors associated with absenteeism:

The same multiple regression model was used to investigate the different variables which were associated with absenteeism (table 25). Days absent from the school (log transformed) was the dependent variable. The result showed that older children had more absenteeism (P <0.01), malnourished children (as indicated by BMI) and those who came from lower SES background (as indicated by school items factor) are more likely to be absent (P <0.05 and <0.01 respectively). School was also significantly associated with absenteeism.

Table 24. Significant regression coefficients and R^2 in multiple regression analysis of the school achievement tests on parasitaemia controlling for age, SES, father's literacy, nutritional variables*

Test	B	SE	P
Mathematics ($R^2 = 0.21$)			
- age	1.2	0.3	< 0.001
- animal	0.40	0.19	<0.05
Reading ($R^2 = 0.13$)			
- age	1.6	0.2	< 0.001
- housing	0.6	0.2	< 0.01
- haemoglobin (< 90 g/L)	- 2.7	1.1	< 0.05

* d.f. : 513- 537

Table 25. Significant regression coefficients and R^2 in multiple regression analysis of absenteeism on parasitaemia controlling for age, SES, father literacy and nutritional variables*

Test	B	SE	P
Days absent from school ($R^2 = 0.29$)			
- age	0.11	0.04	< 0.01
- BMI	- 1.2	0.5	< 0.05
- school items factor	- 0.14	0.05	< 0.01

* d.f. : 286

7.2.3. Measurements at follow-up:

Screening for malaria two weeks after enrollment identified 150 children who were parasitaemic at the baseline and still parasitaemic at follow-up. These were matched for school and grade with 150 who were parasitaemic at the baseline but no longer parasitaemic at follow-up.

A principal component factor analysis was conducted with the final cognitive test scores. The result is shown in table 26. All items were loaded ≥ 0.40. The final tests were loaded in a similar way to the initial tests. The two peg boards and visual search loaded on factor one formed the motor factor. All other cognitive tests loaded in factor two formed the general cognitive factor.

Table 27 shows the initial and final cognitive tests scores by parasitaemia group at the follow-up for those who completed the study. No significant differences were detected between the two groups at either test sessions except that the initial digit span scores were higher among the parasitaemic group at enrollment ($P < 0.05$).

To investigate the effect of the natural cure from parasitaemia on cognitive scores we conducted multiple regression analysis in which we predicted the final cognitive scores controlling for initial scores, and offering age and tester and then forcing the natural cure group (cured / not cured) to enter in the equation in the third block. Offering SES and anthropometry made no difference to the results. No significant effect could be detected for the natural cure.

Table 26. Results of the factor analysis of the cognitive function tests at follow-up of the natural trial

Factor	Test	Loading	Eigen value
Factor one:			
	Motor factor		2.4
	- Peg board dominant	0.89	
	- Peg board non-dominant	0.89	
	- Visual search	0.69	
Factor two:			
	General Cognitive factor		2.0
	- Digit span	- 0.67	
	- Verbal fluency	- 0.44	
	- Picture learning	- 0.42	
	- Stroop indirect	0.72	
	- Symbol-symbol	0.70	

Table 27. Initial and final cognitive tests scores for children who were initially parasitaemic and completed the natural trial by final parasitaemia group.

Test		Non-parasitaemic n= 150 Mean±SD	Parasitaemic n= 150 Mean±SD
Digit span	initial*	5.6 ± 1.1	5.9 ± 1.2
	final	5.8 ± 1.3	6.1 ± 1.4
Peg board dominant (seconds)[a]	initial	133.5 ± 1.2	130.2 ± 1.2
	final	118.6 ± 1.1	116.4 ± 1.1
Peg board non-dominant (seconds)[a]	initial	159.6 ± 1.2	158.0 ± 1.2
	final	140.3 ± 1.6	137.8 ± 1.1
Picture memory	initial	38.7 ± 4.8	38.2 ± 4.9
	final	38.7 ± 4.8	38.5 ± 4.8
Symbol - symbol (seconds) [a]	initial	0.70 ± 1.2	0.67 ± 1.3
	final	0.62 ± 1.2	0.60 ± 1.2
Stroop direct (seconds) [a]	initial	0.68 ± 1.2	0.66 ± 1.2
	final	0.59 ± 1.12	0.58 ± 1.2
Stroop indirect (seconds) [a]	initial	1.01 ± 1.2	0.99 ± 1.2
	final	0.84 ± 1.2	0.82 ± 1.2
Verbal fluency	initial	21.7 ± 6.7	22.5 ± 5.9
	final	22.5 ± 6.6	23.2 ± 6.3
Visual search (seconds) [a]	initial	2.5 ± 1.3	2.5 ± 1.3
	final	2.1 ± 1.2	2.1 ± 1.2
Motor factor	initial	0.1 ± 1.0	0.1 ± 1.0
	final	0.1 ± 1.0	-0.1 ± 1.0
General Cognitive factor	initial	0.1 ± 0.9	-0.01 ± 1.1
	final	0.1 ± 0.9	-0.01 ± 1.0

[a] Geometric mean± geometric SD

* $P < 0.05$ (t-test)

7.2.4. C-Reactive Protein (CRP) assay:

Table 28 shows medians, 25th and 75th percentiles and geometric means ± geometric SD of CRP on enrollment into the natural trial and at follow-up. There was a significant difference in CRP concentrations between parasitaemic and non-parasitaemic groups both on enrollment and at follow-up ($P < 0.001$ for both).

A comparison of those who were no longer parasitaemic at follow-up and those who were non-parasitaemic on enrolment showed no significant difference between CRP concentrations between the two groups ($P > 0.05$).

Table 29 shows the significant correlations with CRP on enrollment. CRP was positively correlated with parasite density and splenomegaly ($P < 0.001$ for both) while it was negatively correlated with haemoglobin and picture memory ($P < 0.001$ and < 0.01 respectively).

To examine the possible association between CRP and cognition and school achievement we conducted multiple regression analysis on the enrollment data. We used the same model we used previously in 7.2.2.4 but substituted CRP for the parasitaemia group coded as dichotomous variable (0= < 2 mg/L, 1= ≥ 2 mg/L). No association was found.

7.2.5 Post Hoc analysis:

We conducted a post hoc analysis to investigate if those who lost their infection were different in any way from those who remained parasitaemic. The two groups were similar in their cognitive test scores except for the digit span where those who remained parasitaemic had higher initial score (table 27). However, we found that they were different in the initial parasite density, CRP concentrations and haemoglobin concentration. Those who became non-parasitaemic had lower initial parasite density: 1204 parasites per μl compared with 1488 parasites per μl among those who remained parasitaemic ($P < 0.01$). They also had lower intial CRP concentrations (table 28). In contrast, they had a higher haemoglobin concentration: 114 ± 15 g/L compared with 107 ± 13 g/L ($P < 0.001$).

We repeated all regression analysis on cognitive tests controlling for the initial parasite density, initial CRP, and haemoglobin. We did not find an effect of losing infection. However, we found that initial parasite density significantly predicted change on the peg board scores ($P < 0.05$), motor factor ($P < 0.01$) and picture memory ($P < 0.05$) .

Table 28. Comparison of CRP concentrations among parasitaemic and non-parasitaemic groups at enrollment and follow-up of the natural trial

		Enrollment				Follow-up			
Group	n	Median	percentiles 25^{th}	75^{th}	geometric mean ± SD	Median	percentiles 25^{th}	75^{th}	geometric mean ± SD
Parasitaemic groups combined	437	0.38	0.15	0.87	0.38 ± 3.9*				
Parasitaemic at follow-up	134	0.43	0.20	0.96	0.47.1± 3.8***	0.33	0.16	0.97	0.38 ± 3.8**
Non-parasitaemic at follow-up	131	0.25	0.09	0.62	0.26± 3. 9	0.15	0.07	0.34	0.16 ± 3.1
Non-parasitaemic on enrollment	125	0.12	0.05	0.32	0.13 ± 2.9				

* Parasitaemic children on enrollment compared with non-parasitaemic group on enrollment (P: <0.001).

**Parasitaemic children at follow-up compared with non-parasitaemic group at follow-up (P: <0.001).

***Values on enrollment of children who were and remained parasitaemic compared with children who were parasitaemic and lost their infection (P: < 0.001).

Table 29. Significant correlations between CRP and parasite density (n=434), Hb, splenomegaly and cognitive tests (n = 559) on enrollment in the natural trial controlling for age

	r	P
Parasite density	0. 46	< 0.001
Splenomegaly	0.31	< 0.001
Hemoglobin	- 0.42	< 0.001
Picture memory	- 0.11	< 0.01

7.2.6. Summary of chapter three: Results:

In this chapter:

- the results of the clinical trial was discussed first but not in details:
  * there was a high prevalence of stunting, underweight and anaemia.
  * in the cross-sectional study parasitaemic children were significantly more anaemic and slower in the fine motor functions.
  * there was no significant difference between the treatment and the placebo group in their performance in the cognitive tests.

- the findings of the natural trial then discussed in details:
  * malnutrition was highly prevalent and approximately half of the children were found to be stunted or underweight and 40% were anaemic.
  * Parasitaemia was strongly associated with anaemia, splenomegaly, malnutrition and poor SES.
  * a significant association was also found between parasitaemia and fine motor function after controlling for age, SES, nutritional status and anaemia.
  * SES was found to be associated with cognition.
  * WAZ was also found to be associated with tests that had an element of fine motor coordination.

* anaemic children conducted worse in three cognitive tests but this was only among those who had Hb below 90 g/L.
* school achievement tests were associated with school, SES and anaemia.
* older children, those from poor SES and malnourished children found to be more likely to be absent from school.
* CRP levels differed significantly between parasitaemic and non-parasitaemic groups and it was positively correlated with parasite density and splenomegaly but was negatively correlated with haemoglobin and picture memory test.
* No significant difference was found in change in performance over the study period between those who were naturally cured and those who remained parasitaemic.
* CRP levels among parasitaemic who became non-parasitaemic returned back to base line levels in two weeks time.
* a post hoc analysis showed that the children who became non-parasitaemic at follow-up had lower initial parasite density and lower initial CRP but had a higher haemoglobin level. Although after controlling for these differences there was no effect for losing infection, children who initially had the most intense infection improved the most in a fine motor tests and a picture memory test.

Chapter four

Discussion

8. Discussion

The association between malaria parasitaemia and cognitive function of schoolchildren was examined through a case control study in which we compared the cognitive function of a group of schoolchildren with asymptomatic parasitaemia with those of a group who were non-parasitaemic, matched for school and grade. Two weeks later, the parasitaemic group was screened for parasitaemia and those who remained parasitaemic were compared with children matched for grade and school who were no longer parasitaemic. The findings of the case control study will be discussed first then the findings from the natural experiment. Since the clinical trial was abandoned due to rapid unforeseen clearance of parasitaemia, I will not discuss the findings in details.

8.1. Discussion of the case control study:

8.1.1. Validity of the results:

Before I discuss the findings from the case-control study it is important to look first at the validity of these results. Validity means that the results reflect the true situation. In any epidemiological research, validity is a big concern. It has two components: the internal and the external validity. By the internal validity we mean to what extent the study succeeded in proper selection of the study groups and in avoiding any error in design, measurements and statistical analysis. External validity (or generalizability) refers to what extent we are able to extrapolate from the study population to those who are outside that population.

8.1.1.1. Internal validity:

Greenland (1997) identified three conditions that need to be fulfilled to guarantee the internal validity of results of any case-control study. These conditions are: comparison validity, specification validity and measurement validity.

Comparison validity:

In any study it is important to be sure that the study groups are comparable. Our study groups, the parasitaemic and non-parasitaemic were chosen from the same school population and were matched for grade and school. They were similar regarding their age, sex, nutritional status and socioeconomic characteristics except for the school items factor (table 14). There are some known confounders (e.g. age, nutritional status and socioeconomic status) which influence the outcome in cognitive and school achievement tests (Grantham-McGregor, 1993). We measured a large number of possible confounders and controlled for them in the analysis. In spite of the possibility that there may be some other unrecognized confounders, it is unlikely that confounders which were unmeasured biased the results to any marked extent.

There are reports from the Yemen of a high prevalence of schistosomiasis among schoolchildren with a patchy distribution (Hazza, Arfaa, Haggar, 1983; Schaap, Den-Dulk, Polderman, 1992). In the area where the clinical trial was conducted, a previous pilot study had shown that 36% of children were infected with *Schistosoma haematobium* while *Schistosoma mansoni* was very rare. The same pilot showed that

for *S. haematobium* a positive result on a strip test for haematuria was highly correlated with a positive result on microscopy. Therefore, we excluded all children who had haematuria on a strip test. In the area where the natural trial was conducted, due to logistic and time constraints it was not possible to do urine and stool analysis to exclude those with schistosomiasis. However, we excluded schools where previous surveys had found a high prevalence of schistosomiasis but there remained a possibility that some of the children in our sample were infected. There is no good evidence that schistosomiasis detrimentally affects children's cognitive function and school achievement. Published studies have given inconsistent results and the study design have had many problems (Loveridge, Ross, Blair, 1948; Clarke, Blair, 1966; Ekanem *et al.* 1994; Bell *et al.* 1973; Kimura *et al.* 1992; Connolly, Kvalsvig, 1993). The studies of impact of intestinal parasites on cognition also have given inconsistent results. Although some treatment trials have shown an effect on cognitive function (e.g. Nokes *et al.* 1992), findings from recent robust trials did not support a casual relationship in children with light to moderate infection (Simeon *et al.* 1995a, Simeon *et al.* 1995b). I am unaware of any study that found that the prevalence of schistosomiasis or intestinal parasites have occurred with different frequencies between parasitaemic and non-parasitaemic children. Also the infection status with schistosomiasis or intestinal parasites is unlikely to have changed during the relatively short time of this study. Therefore, it is unlikely that they would biased the results.

Specification validity (Statistical-conclusion validity):

Most statistical methods depend on the assumption that sampling model or error distribution are valid. When the sampling model or the statistical methods used for analysis are incorrect the results may be biased. The researcher cannot then conclude that any statistical relationship found between the dependent and independent variables truly exists. Low statistical power, and use of inappropriate or multiple statistical tests are major threats for this type of validity. Low statistical power increases the chance of false negative result (type II error) while use of inappropriate or multiple statistical tests may increase the chance for false positive findings (type I error). To overcome the first threat it is important to have a large enough sample size. Our study had a large sample size which was calculated to be able to find assignment effect if a difference existed with 95% confidence interval and 80% power. To avoid the second threat before conducting any statistical tests the assumptions necessary for these tests e.g. normal distribution and equality of variance were satisfied. However, we used many tests which may increased the chance of type I error i.e. false positive findings, but this was necessitated by the research hypothesis and the different cognitive tests we have to use to measure different cognitive functions which might be selectively affected.

Measurement validity:

The conclusion of any study can be violated by errors in measuring the study variables. Possible errors in measurement include procedure errors, proxy variable errors and construct errors:

i. Procedure errors: this means that defects or mistakes had occurred with measurement procedure. The reliability for each measurement was ascertained before we started the field work and throughout the study. For cognitive tests, the test-retest reliability was good ($r \geq 0.70$). For anthropometric measurements the $r \geq 0.98$. In addition, all cognitive measurements on a child were carried out by the same person and following a standard format. We frequently checked our instruments (e.g. weight scale and HemoCue apparatus) against known standard throughout the field work. Moreover, due to the sensitivity of cognitive tests and to ensure that testers did not bias the cognitive tests scores, I controlled statistically for testers in the regression model.

ii. Proxy variable errors: this may arise from using a proxy variable instead of actual variable. The only proxy variable we used was CRP as a proxy for cytokines. We hypothesized that cytokines may affect cognition in a direct but a transient way. Since cytokines have a very short half-life and are difficult to measure we used CRP as proxy for them. However, information about the precise time relation between cytokines and CRP is scarce, especially in asymptomatic malaria. The very limited available information comes from patients who had clinical malaria and were under treatment (Gillespie *et al.* 1991; Harpaz *et al.* 1992) or from cancer patients who received cytokines for treatment (Banks *et al.* 1995). No similar information is available from

asymptomatic parasitaemic subjects. Therefore, the extent to which CRP reflects the cytokines is not well understood and there is a possibility that CRP it is not the best proxy.

Before considering the third type of errors i.e. construct errors, I will discuss first the sensitivity of the method we used to diagnose parasitaemia and to define our study groups i.e. parasitaemic and non-parasitaemic groups.

Sensitivity of microscopical diagnosis of infection with plasmodium spp and possibility of missing infections:

Although the microscopical examination of the blood remains the main method for diagnosis of parasitaemia there are some indications that this method is not very sensitive. There is some evidence that microscopical examination may miss very low density infections.

Previous studies showed that the sensitivity of microscopical diagnosis increased as the number of high power fields (HPF) examined increased. In Nigeria, Dowling and Shute (1965) showed that among adults only 43% of infections were detected by examining 200 HPF compared with 61% and 70% by examining of 600 and 1000 HPF respectively. Another study showed that by examining 400 HPF compared with 200 HPF the prevalence of infection with *P.falciparum* was increased by 10% (Molineaux, Gramiccia, 1980).

However, time constraints made it impractical in many studies to examine more than 100 fields. In a study of 245 schoolchildren aged 6 to 16 years conducted in Congo the prevalence was 76% for 100 HPF compared with 80% for 200 HPF. Only 10 who were negative after examination of 100 HPF were found to be positive after examination of 200 HPF (Trape, 1985).

Therefore, a more sensitive method is needed and polymerase chain reaction (PCR) appears to have the greatest sensitivity. Snounou (1993) has shown that PCR provides 200 fold increase in sensitivity compared with microscopy. In a recent study, it was shown that two thirds of individuals found to have no infection on microscopy were found to be infected with PCR. The study concluded that PCR can detect parasitaemia at an intensity at least 100 to 1000 times lower than microscopical examination can (Bottius et al, 1996).

iii. Construct errors: these errors can arise from unclearly defined variables. I am aware that our definition of the control group i.e. the non-parasitaemic group, which depended on finding no parasites in 100 high power field (HPF) in a stained thick blood smear was not ideal. If we had the facility to use a more sensitive method such as polymerase chain reaction (PCR) some of the non-parasitaemic children may have been found to be parasitaemic (Snounou, 1993, Bottius *et al.* 1996). However, most of the research concerning malaria relies upon this diagnostic method. In an attempt to improve our sensitivity and

specificity we did not depend on only one malaria smear but instead we examined two malaria smears taken 24 hours apart for each subject. I also limited the definition of parasitaemic group to those who had parasite density equal to or above 750 parasites per μl to increase the difference between parasitaemic and non-parasitaemic groups. The fact that both groups were significantly different in their CRP concentrations indicates that our groups were clearly different in infection status.

In conclusion we have a fairly reasonable internal validity but we admit that we had a problem in defining the non-parasitaemic group.

8.1.1.2. External validity:

A study is externally valid when it allows unbiased inferences regarding those beyond the subjects in the study. Once the internal validity of a study has been established, the external validity should be considered. Although representativeness of the sample is an important requirement for external validity, due to resource limitations it was not possible to use random sampling to ensure representativeness. In this study, both parasitaemic and non-parasitaemic groups were chosen from rural school population in an area which had endemic malaria. Our study was limited to boys who were attending school in grade four to six only. There is a possibility that girls and boys in other age groups may react to the asymptomatic parasitaemia in a different way. In addition it is well known that cognitive development is influenced by many factors

such as the children's nutritional status, and their SES background. Furthermore, these factors may interact with each other in their effect on children's cognition. Thus findings may be different in different types of populations. At the moment these findings may be extrapolated to Yemeni boys of same age, social background and nutritional status. Our study was conducted in the schools i.e. in the same circumstances under which the target population usually function, and this supports its ecological validity. We also have limited evidence from the cross-sectional comparisons of both trials that were conducted in two different populations at two different times that there was some impairment in fine motor function. The consistency of these findings supports the external validity of the study to some extent. Nonetheless, there are obvious limitations regarding the external validity of the study. Therefore, it is important that similar studies should be replicated in other populations to determine whether the findings can be replicated.

8.1.2. Findings of the case-control study:

We compared a group of parasitaemic children with a group of non-parasitaemic children on their performance on cognitive and school achievement tests. Some other known factors that affect cognition such as nutritional status, short-term hunger and socioeconomic status (SES) were also measured and taken into account. I am going to discuss the findings in relation to the sample general characteristics then the results of cognitive and school achievement tests and factors associated with them. Finally, the results of the C-reactive protein will also be discussed.

8.1.2.1. General characteristics of the sample:

Nutritional status:

Very little information is available about nutritional status of schoolchildren in Yemen. Our study showed that the malnutrition was common and that the prevalence of stunting and underweight is high (51% and 55% respectively). The fact that the clinical trial showed a similar prevalence although it was conducted in a different area support the finding that malnutrition is highly prevalent among rural Yemeni school-children. It is interesting that the figures are similar to those reported from the Yemen Republic among children under five. The Multiple Indicators Cluster Survey (MICS) conducted in 1996 found that about 40% of children under five years were stunted or underweight (Zein, 1997). Among schoolchildren we only found one published study which was conducted by Haithami and colleagues (1989). It reported a much lower prevalence of stunting and underweight (22.2% and 22.8% respectively).

However, this apparent discrepancy is probably due to the fact that the later study was conducted in the capital of the South Yemen which has one of the most affluent and educated population in the country.

Our prevalence of malnutrition is much higher than those reported from neighboring countries and from many other developing countries. Al-Frayh and Bamgboye (1993) reported a much lower figure from neighboring Saudi Arabia where only 20% of schoolchildren were below -2 SD scores of the reference population for weight-for-height and weight-for-age. This reflects the wide gap in socioeconomic and health

status between the two neighboring countries. Ukoli and colleagues (1993) found that only 14% of Nigerian schoolchildren were stunted and 20% were underweight. It was also found that the prevalence of underweight among Zanzibari schoolchildren was 10% or less (Stoltzfus *et al.* 1997c). Nonetheless, figures similar to the ones we have shown for stunting have been reported from some other developing countries.

Pegelow and colleagues (1997) found that 51% of Indonesian primary schoolchildren were stunted. In Zanzibar, different prevalences of stunting were reported among different age groups varying from 14% in 7-year-old children to 83% in 13-year-old children (Stoltzfus *et al.* 1997c). Finally, Surveys of schoolchildren in Ghana, Tanzania, India, Vietnam, and Indonesia found that 51% were stunted, 19% severely stunted, 48% were underweight, and 6% severely underweight (Partnership for Child Development, 1998).

In conclusion, it seems that the Yemen may be similar to some other developing countries regarding the chronic malnutrition as reflected by stunting. However, it has a uniquely high prevalence of underweight. The high prevalence of malnutrition among Yemeni children, as else where, is due to inadequate dietary intake and infectious diseases. It has been shown that the diet of Yemeni families is not balanced and it lacks important food items such as vegetables, fruit and animal products e.g. milk and meat (de Regt, 1994). On the other hand, infectious diseases are highly prevalent in Yemen. In the two weeks preceding the Yemen Demographic Maternal and Child Health Survey (Central Statistical Organization, 1994). 49%, 46% and 34% of children under five had cough, fever or diarrhea respectively.

Anaemia:

Anaemia has been recognized as a major public health problem in the Yemen (Karrar, 1993). However, very little has been published about the size of the problem and nothing about the prevalence among schoolchildren. The National Nutrition Survey (1979) found that 66% of the rural and 17% of the urban pre-school children were anaemic. We found that the mean haemoglobin concentration was 113 ± 14 g/L and the range was 66 to 150 g/L. 41% had a Hb below 110 g/L which is the WHO cut-off value for definition of anaemia (WHO, 1996a). Six per cent of our sample had a Hb below 90 g/L. The results from the clinical trial showed an even higher prevalence with 55% below 110 g/L. These figures are much higher than figures reported among schoolchildren from other Arabic countries e.g. 3.2% in Libyan schoolchildren (Bredan, Kumar, Bshiwah, 1983). It is also higher than figures reported from some other developing countries e.g. 14.7% among Jamaican schoolchildren (Hutchinson *et al.* 1997), 6.3% in South Africa (Westhuyzen, Steyn, 1992), and 13% in Indonesian schoolchildren (Pegelow *et al.* 1997). Nevertheless, similar figures to ours have been reported from some developing countries. Stoltzfus and colleagues (1997c) reported that among Zanzibari schoolchildren 63% had a haemoglobin below 110 g/L. Also, biomedical surveys of 8-12 years old schoolchildren in Ghana, Tanzania, India, Vietnam, and Indonesia found that 47-81% were anaemic (Hb < 120 g/L) (Partnership for Child Development, 1997).

The causes of anaemia are multiple. Low dietary intakes, low bioavailability of iron in the diet and iron loss due to intestinal parasites are the major causes. Malaria can be another contributing factor (Brabin, 1992). In Yemen, although the per capita intake was found to be adequate, iron absorption may be inhibited by phyates in whole cereals, polyphenols in tea and poor vitamin C intake (Zein, 1997).

Parasitaemia and parasite density:

We found that the prevalence of asymptomatic malaria parasitaemia among schoolchildren was high but it varied from school to another from 20% to 60%. Similar prevalence was reported from other studies among African schoolchildren (Trape *et al.* 1994; Hogh *et al.* 1995; Albonico *et al.* 1997). However, most of the asymptomatic parasitaemic children had a very low parasite density and only 25% had a parasite density above 1,700 /µl and 10% were above 2,400/µl. During screening we found that only 1% of children had parasite density above 5,000 /µl and these children were treated immediately. Stoltzfus and colleagues (1997d) also found that although 61% of Zanzibar schoolchildren were parasitaemic, less than 1% of children had densities above 5000 parasites/µl blood. In a similar age group living in a holoendemic area in Senegal, parasite density above 5,000 /µl was found in less than 5% of the sample (Trape *et al.* 1994).

It is noteworthy to mention that 64% of parasitaemic children who were in the placebo group in the clinical trial were no longer found be infected after two weeks of the first examination. This may indicate that children in hyperendemic areas have high

immunity so they can get rid of infection quickly. Another possibility is the cyclical nature of asymptomatic parasitaemia as found in other studies (Daubersies *et al.* 1996).

Splenomegaly:

A high proportion of children living in malaria endemic areas have splenomegaly (Greenwood, 1987a). We found that 35% of children had splenomegaly. The degree of splenomegaly was found to be a negatively correlated with haemoglobin ($r = -0.33$, $n=580$, $P < 0.001$), the same finding reported by Sturrock (1996). Hypersplensim with destruction of red blood corpuscles (RBC) is a possible mechanism for anaemia (Greenwood, 1987a).

8.1.2.2. Comparison between parasitaemic and non-parasitaemic groups:

Four hundred and forty-five parasitaemic and 142 non-parasitaemic children were compared in terms of their performance on cognitive and school achievement tests. The two study groups were chosen from the same school population and were matched for school and grade. The mean age of the two groups was similar.

Nutritional status:

The interaction between malaria and malnutrition is complex (McGregor, 1982). We did not find a difference between means of the nutritional indices of the parasitaemic and non-parasitaemic groups. However, among the parasitaemic groups the parasite density was negatively correlated with WAZ ($r=-0.11$, P: <0.05, $n=434$). Wenlock

(1979) found that parasitaemic children had lower WAZ ($P < 0.01$). After a bed net trial, D'Alessandro and colleagues (1995) reported that the WAZ was higher in children protected by impregnated bed nets than those from untreated villages. Adelekan and colleagues (1997) found that WAZ was lower in those with malaria. In contrast, Ahmad and colleagues(1985) showed a positive correlation between parasite density and WAZ and others found no relation (Snow *et al.* 1991).

We also found that the parasite density was negatively correlated with HAZ ($r= -0.10$, $P< 0.05$, $n=434$). Sharp and Harvey (1980) found that there was a greater proportion of stunted under five children in a highly malarious area (46%) than in an area with low malaria (36%).

Anaemia:

We found that the parasitaemic group had a significantly lower haemoglobin than non- parasitaemic group with a mean Hb of 111 ± 14 *versus* 120 ± 14 g/L respectively ($P<0.001$). A similar finding was also found in the clinical trial. This association has been found among schoolchildren from other malarious areas (e.g. Stoltzfus *et al.* 1997d; Tatala, Svanberg, Mduma, 1998) and among other age groups (e.g. Reed, Wirima, Steketee, 1994; Premji *et al.* 1995; Achidi *et al.* 1996; Newton *et al.* 1997; Cornet *et al.* 1998). Only a few studies failed to show an association between asymptomatic malaria and anaemia (e.g. Akenzua *et al.* 1985).

We found that parasite density was negatively correlated with haemoglobin (r= - 0.23, n=434, P< 0.001) (fig. 8). This was also has reported by other investigators (e.g. Achidi, *et al.* 1996; Kitua *et al.* 1997). However, some studies did not find a correlation (e.g. Beales, 1997; Cornet, *et al.* 1998, Stoltzfus, 1997d).

Splenomegaly:

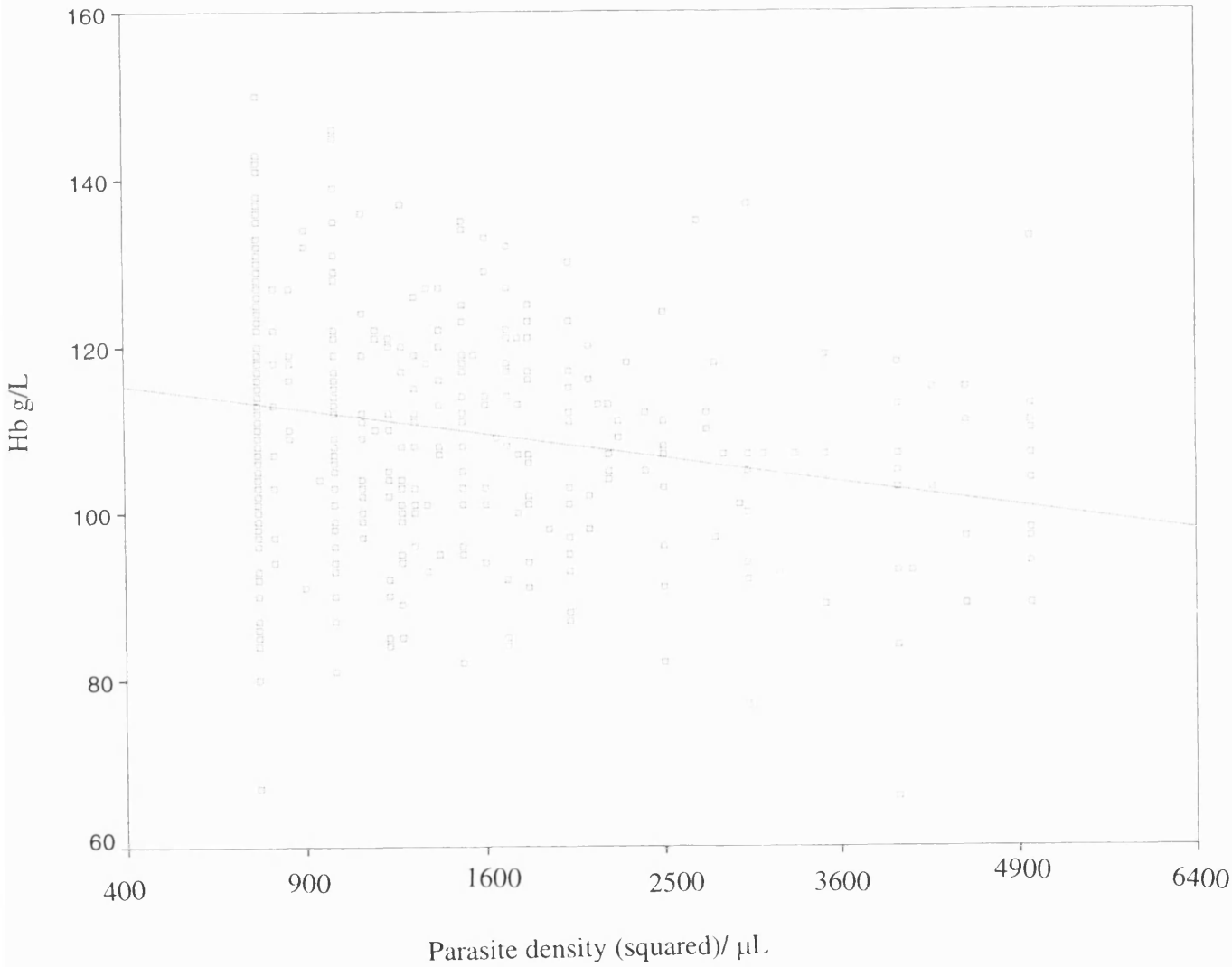
Splenomegaly is one of clinical characteristic of asymptomatic parasitaemia (Greenwood, 1987a). We found that the prevalence of splenomegaly was significantly higher among the parasitaemic group (41%) compared with non-parasitaemic group (16%). This was also found by many other studies (e.g. Luxemburger *et al.* 1998; Schaefer *et al.* 1995). In Papua New Guinea it was found that the prevalence of splenomegaly matched the pattern of age related parasite density (Genton *et al.* 1995).

Socioeconomic status (SES):

Little is known about the association between malaria and socioeconomic status (Koram *et al.* 1995). We found that the parasitaemic children tend to be from a lower socioeconomic background as indicated by school items factor (P< 0.05). In addition fathers of the parasitaemic children were more likely to be farmers and illiterate. Low educational levels have been shown to be associated with malaria in previous studies (Fungladda *et al.* 1987; Butraporn, Sornmani, Hungsapruek, 1986). It may be that educated people know more about malaria and its transmission so they may use some sort of protection e.g. coils, nets etc. In Gambia, Koram and colleagues (1995) found that malaria was associated with poor quality housing and crowding. However in this

fig. 8. Correlation between haemoglobin (Hb) and parasite density* in the natural trial at baseline

(r= - 0.23, n=434, P< 0.001)



* Square root transformation

Gambian study no association was found between the risk of malaria and the overall education level of parents. Better housing quality may reduce entry of mosquitoes due to the use of screens on windows and doors and presence of ceilings. As a long-term measure it seems that improving housing quality and parental education may be one of the control strategies.

8.1.2.3. Factors associated with cognition:

Age:

All cognitive test scores were associated with age except the digit span. This validates the use of the tests in this population to some extent.

SES:

Many studies have shown the importance of SES backgrounds in child development (Waber *et al.* 1981; Powell, Grantham-McGregor, 1985). We found that performance in cognitive tests of children who came from lower SES background was less than the performance of their classmates who came from better SES background. Children who came from wealthy families did better in four out of eight tests and in the general cognitive factor. We also found that children whose fathers were literate did significantly better in the visual search and the peg board dominant tests. Sigman and colleagues (1989) found that Kenyan children with literate parents had higher scores on cognitive tests. Unfortunately, we could not examine the impact of maternal literacy on child development due to the very high maternal illiteracy rates in the

Yemen, especially in rural areas, where 90% are illiterate (Central Statistical Organization, 1992). However the importance of mother's education in relation to both general child well-being and cognitive development is well established. In India it was found that malnutrition was more predominant among the children of illiterate mothers and food consumption patterns were better in children with literate mothers (Arya, Devi, 1991). Therefore, it is not surprising that many studies have found that mother's education significantly influences the child development (e.g. Powell, Grantham-McGregor, 1985; Sigman *et al.* 1989).

Nutritional status:

There is now reasonably strong evidence that nutritional status influences cognitive function and child development (Pollitt, 1990; Grantham-McGregor, 1995). We found that the children with lower WAZ were slower in the visual search test and the motor factor. Some studies found that WAZ is associated with cognitive tests scores (Moock, Lesile, 1986) while others found no association (e.g. Grantham-McGregor *et al.* 1997). We did not find an association between HAZ and cognition which was unexpected. Several studies have shown that HAZ is far more important in predicting cognitive tests scores than WAZ (e.g. Powell, Grantham-McGregor, 1985; Grantham-McGregor *et al.* 1991; Grantham-McGregor *et al.* 1996). Our measurement of height was well standardized and has a high reliability index ($r \geq 0.98$) however, age may be less accurate. Birth certificates were not introduced to the Yemen until recently so we used the school's register to determine the age of the child, which may be less precise.

However we found that age was significantly associated with other outcomes (e.g. cognitive and school achievement tests) which indicates that it has some validity.

Parasitaemia:

The multiple regression analysis showed that parasitaemic children were significantly slower in the peg board dominant test ($P < 0.05$). It shows also that the parasitaemic group was slower in the peg board non-dominant test but this was only approaching significance ($P = 0.05$). The fact that we found in the clinical trial that the parasitaemic children were slower in the peg board non-dominant test than the non-parasitaemic group ($P< 0.05$) indicates some consistency in the finding. Previous studies have shown that motor function was impaired in both naturally-occurring and experimentally induced cold (Smith *et al.* 1987a; Smith *et al.* 1988; Hall, Smith, 1996a). In a double blind controlled trial subjects challenged with IFN-α were also found to be slower on a peg board task (Smith *et al.* 1991). The effect of IFN-α on the peg board test in our study was not mediated via anemia because after controlling for haemoglobin concentration it remained statistically significant.

In cross-sectional studies finding an association does not imply casualty. Hill (1965) proposed nine criteria that should be fulfilled before deciding that the association is causal:

Strength:

It has been suggested that strong association is more likely to be casual. Our finding of an association between malaria and impaired performance in fine-motor task is fairly strong.

Consistency;

Consistency means that the observed association has been repeatedly reported from different places by different investigators at different times. As we previously mentioned, there were no similar studies about the impact of asymptomatic parasitaemia on cognitive function and school achievement. Nevertheless, we have evidence from cross-sectional comparisons in both trials that we conducted in two different populations at two different times that there was an impairment in the fine motor function which supports, to some extent, the consistency of the finding. This merits more research.

Specificity:

This means that a cause leads to a single effect and not multiple effects. Other statisticians (e.g. Rothman, Greenland, 1997) do not agree with Hill about this criterion since in many circumstances a single event can lead to different effects. Our study shows that malaria affects motor function but not the general cognitive function. However, as we mentioned previously this needs to be repeated.

Temporality:

This refers to the fact that the cause should precede the effect. In our research, as in many other retrospective studies- it may be difficult to say this since there was no information available about performance of those children before they got infected. However, this can be an area for future research.

Biological gradient:

By this we mean whether the association that was found reveals any dose response relationship. In this study we are unable to show a linear relationship between parasitaemia and beg board tasks. So the dose response relationship was not apparent.

Plausibility:

This refers to the biological plausibility of the hypothesis. We have been hypothesizing that malaria can affect cognition through cytokines and its acute phase response. The fact that Smith and colleagues (1991) were able to show that after injecting volunteers with IFN-α they developed impairment in a peg board task - which is the task that we found it impaired with malaria- is good support for our hypothesis.

Coherence:

This refers to the fact that our interpretation of the causal effect does not conflict in any way with what we know about the disease. We can not think about contradiction between our knowledge about malaria and the possibility that malaria may impair fine motor function.

Experimental evidence:

It will be greatly support to our finding if future research can show that protection against malaria will prevent development of an impairment in motor function compared with an unprotected group. This will be discussed further in the recommendations for further research.

Analogy:

The presence of analogous findings helps to infer causality. We have strong evidence from studies of viral infections (Smith *et al.* 1987a; Smith *et al.* 1988; Hall, Smith, 1996a) which showed that an impairment of fine motor function was consistently found in both naturally-occurring and experimentally-induced cold. Other important evidence came from IFN-α experiment that showed that volunteers who were challenged with INF-α had a similar impairment to their motor function. Cytokines were found to be elevated in malaria (e.g. Grau *et al.*1989; Harpaz *et al.* 1992; Jakobsen *et al.* 1995). Therefore, by analogy, malaria could impair motor function in a similar way.

In conclusion there is limited evidence supporting that the impairment of fine motor function is casually related to malaria. However, cross-sectional studies alone can not prove causation and treatment trials are needed.

Haemoglobin:

Since anaemia could be the mechanism through which parasitaemia may affect cognition and because we did not include haemoglobin in the first analysis we then repeated all the regression analyses offering haemoglobin group as an explanatory variable instead of parasitaemia group. We found that haemoglobin had a significant association with three cognitive tests. However, the relation was not linear and only those with haemoglobin below 90 g/L showed an effect. Iron deficiency anaemia has been found to have a detrimental effect on cognitive function in many observational and experimental studies (Pollitt, 1990; Lozoff, Jimenez, Wolf, 1991; Idjradinata, Pollitt, 1993). However, a few studies have failed to find an association between haemoglobin and cognitive tests score (e.g. Agarwal *et al.* 1989).

Splenomegaly:

Since splenomegaly is one of the main characteristics of asymptomatic malaria we investigated its relationship with cognitive tests scores. No significant association was found. We are unaware of any published study that investigated this relation.

However, since splenomegaly was highly correlated with anemia, as we have shown in this study, splenomegaly may affect cognition indirectly through anaemia.

Short-term hunger:

Although 62% of children mentioned that they were ever hungry before break, 12% said they went bed hungry during last week and five per cent had no breakfast during last week, no association was found with cognitive tests scores. Simeon and Grantham-McGregor (1989) found that when undernourished children missed breakfast their performance on tests of verbal fluency, auditory short-term memory, and perceptual speed was detrimentally affected. They also found that breakfast improved performance of those children. It may be that the children's ability to report hunger was not good in the Yemeni population.

8.1.2.4. Factors associated with school achievement:

Although school achievement can be influenced by many factors such as quality of schooling and SES background, poor health can be also an important factor. In general, school achievement at all grade levels was poor and many of the children at grade six had scores appropriate for grade four. We found that the school achievement was significantly influenced by school, age, haemoglobin and housing. It has been recognized that school influences the school achievement to a great extent (Grantham-McGregor, Walker, 1998). Children from better SES backgrounds scored better. Many studies have shown that children from better SES background have better school achievement scores (e.g. Clark, Grantham-McGregor, Powell, 1991; Walker *et al.* 1996; Hutchinson *et al.* 1997). We also found that children who had haemoglobin

below 90 g/L had lower school achievement scores. This was significant after controlling for age, socioeconomic background and nutritional status. Many other studies have shown that anaemia was associated with poor school performance (e.g. Popkin, Lim-Ybanez, 1982; Agarwal *et al.* 1987; Florencio, 1988 ; Walker *et al.* 1996). After iron supplementation an improvement in school achievement has been found in several studies (Soemantri, Pollitt, Kim, 1985; Soemantri, 1989).

It is surprising that we did not find that nutritional status was associated with school achievement which was reported by several studies (e.g. Agarwal *et al.* 1987; Florencio, 1988; Hutchinson *et al.* 1997). This may again reflect that age was not precise.

8.1.2.5. Factors associated with Absenteeism:

The study showed that older children were more likely to be absent from the school. This is may be because they are the ones more able to help their families in field work or in looking after animal herds. There are no enough statistics about the drop-out rate from the Yemen Republic however, a recent report showed that by the end of the primary school over half of both girls and boys have dropped out of school and by the end of the preparatory school three quarters of children who started school no longer attend (World Bank, 1998). We found that malnourished children (as indicated by low BMI) had higher absenteeism. This may be due to their poor health and nutritional status so they could not attend regularly. Clark and colleagues found that WHZ

predicted attendance (Clark, Grantham-McGregor, Powell, 1991). We also found that children who came from lower SES backgrounds and who had fewer school items had poorer attendance. In Jamaica, Hutchinson and colleagues (1997) found that social background variables were significantly associated with attendance. Clark and colleagues also found that school-uniform rating was the best predictor of attendance (Clark, Grantham-McGregor, Powell, 1991). This may reflect both the socioeconomic status and the value family places on education.

We did not find an association between malaria and absenteeism although some other studies have found an association (e.g. Trape, Zoulani, Quinet, 1987, Trape *et al.* 1993). Attendance has improved after malaria control with chemoprophylaxis (Colbourne, 1955) or using impregnated bed nets (Shiff *et al.* 1996). Also, an association between infection and absenteeism has been found by other researchers. Nokes and Bundy (1993) found that children infected with heavy to moderate *T. trichiura* infection were absent from school more often than their uninfected counterparts. Parasitic infection also predicted poorer school attendance in another Jamaican study (Hutchinson *et al.* 1997). After treatment of parasitic infections attendance has been improved (e.g. Simeon *et al.* 1995b).

In this study anaemia, although it was highly prevalent, did not predict school attendance, unlike other studies (e.g. Hutchinson *et al.* 1997). Both anaemia and parasitic infections are common among Yemeni schoolchildren (Karrar, 1993; el-Qirbi, Sadek, Ghaly, 1987)

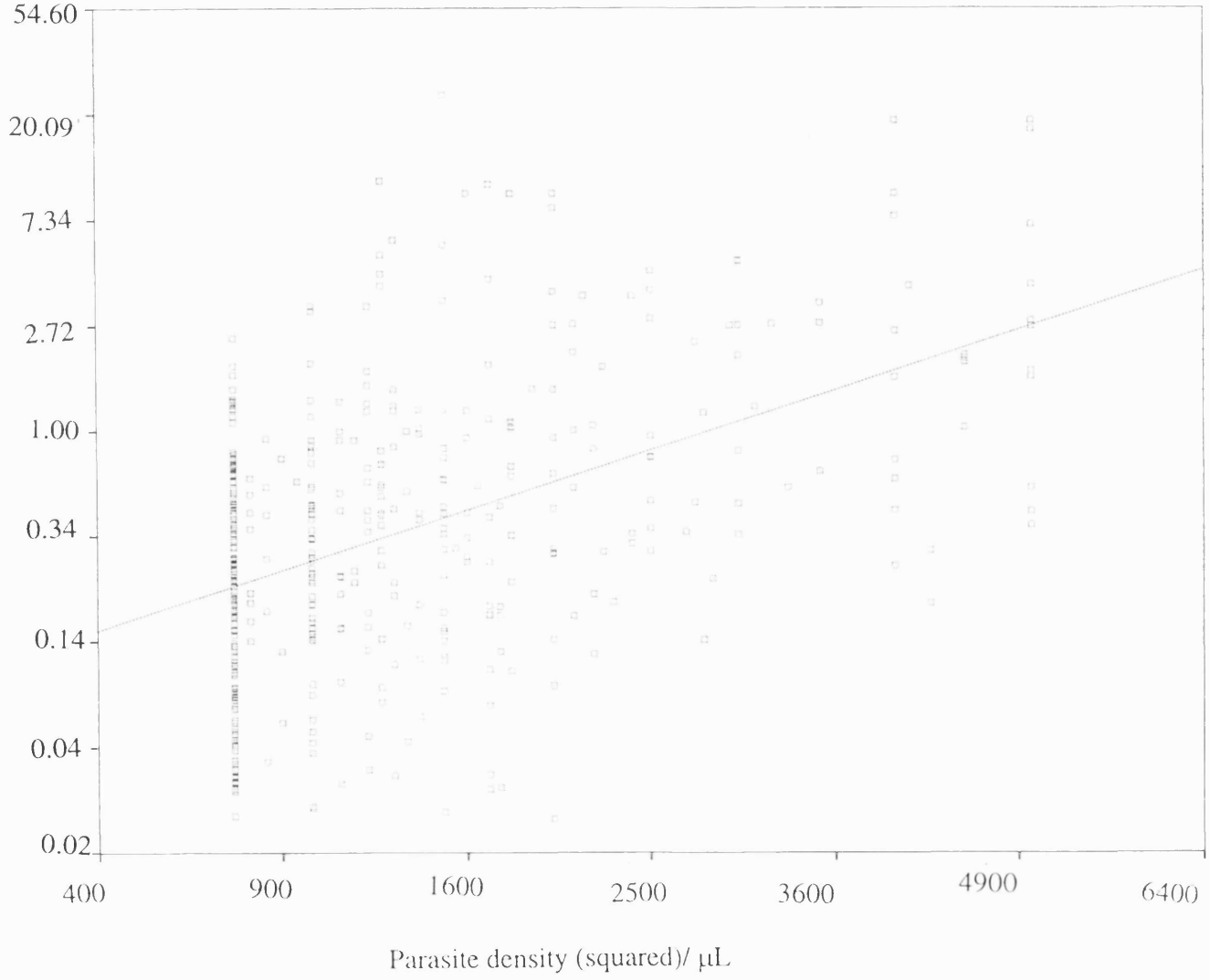
8.1.3. Results of C-reactive protein assay:

We hypothesized that cytokines would affect cognition in a direct but a transient way. Since cytokines have a very short half-life and are difficult to measure we used CRP as a proxy. We found that CRP concentration were relatively low among our sample. The median and the 75th percentile were 0.38 and 0.87 mg/L respectively. These concentrations are similar to those found in neighboring Ethiopia where CRP concentrations among schoolchildren were low and the median and the 75th percentile were 0.0 and 0.95 mg/L respectively (Wolde-Gebriel *et al.* 1993). However, these concentrations are much lower than those found in Gambia among asymptomatic parasitaemic under five year old children where 37% were above eight mg/L (McGuire *et al.* 1996). It is also lower than concentrations found in Tanzania among asymptomatic parasitaemic children under 15 years where the median was 3.3 mg/L (Hurt *et al.* 1994). This may reflect normal variation among different populations or it may reflect different levels of infection and consequent immunolgical response. However, most of these developing countries have similar infection patterns.

We found that CRP concentrations differed significantly between the parasitaemic and non-parasitaemic children. This finding has been reported by many studies (e.g. McGuire *et al.* 1996; Hurt *et al.* 1994; Jakobsen *et al.* 1998). CRP was also positively correlated with parasite density ($r= 0.46$, $n= 434$, $P <0.001$) (fig. 9) and splenomegaly but negatively correlated with haemoglobin ($r= - 0.42$, $n= 559$, $P <0.001$) (fig. 10). Jakobsen and colleagues (1998) found that CRP correlated with parasite density. McGuire and colleagues (1996) found that an elevated CRP was significantly

fig. 9 Correlation between C-reactive protein (CRP)* and parasite density in the natural trial at baseline**

(r= 0.46 , n=434, P< 0.001)

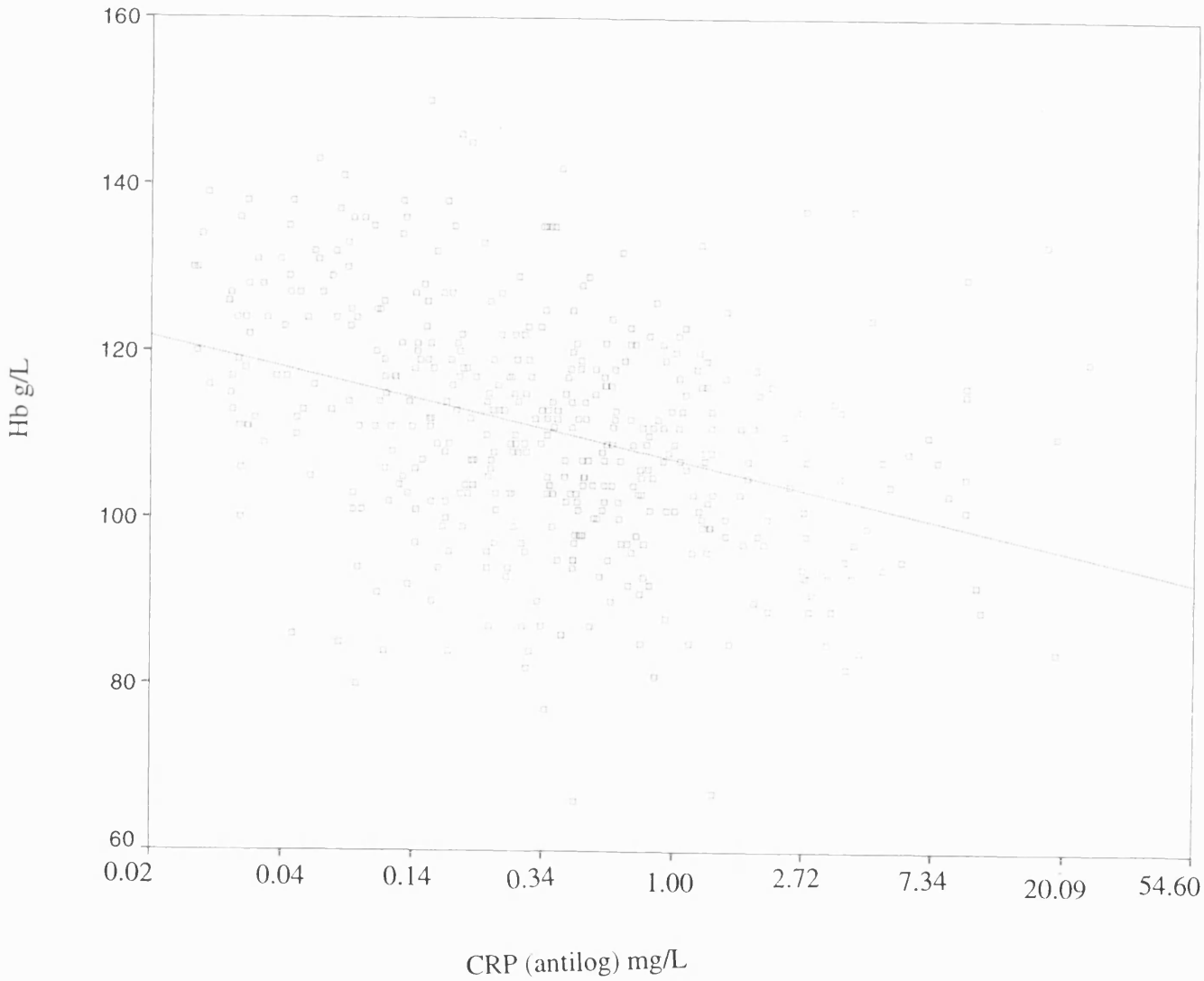


* Log transformation

** Square root transformation

fig. 10. Correlation between haemoglobin (Hb) and C-reactive protein* (CRP) in the natural trial at baseline

(r= - 0.42 , n= 559, P< 0.001)



* Log transformation

associated with the presence of anaemia and splenomegaly. Finally, we found that CRP was negatively correlated with one of the cognitive tests (picture memory), an important finding which may indicate that cognition can be impaired by acute phase response as we hypothesized. This has not been investigated before and merits a further research.

8.2. Discussion of natural experiment:

Cross-sectional studies have limitations because their findings can be confounded by different factors. A more effective method to overcome this problem and to establish a causal relationship is to use the randomised controlled trials in which the two study groups differ only in the treatment they receive. Although the design of the clinical trial was stronger regarding the randomization of treatment and blindness of the subjects and interviewers it was not attainable due to rapid loss of parasitaemia among the placebo group. This loss of parasitaemia in the placebo group severely reduced the power of the study to show differences between the treated and placebo group and it was not surprising that there was no treatment effect. Therefore, we conducted a natural trial where we re-screened those who were parasitaemic at recruitment after two weeks and we matched for grade and school 150 children of those who remained parasitaemic with 150 children who were previously parasitaemic but no longer parasitaemic and we re-tested them using the same battery of cognitive tests. However, this design also has limitations. The premise on which the natural trial was based is that all infected children were similar initially and any difference found at

post-testing was due to the loss of infection in one group. However, it may be that those who became non-parasitaemic differed from those who remained parasitaemic in some of their initial characteristics that may also affect cognition which would have confounded the results.

Nevertheless, comparison of those who remained parasitaemic and those who became non-parasitaemic after two weeks showed they were not different from each other regarding the other known confounders of the study outcomes e.g. age, nutritional status and socioeconomic status. However, there is a possibility that there are some unrecognized confounders which affected cure and cognition.

8.2.1. Cognitive tests scores at follow-up:

A comparison of the test scores between the two groups did not show a difference. The results showed no significant difference in change in cognition from the initial to final tests between those who were cured and those who remained parasitaemic.

8.2.2. Validity of the results at the follow-up:

It is important to be sure that the above mentioned results of the natural experiment are valid. Beside the criteria we have already discussed about the internal validity of the case control study, the following important questions need to be addressed before internal validity can be ensured (Fairchild, Haas, Habicht, 1989):

8.2.2.1. Were the tests used able to detect differences if there were any?

The choice of the tests we used in this study was influenced by two main considerations. First, we had to use simple tests that required little equipment as the testing conditions were difficult and we had to test in different schools. Second, we hypothesized that children with parasitaemia would be less efficient in several cognitive functions (e.g. speed of processing, attention and working memory). Therefore, we used a wide range of tests to cover these functions. These tests have previously been used in similar studies to detect the effect of infections such as the common cold in adults in the UK, and they have been used in children of a similar age in developing countries to detect the effect of geohelminthic infections. In all situations they were sensitive enough to show effect in cross-sectional designs and after treatment trials. So we are confident that our tests were able to detect a difference if there was any.

8.2.2.2. Was the initial classification by study groups correct?

It is important to rule out the effect of misclassification. As I mentioned before because we depended on malaria smears, there was a possibility of misclassification. However, we had no other alternative diagnostic method and the method is widely used and accepted in the malaria field. Nonetheless, we did not depend on one malaria smear and instead we examined two smears 24 hours apart and limited our definition of parasitaemic group to those who had parasite density equal or above 750 parasites per μl. It is therefore unlikely that the infected group was misclassified. However, there is still a possibility

that the non-parasitaemic group may be missclassified due to the low sensitivity of microscopical diagnosis (Snounou, 1993, Bottius *et al.* 1996).

8.2.2.3. Were there any confounders that prevented an existing effect being detected?

We controlled for the initial tests scores, age and testers in the analysis and the children were also matched for school and grade level. The fact that controlling for other known confounders such as nutritional status and SES did not change the results indicates that they were well matched internally and it was unlikely that other variables affect the change in cognition within two weeks once initial score was controlled. One possible explanation for having no effect on cognition was that CRP concentration may still have been high at follow-up in the children who we were parasitaemic at the beginning but non-parasitaemic at the follow-up. We hypothesised that malaria affects cognition transiently through cytokines, and CRP was a proxy for them. It was possible that two weeks was too short for a drop in CRP to occur and hence to detect a change in cognition. However, we showed that this was not the case. CRP concentration among those who were parasitaemic at baseline and became non-parasitaemic at follow-up returned to normal concentrations at the follow-up. At this time they were significantly lower than the concentrations of the comparison group which remained parasitaemic. However, the initial CRP concentrations and initial parasite density of the children who lost their infection were lower than those of the children who remained infected and their Hb concentrations were higher.

This finding is troubling and may have reduced the ability to detect differences attributable to the loss of parasitaemia. Therefore we controlled statistically in the analysis for the initial CRP concentrations, Hb concentrations, and parasite density. We found that those who lost the infection did not benefit in cognitive function.

It may also be that two weeks was a short time and there was some other acute phase response still going on. Another possibility is that those who were non-parasitaemic at follow-up still had a low parasite density which could not be detected by microscopy (Snounou, 1993, Bottius *et al.* 1996).

Nevertheless, we found that the initial parasite density significantly influenced the change in the score and those who were heavily parasitaemic improved the most in both peg board tests, the motor factor and the picture memory test.

8.2.2.4. Was the sample size large enough to provide the required statistical power to detect differences if there were any?

The sample size provided enough power to detect relatively small differences in cognitive tests scored between the two groups. The sample size was calculated to be able to detect a difference of 10-15% in cognitive function tests scores, at 90% power and 5% level of significance. The required sample size was 120 children and we increased our sample size to 150 children to give more power and to allow for any unplanned drop out.

In conclusion, although the tests used were sensitive, the children were reasonably well classified as parasitaemic and non-parasitaemic, and sample size was adequate, it is possible that any ability to detect differences may have been limited. We therefore recommend that other studies with different designs be done to prove or disprove the findings.

8.2.3. Conclusion:

Our ability to detect changes in the natural trial was reduced due to inequalities between the groups in CRP concentrations, parasite density and Hb concentrations on enrollment. In view of persistent association between parasitaemia with fine motor function in both cross-sectional studies (clinical and natural trial) and that initial parasite density predicted change in the scores of both peg boards and motor factor it seems likely that parasitaemia may affect fine motor function. It is therefore important that an alternative approach to examine this problem is pursued in further studies (see Recommendations for further research).

8.2.4. Summary of chapter four: Discussion:

In this chapter:

- we first considered the validity of the results of the case control study and showed that we have a fairly reasonable internal validity in spite of the difficulties we had in defining the non-parasitaemic group. Regarding external validity, although there is some consistency in the findings of both trials, there are obvious limitations and the results need to be replicated in other population.
- the prevalence of malnutrition and anaemia among our sample was compared to the prevalence among schoolchildren from other developing countries.
- the associations found between parasitaemia and nutritional status, anaemia, splenomegaly, and SES was discussed and compared to the findings from other studies. Factors that found to be associated with cognition, school achievement, and absenteeism in this study compared to previous research findings.
- the association that was found in this study between parasitaemia and fine motor functions was discussed in relations to the criteria that should be fulfilled before causality can be ascertained.
- results of CRP assay has been discussed and compared with the findings of previous research.

- we then discussed the validity of the findings of the natural trial. Although, the tests we have used were sensitive enough to be able to detect a difference if there was any and the sample size was large enough to provide the required statistical power to detect differences if there were any, we have strong limitation by relying upon the microscopy on diagnosis of parasitaemia and therefore the non-parasitaemic group may be misclassified.
- finally possible reasons that prevented an existing effect to be detected were identified and the need for more research was stressed.

Chapter Five

Summary and recommendations

9. Summary

The main findings of this study can be summarized as following:

9.1. Summary of the general characteristics of the sample:

Among Yemeni rural primary schoolchildren we found that:

- Malnutrition was highly prevalent and approximately half of the children were found to be stunted or underweight.
- Anaemia was also a major health problem among rural schoolchildren 40 to 55% of whom had Hb below the WHO cut-off value for anaemia.
- Asymptomatic parasitaemia was a common findings in most schools we screened. The prevalence varied from one school to another between 20% to 60%.
- The school achievement level in all grades was poor and many of the children at grade six had scores which are expected in the grade four.

9.2. Summary of the findings from the case-control study:

9.2.1. Parasitaemia:

- Parasitaemia was strongly associated with anaemia.
- Parasitaemia was associated with splenomegaly.
- Parasitaemia was associated with malnutrition.
- Poor socioeconomic status as shown by illiteracy of the father and lower. scores in school items was associated with parasitaemia.

9.2.2. Factors associated with cognition:

- A significant association was found between parasitaemia and fine motor function. This effect was still significant after controlling for age, SES, nutritional status and anaemia.
- Socioeconomic status was associated with some cognitive function test scores where the children from lower SES were far behind their classmates who came from better SES background.
- Nutritional status also affected some of the cognitive function. Malnourished children's performance was worse especially in tests that had an element of fine motor coordination.
- Anaemia was associated with lower performance in some of cognitive tests. The relation was not linear and only children with haemoglobin below 90 g/L showed an affect.
- Cognition was not associated with splenomegaly or reported short-term hunger.

9.2.3. Factors associated with school achievement

- School influenced school achievement to a great extent
- Socioeconomic status was associated with school achievement scores and children from lower SES had lower scores
- Anaemia was associated with lower school achievement scores

9.2.4. Factors associated with absenteeism

- Older children were more likely to be absent from school
- Socioeconomic status was associated with absenteeism and children from lower SES homes had more days absent
- Nutritional status also associated with absenteeism and malnourished children were more likely to be absent

9.2.5. C-reactive protein (CRP)

- CRP concentrations differed significantly between parasitaemic and non-parasitaemic groups
- CRP concentrations was positively correlated with parasite density and splenomegaly while it was negatively correlated with haemoglobin
- CRP concentrations were correlated negatively with one of the cognitive tests (picture memory)

9.3. Findings from the natural trial

- No significant difference was found in change in performance over the study period between those who were naturally cured and those who remained parasitaemic.
- CRP concentrations among parasitaemic who became non-parasitaemic returned back to baseline concentrations in two weeks time.
- Initial parasite density predicted change on peg board scores, motor factor score and picture memory.

10. Recommendations

10.1. General recommendations:

I- This is one of very few studies which were conducted among schoolchildren in Yemen. Considering the above results I would like to make the following recommendations:

- Schoolchildren should be a priority for further research in the Yemen Republic and the government and aid organizations should invest in the health of schoolchildren to enable them to become useful citizens. A better educated population should lead to improved national development. For children to be able to benefit from education they need to be healthy. It has been well known that poor nutrition and health can hinder the children's ability to learn in schools.

- Some other health problems which may be important that we were not able to investigate in this study should be explored in further research e.g. geohelmnthic infections and iodine deficiency. Possible strategies for control of such common health problems should be implemented.

- Due to the high prevalence of anaemia and the detrimental effect on cognition we have shown, we recommend piloting small-scale iron supplementation trials to find the best, culturally acceptable and economically affordable interventions. There is evidence that the prevalence of anaemia shows a marked decline following iron supplementation for schoolchildren. There is also evidence of an improvement in growth and intellectual performance with iron treatment. The fact that weekly iron supplementation has been found to be as efficacious as daily iron supplementation in improving iron status and correcting iron deficiency anemia among schoolchildren is encouraging.

- Due to the high prevalence of malnutrition among schoolchildren we have found and its well-known detrimental effect on cognition together with high prevalence of short-term hunger which has been shown to impair the ability of children to learn at school, we highly recommend piloting small scale school breakfast programs. Learning from experience of other developing countries to find the best way of implementing such programmes on a larger scale is important.

- In general the students had very low levels of school achievement and it is unlikely that improved health and nutrition alone is sufficient to improve the children achievement levels. The condition of the schools with lack of materials, furniture and overcrowding call for immediate action.

- Health education should be an important part of the school curriculum and should focus on the highly prevalent health problems among schoolchildren and the community e.g. malaria, anaemia, malnutrition, parasitic infection etc.. Both WHO and World Bank have found that school health education is one of the most cost-effective investments to improve health (James, Kickbusch, O'Byrne D, 1995).

II- Since this study investigated the unexplored area of impact of asymptomatic parasitaemia on cognitive function of schoolchildren and due to the limitations we discussed above we would like to recommend that more research should be conducted on this topic. Other possible effects of asymptomatic parasitaemia on health e.g. anaemia, acute phase response etc. should also be investigated further and proper control measures should be implemented.

10.2. Recommendations for further research:

Since the case-control study we have conducted showed that parasitaemia was associated with the fine-motor function while the controlled experiment did not show an effect, there is a great need for further research in this virgin research field. The internal validity of the future research could be improved more possibly by using more sensitive tests such as the computerized cognitive tests and by using more sensitive diagnostic tests in defining the selection criteria of the study groups e.g. PCR. However, applicability of such methods should be considered carefully especially at the field setting, in developing countries. The external validity would be improved by randomization to treatment group through a design similar to that we used in the clinical trial. However, the sample size needs to be extremely large to be sure that we would end up with enough children who remain infected among the placebo group.

A more feasible design may be to conduct a chemoprophylaxis trial during the rainy season where the children are randomized within the class to either monthly prophylaxis with Fansidar or placebo and followed up over a larger period of time for their cognition, school achievement, absenteeism, haemoglobin, and nutritional status.

Another possibility is to conduct a prospective study, to examine the impact of becoming parasitaemic among those who are initially non-parasitaemic. This group should be compared with another group who were non-parasitaemic and continues to be non-parasitaemic to control for any practice effect. Also, more research is needed on the relation of acute phase response to cognition, since not enough data is available.

10.3. Summary of chapter five: summary and recommendations:

In this chapter:

- the findings of this study were summarized.
- as this research is one of very few studies which were conducted among schoolchildren in Yemen we recommend that Yemeni schoolchildren should be a priority for further research.
- since we have shown that both anemia and malnutrition were highly prevalent among our sample and had an impact on cognition and school achievement we recommend piloting small-scale interventions for these two problems.
- we recommend that other health problems which we were not be able to investigate in this study need to be investigated in further research.

11. References

Abdalla S, Weatherall DJ, Wickramasinghe SN, Hughes M. The anaemia of *P. falciparum* malaria. *Br J Haematol* 1980; **46**; 171-188.

Abdalla SH. Iron and folate status in Gambian children with malaria. *Ann Trop Paediatr* 1990; **10**: 265-272.

Abdalla A, Badran A, Galal S. Effect of bilharziasis on the mental power and scholastic achievement of school children. *J Egyp Public Health Assoc* 1964; **40**: 135-146.

Achidi EA, Salimonu LS, Asuzu MC, Berzins K, Walker O. Studies on *Plasmodium falciparum* parasitaemia and development of anemia in Nigerian infants during their first year of life. *Am J Trop Med Hyg* 1996; **55**: 138-143.

Adams F, Quesada JR, Gutterman JU. Neuropsychiatric manifestations of human leukocyte interferon therapy in patients with cancer. *JAMA* 1984; **252**: 938-941.

Adelekan DA, Adeodu OO, Thurnham DI. Comparative effects of malaria and malnutrition on plasma concentrations of antioxidant micronutrients in children. *Ann Trop Paediatr* 1997; **17**: 223-227.

Agarwal DK, Upadhyay SK, Tripathi AM, Agarwal KN. Nutritional status, physical work capacity and mental function in school children. Nutrition Foundation of India Scientific report, no. 6, 1987.

Agarwal DK, Upadhyay SK, Agarwal KN, Singh RD, Tripathi AM. Anaemia and mental functions in rural primary school children. *Ann Trop Paediatr* 1989; **9**: 194-198.

Ahmad SH, Moonis R, Shahab T, Khan HM, Jilani T. Effect of nutritional status on total parasite count in malaria. *Indian J Pediatr* 1985; **52**: 285-287.

Akenzua GI, Ihongbe JC, Imasuen IW, Nwobi BC. Anaemia in children: a survey in (Obadan) a rural community in the rain forest zone of Nigeria. *J Trop Pediatr* 1985; **31**: 20-24.

Albonico M, Chwaya HM, Montresor A, Stolzfus RJ, Tielsch JM, Alawi KS, Savioli L. Parasitic infections in Pemba Island school children. *East Afr Med* J 1997; **74**: 294-298.

al-Frayh AS, Bamgboye EA. The growth pattern of Saudi Arabian pre-school children in Riyadh compared to NCHS/CDC reference population. *J R Soc Health* 1993 ; **113**: 234-239.

Al Serouri AW. The first school health survey in Aslam district. School Health Programme, International Cooperation for Development, Yemen. 1995.

Alleyne GAO. Preface. In: Grantham-McGregor SM (Ed). Nutrition, health, and child development. Research advances and policy recommendations. Pan American Health Organisation Scientific Publications, Washington DC, 1998.

Armitage P, Berry G. Statistical Methods in Medical Research. Third ed. Oxford: Blackwell Scientific publications,. 1994.

Arya A, Devi R. Influence of maternal literacy on the nutritional status of preschool children. *Indian J Pediatr* 1991; **58**: 265-268.

Arthur P, Kirkwood B, Ross D, Morris S, Gyapong J, Tomkins A, Addy H. Impact of vitamin A supplementation on childhood morbidity in northern Ghana [letter]. *Lancet* 1992; **339**: 361-362.

Azubuike JC, Izuora GI, Obi GO. Anaemia in non-sickling Nigerian children around Enugu. *Trop Geogr Med* 1977; **29**: 365-368.

Aukett MA, Parks YA, Scott PH, Wharton BA. Treatment with iron increases weight gain and psychomotor development. *Arch Dis Child* 1986, **61**: 849-857.

Baddeley A. Cognitive function and whipworm infection. *Parasitol Today* 1992; **8**: 394-395.

Baddeley, Gardner JM, Grantham-McGregor S. Cross-cultural cognition: Developing tests for developing countries. *Applied Cognitive Psychology* 1995; **9**: S173-S195.

Banks RE, Forbes MA, Storr M, Higginson J, Thompson D, Raynes J, Illingworth JM, Perren TJ, Selby PJ, Whicher JT. The acute phase protein response in patients receiving subcutaneous IL-6. *Clin Exp Immunol* 1995; **102**: 217-223.

Bartel P, Griesel R, Burnett L, Freiman I, Rose E, Geefhysen J. Long-term effects of kwashiorkor on psychomotor development. *S Afr Med J* 1978; **35**: 360-362.

Bates CJ, Evans PH, Dardenne M, Prentice A, Lunn PG, Northrop Clewes CA, Hoare S, Cole TJ, Horan SJ, Longman SC, *et al.* A trial of zinc supplementation in young rural Gambian children. *Br J Nutr* 1993; **69**: 243-255.

Bayley N. Bayley Scales of infant development. New York. Psychological corporation, 1969.

Beales PF. Anaemia in malaria control: a practical approach. *Ann Trop Med Parasitol* 1997; **91**: 713-718.

Bell RM, Daly J, Kanengoni E, Jones JJ. The effects of endemic schistosomiasis and of hycanthone on the mental ability of African school children. *Trans R Soc Trop Med Hyg* 1973; **67**: 694-701.

Binda-ki-Muaka P, Nzita M, Eeckels R. Malaria, anaemia, and HIV 1 transmission in central Africa [letter]. *Lancet* 1995; **346**: 1294-1295.

Birch HG, Pineiro C, Alcalde E, Toca T, Cravioto J. Relation of kwashiorkor in early childhood and intelligence at school age. *Pedatric Research* 1971; **5**: 579-585.

Black MM. Zinc deficiency and child development. *Am J Clin Nutr* 1998; **68** (Suppl): 464S-469S.

Bloland PB, Lackritz EM, Kazembe PN, Were JB, Steketee R, Campbell CC. Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *J Infect Dis* 1993; **167**: 932-937.

Boivin MJ, Giordani B, Ndanga K, Maky MM, Manzeki KM, Ngunu N, Muamba K. Effects of treatment for intestinal parasites and malaria on the cognitive abilities of schoolchildren in Zaire, Africa. *Health Psychol* 1993; **12**: 220-226.

Bondi FS. The incidence and outcome of neurological abnormalities in childhood cerebral malaria: a long-term follow-up of 62 survivors. *Trans R Soc Trop Med Hyg* 1992; **86**: 17-19.

Bottius E, Guanzirolli A, Trape JF, Rogier C, Konate L, Druilhe P. Malaria: even more chronic in nature than previously thought; evidence for subpatent parasitaemia detectable by the polymerase chain reaction. *Trans R Soc Trop Med Hyg* 1996; **90**: 15-19.

Bouvier P, Rougemont A, Breslow N, Doumbo O, Delley V, Dicko A, Diakite M, Mauris A, Robert CF. Seasonality and malaria in a west African village: does high parasite density predict fever incidence? *Am J Epidemiol* 1997; **145**: 850-857.

Brabin BJ. The role of malaria in nutritional anemias. In: Fomon SJ, Zlotkin S. nutritional anemias. *Nestle Nutrition Workshop series 1992*; **30**: 65-80.

Bradley-Moore AM, Greenwood BM, Bradley AK, Akintunde A, Attai ED, Fleming AF, Flynn FV, Kirkwood BR, Gilles HM. Malaria chemoprophylaxis with chloroquine in young Nigerian children. IV. Its effect on haematological measurements. *Ann Trop Med Parasitol* 1985a; **79**: 585-595.

Bradley-Moore AM, Greenwood BM, Bradley AK, Kirkwood BR, Gilles HM Malaria chemoprophylaxis with chloroquine in young Nigerian children. III. Its effect on nutrition. *Ann Trop Med Parasitol* 1985b; **79**: 575-584.

Bredan AS, Kumar NS, Bshiwah SM. Hematologic values and prevalence of anaemia in Libyan school children. *Trop Geogr Med* 1983; **35**: 357-361.

Brewster DR, Kwiatkowski D, White NJ. Neurological sequelae of cerebral malaria in children. *Lancet* 1990; **336**: 1039-1043.

Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. *Lancet* 1996; **348**: 992-996.

Bruce MC, Walmsley M, Packer MJ, Lagog M, Gibson N, Alpers M, Day KP, Walliker D. Dynamics of *Plasmodium falciparum* genotypes within individuals in Papua New Guinea. *Trans R Soc Trop Med Hyg* 1996; **90**: 3-4.

Bundy DAP, Cooper ES. Trichuris and trichuriasis in humans. *Adv Parasitol* 1989; **28**: 107-173.

Bundy DAP. Immunoepidemiology of intestinal helminthic infections. 1. The global burden of intestinal nematode disease. *Trans R Soc Trop Med Hyg* 1994; **88**: 259-261.

Butraporn P, Sornmani S, Hungsapruek T. Social, behavioural, housing factors and their interactive effects associated with malaria occurrence in east Thailand. *Southeast Asian J Trop Med Public Health* 1986; **17**: 386-392.

Callender JE, Grantham-McGregor S, Walker S, Cooper ES. *Trichuris* infection and mental development in children. *Lancet* 1992; **339**: 181.

Callender JE, Grantham-McGregor SM, Walker SP, Cooper ES. Treatment effects in *Trichuris* dysentery syndrome. *Acta Paediatr* 1994; **83**: 1182-1187.

Castle WM, Clarke VDV, Hendrikz E. The effects of subclinical bilharziasis on mental ability in schoolchildren. *S Afr Med J* 1974; **48**: 2035-2038.

CDC. Atlanta. Epi Info. version 6. 1994.

Central Statistical Organization. The Yemen Demographic Maternal and Child Health Survey. 1992.

Central Statistical Organization. Final results of 1994 census. The Yemen Republic. 1994.

Champakam S, Srikantia SG, Gopalan C. Kwashiorkor and mental development. *Am J Clin Nutr* 1968; **21**: 844-852.

Chandler AM, Walker SP, Connolly K, Grantham-McGregor SM. School breakfast improves verbal fluency in undernourished Jamaican children. *J Nur* 1995; **125**: 894-900.

Chavez A, Martinez C. Neurological maturation and performance on mental tests. Growing up in a developing community. INCAP, Guatemala City. 1982.

Chavez A, Martinez C, Soberanes B, Dominguez L, Avila A. Early nutrition and physical and mental development in Mexican rural adolescent females. Nutrition of adolescent girls research Program, research report Series No. 4, Washington, DC: International Center for Research on Women. 1994.

Chizzolini C, Trottein F, Bernard FX, Kaufmann MH. Isotypic analysis, antigen specificity, and inhibitory function of maternally transmitted *Plasmodium falciparum* specific antibodies in Gabonese newborns. *Am J Trop Med Hyg* 1991; **45**: 57-64.

Clark, Grantham-McGregor, Powell. Health and nutrition predictors of school failure in Kingston, Jamaica. *Ecol Food Nutr* 1991; **26**: 47-57.

Clarke V-de D, Blair DM. The prevalence of bilharziasis in European schoolboys at Salisbury, Rhodesia. *Cent Afr J Med* 1966; **12**: 25-28.

Colbourne MJ. The effect of malaria suppression in a group of Accra school children. *Trans R Soc Trop Med Hyg* 1955; **49**: 356-369.

Connolly KJ, Kvalsvig JD. Infection, nutrition and cognitive performance in children. *Parasitology* 1993; **107** (Suppl): S187-S200.

Cornet M, Le-Hesran JY, Fievet N, Cot M, Personne P, Gounoue R, Beyeme M, Deloron P. Prevalence of and risk factors for anaemia in young children in southern Cameroon. *Am J Trop Med Hyg* 1998; **58**: 606-611.

Cot M, le-Hesran JY, Miailhes P, Roisin A, Fievet N, Barro D, Etya'Ale D, Deloron P, Carnevale P, Breart G. Effect of chloroquine prophylaxis during pregnancy on maternal haematocrit. *Ann Trop Med Parasitol* 1998; **92**: 37-43.

Cravioto J, DeLicardie E, Birch H. Nutrition, growth and neuro-integrative development: an experimental and ecological study. *Pediatrics* 1966; **38**: 319-372.

Crawley J, Smith S, Kirkham F, Muthinji P, Waruiru C, Marsh K. Seizures and status epilepticus in childhood cerebral malaria. *QJM* 1996; **89**: 591-597.

D'Alessandro U, Olaleye BO, McGuire W, Langerock P, Bennett S, Aikins MK, Thomson MC, Cham MK, Cham BA, Greenwood BM. Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bed net programme. *Lancet* 1995; **345**: 479-483.

Dallman PR, Yip R, Oski FA. Iron deficiency and related nutritional anaemias. In: Nathan DG, Oski FA (Ed.). Hematology of infancy and childhood. Fourth ed., W.B. Saunders Company, 1993.

Daubersies P, Sallenave Sales S, Magne S, Trape JF, Contamin H, Fandeur T, Rogier C, Mercereau Puijalon O, Druilhe P. Rapid turnover of *Plasmodium falciparum* populations in asymptomatic individuals living in a high transmission area. *Am J Trop Med Hyg* 1996; **54**: 18-126.

de-Andraca I, Walter T, Castillo M, Pino P, Rivera P, Cobo C. Iron Deficiency anemia and its effects upon psychological development at preschool age: a longitudinal study. *Nestle Foundation Nutrition Annual report* 1990; 53-62.

DeMaeyer EM. Prevalence and controlling iron deficiency anaemia through primary health care. World Health Organization, 1989.

Demetri GD, Spriggs DR, Sherman ML, Arthur KA, Imamura K, Kufe DW. A phase I trial of recombinant human tumor necrosis factor and interferon gamma: effects of combination cytokine administration *in vivo*. *J Clin Oncol* 1989; **7**: 1545-1553.

De Regt M. Nutritional habits and child feeding practices among Hodeida's poor. Hodeida standards of living survey. HUPHCP, 1994.

Dolan G, ter-Kuile FO, Jacoutot V, White NJ, Luxemburger C, Malankirii L, Chongsuphajaisiddhi T, Nosten F. Bed nets for the prevention of malaria and anaemia in pregnancy. *Trans R Soc Trop Med Hyg*. 1993; **87**: 620-626.

Dowling MAC, Shute GT. The value of routine thick film examination in the diagnosis of scanty parasitaemia in adult, semi-immune African. WHO/MAL/485-65.

Draper CC. Effect of malaria control on haemoglobin levels. *BMJ* 1960; **1**: 1480-1483.

Draper KC, Draper CC. Observation on growth of African infants with special reference to the effect of malaria control. *J Trop Med Hyg* 1960; **63**: 165-171.

Dugbartey AT, Spellacy FJ. Simple reaction time and cognitive information processing efficiency after cerebral malaria in Ghanaian children. *Neurol Infect Epidemiol* 1997; **2**: 141-144.

Dugbartey AT, Dugbartey MT, Apedo MY. Delayed neuropsychiatric effects of malaria in Ghana. *J Nerv Ment Dis* 1998; **186**: 183-186.

Dugbartey AT, Spellacy FJ, Dugbartey MT. Somatosensory discrimination deficits following pediatric cerebral malaria. *Am J Trop Med Hyg* 1998; **59**: 393-395.

Editorial. Malaria and anaemia. *Rev Soc Bras Med Trop* 1991; **24**: 131-136.

Ekanem EE, Asindi AA, Ejezie GC, Antia Obong OE. Effect of *Schistosoma haematobium* infection on the physical growth and school performance of Nigerian children. *Cent Afr J Med* 1994; **40**: 38-44.

el-Qirbi AA, Sadek MS, Ghaly-MY. A preliminary survey for intestinal and blood parasites among school children at Sana'a Governorate, Yemen. *J Egypt Soc Parasitol* 1987; **17**: 495-501.

El Samani FZ, Willett WC, Ware JH . Nutritional and sociodemographic risk indicators of malaria in children under five: a crosssectional study in a Sudanese rural community. *J Trop Med Hyg* 1987; 90: 69-78

Evans DE, Moodie AD, Hansen JD. Kwashiorkor and intellectual development. *S Afr Med J* 1971; **45**: 1413-1426.

Evans D, Hansen JD, Moodie AD, van-der-Spuy HI. Intellectual development and nutrition. *J Pediatr* 1980; **97**: 358-363.

Eysenck. MW. A handbook of cognitive psychology. A. Wheaton & Co. Ltd., Exeter. Third ed. 1988.

Faich GA, Mason J. The prevalence and relationships of malaria, anemia, and malnutrition in a coastal area of El Salvador *Am J Trop Med Hyg* 1975; **24**: 161-167.

Fairchild MW, Haas JD, Habicht JP. Iron deficiency and behavior: criteria for testing causality. *Am J Clin Nutr* 1989; **50**(Suppl): 566-574.

Farnert A, Snounou G, Rooth I, Bjorkman A. Daily dynamics of *Plasmodium falciparum* subpopulations in asymptomatic children in a holoendemic area. *Am J Trop Med Hyg* 1997; **56**: 538-547.

Fasan PO. Malaria in the school children of Lagos city and Lagos state. *West Afr Med J* 1969; **18**:176-180.

Filteau SM, Morris SS, Abbott RA, Tomkins AM, Kirkwood BR, Arthur P, Ross DA, Gyapong JO, Raynes JG. Influence of morbidity on serum retinol of children in a community based study in northern Ghana. *Am J Clin Nutr* 1993; **58**: 192-197.

Filteau SM, Tomkins AM. Micronutrients and tropical infections. *Trans R Soc Trop Med Hyg* 1994; **88**: 1-3.

Fleming AF, Werblinska B. Anaemia in childhood in the guinea savanna of Nigeria. *Ann Trop Paediatr* 1982; **2**: 161-173.

Florencio C. Nutrition, health and other determinants of academic achievement and school-related behavior of grades one to six pupils. Quezon City, Philippines: University of Philippines, 1988.

Freeman E, Klein RE, Townsend JW, Lechtig A. Nutrition and cognitive development among rural Guatemalan children. *Am J of Pub Health* 1980; **70**: 1277-1285.

Friis H, Mwaniki D, Omondi B, Muniu E, Magnussen P, Geissler W, Thiong'o F, Michaelsen KF. Serum retinol concentrations and Schistosoma mansoni, intestinal helminths, and malarial parasitemia: a cross-sectional study in Kenyan preschool and primary school children. *Am J Clin Nutr* 1997; **66**: 665-671.

Frood JD, Whitehead RG, Coward WA. Relationship between pattern of infection and development of hypoalbuminaemia and hypo-beta-lipoproteinaemia in rural Ugandan children. *Lancet* 1971; **2**: 1047-1049.

Fungladda W, Sornmani S, Klongkamnuankarn K, Hungsapruek T. Sociodemographic and behavioural factors associated with hospital malaria patients in Kanchanaburi, Thailand. *J Trop Med Hyg* 1987; **90**: 233-237.

Galler JR, Ramsey F, Solimano G, Lowell WE, Mason E. The influence of early malnutrition on subsequent behavioral development. I. Degree of impairment in intellectual performance. *J Am Acad Child Psychiatry* 1983; **22**: 8-15.

Galler JR, Ramsey F, Solimano G, Kucharski LT, Harrison R. The influence of early malnutrition on subsequent behavioral development. IV. Soft neurologic signs. *Pediatr Res.* 1984; **18**: 826-832.

Gardner JM, Grantham-McGregor S, Baddeley A. *Trichuris trichiuria* infection and cognitive function in Jamaican school children. *Ann Trop Med Parasitol* 1996; **90**: 55-63.

Genton B, al Yaman F, Beck HP, Hii J, Mellor S, Narara A, Gibson N, Smith T, Alpers MP. The epidemiology of malaria in the Wosera area, East Sepik Province, Papua New Guinea, in preparation for vaccine trials. I. Malariometric indices and immunity. *Ann Trop Med Parasitol* 1995; **89**: 359-376.

Gibson RS, Heywood A, Yaman C, Sohlstrom A, Thompson LU, Heywood P. Growth in children from the Wosera subdistrict, Papua New Guinea, in relation to energy and protein intakes and zinc status *Am J Clin Nutr* 1991; **53**: 782-789.

Gibson RS, Huddle JM. Suboptimal zinc status in pregnant Malawian women: its association with low intakes of poorly available zinc, frequent reproductive cycling, and malaria. *Am J Clin Nutr* 1998; **67**: 702-709.

Gillespie SH, Dow C, Raynes JG, Behrens RH, Chiodini PL, McAdam KP. Measurement of acute phase proteins for assessing severity of *Plasmodium falciparum* malaria. *J Clin Pathol* 1991; **44**: 228-231.

Gordon RM. The effect of *Ancylostome, Ascaris and Trichuris* infections on the health of the West African native. *Ann Trop Med Parasitol* 1925; **19**:429-460.

Gorman KS. Malnutrition and cognitive development: evidence from experimental/ quasi-experimental studies among the mild-to-moderately malnourished. *J Nutr* 1995; **125** (Suppl): 2239S-2244S.

Graham GG, Adrianzen BT. Status in school of Peruvian children severely malnourished in infancy. In: Brooke J. (Ed.). Behavioral effects of energy and protein deficits. DHEW (NIH) Publication no. 79-1906. 1979; pp. 185-194.

Granham, PCC. Malaria Parasites and other *Haemosporidia*. Oxford: Blackwell,1966.

Graninger W, Thalhammer F, Hollenstein U, Zotter GM, Kremsner PG. Serum protein concentrations in *Plasmodium falciparum* malaria. *Acta Trop* 1992; **52**: 121-128.

Grantham-McGregor SM. Field studies in early nutrition and later achievement. In: Dobbing J. (Ed). Early nutrition and later achievement. Academic Press, London. 1987. pp. 128-174.

Grantham-McGregor SM, Powell CA, Walker SP, Himes JH. Nutritional supplementation, psychological stimulation and mental development of stunted children: the Jamaican study. *Lancet* 1991; **338**: 1-5.

Grantham-McGregor SM. Assessments of the effects of nutrition on mental development and behavior in Jamaican studies. *Am J Clin Nutr* 1993; **57** (Suppl): 303S-309S.

Grantham-McGregor SM. Review of studies of the effect of severe malnutrition on mental development. *J Nutr* 1995; **125** (Suppl): 2233S-2238S.

Grantham-McGregor SM, Walker SP, Himes JH, Powell CA. Stunting and mental development in children. *Nutr Res* 1996; **16**: 1821-1828.

Grantham-McGregor SM, Walker SP, Chang SM, Powell CA. Effect of early childhood supplementation with and without stimulation on later development in stunted Jamaican children. *Am J Clin Nutr* 1997; **66**: 247-253.

Grantham-McGregor SM, Walker S. Health and nutrition determinants of school failure. In: Grantham-McGregor SM (Ed.). Nutrition, health, and child development. Research advances and policy recommendations. Pan American Health Organisation Scientific Publications, Washington DC, 1998.

Grau GE, Taylor TE, Molyneux ME, Wirima JJ, Vassalli P, Hommel M, Lambert PH. Tumor necrosis factor and disease severity in children with falciparum malaria. *N Engl J Med* 1989; **320**: 1586-1591.

Gravenor MB, McLean AR, Kwiatkowski D. The regulation of malaria parasitaemia: parameter estimates for a population model. *Parasitology* 1995; **110**: 115-122.

Greenland S. Concept of validity in epidemiological research. In: Detels R, Holland WW, McEwen J, Omenn GS. (Ed.). Oxford Textxtbook of Public Health. Volume 2: The methods of Public Health. Oxford Universty Press. Third edition, 1997; pp. 597-615.

Greenwood BM. Asymptomatic malaria infections - do they matter? *Parasitol Today* 1987a; **3**: 206-214.

Greenwood BM, Bradley AK, Greenwood AM, Byass P, Jammeh K, Marsh K, Tulloch S, Oldfield FS, Hayes R. Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. *Trans R Soc Trop Med Hyg* 1987b; **81**: 478-486.

Greenwood BM, Greenwood AM, Bradley AK, Snow RW, Byass P, Hayes RJ, N'Jie AB. Comparison of two strategies for control of malaria within a primary health care programme in the Gambia. *Lancet* 1988; **1**: 1121-1127.

Greenwood BM, Greenwood AM, Snow RW, Byass P, Bennett S, Hatib-N'Jie AB. The effects of malaria chemoprophylaxis given by traditional birth attendants on the course and outcome of pregnancy. *Trans R Soc Trop Med Hyg* 1989; **83**: 589-594.

Greenwood BM, Armstrong JR. Comparison of two simple methods for determining malaria parasite density. *Trans R Soc Trop Med Hyg* 1991; **85**: 186-188.

Greenwood M, Marsh K, Snow R. Why do some African children develop severe malaria? *Parasitol Today* 1991; **7**: 276-281.

Grooved peg board test. Lafayette instruments company. Indiana, 1989.

Haas JD, Fairchild MW. Summary and conclusion of the International Conference on Iron Deficiency and Behavioral Development, October 10-12, 1988. *Am J Clin Nutr* 1989, **50**: 703-705.

Hackett LW.: Spleen measurement in malaria. *J Nat Malaria Soc* 1944; **19**: 121-133.

Hagos B, Khan B, Ofulla AVO, Kariuki D, Martin SK. Resopnse of falciparum malaria to choloroquine and three second line antimalarial drugs in a Kenyan coastal school age population. *East Afr Med J* 1993; **70**: 620 - 623.

Hall S, Smith A. Investigation of the effects and aftereffects of naturally occurring upper respiratory tract illnesses on mood and performance. *Physiol Behav* 1996a; **59:** 569-577.

Hall SR, Smith AP. Behavioural effects of infectious mononucleosis. *Neuropsycho-biology* 1996b; **33**: 202-209.

Halloran ME, Bundy DAP, Pollitt E. Infectious Disease and the UNESCO Basic Education Initiative. *Parasitol Today* 1989; **5**: 359-362.

Haithami SS, Gabal MS, Masher AA, el Nofely A. Anthropometric assessment of nutritional status and growth of children in Democratic Yemen. *J Egypt Public Health Assoc* 1989; **64**: 431-444.

Harpaz R, Edelman R, Wasserman SS, Levine MM, Davis JR, Sztein MB. Serum cytokine profiles in experimental human malaria. Relationship to protection and disease course after challenge. *J Clin Invest* 1992; **90**: 515-523.

Hazza YA, Arfaa F, Haggar M. Studies on schistosomiasis in Taiz Province, Yemen Arab Republic. *Am J Trop Med Hyg* 1983; **32**: 1023-1028.

Hedberg K, Shaffer N, Davachi F, Hightower A, Lyamba B, Paluku KM, Nguyen Dinh P, Breman JG. *Plasmodium falciparum* associated anemia in children at a large urban hospital in Zaire. *Am J Trop Med Hyg* 1993; **48**: 365-371.

Hendrickse RG, Hasan AH, Olumide LO, Akinkunmi A. Malaria in early childhood. An investigation of five hundred seriously ill children in whom a "clinical" diagnosis of malaria was made on admission to the children's emergency room at University College Hospital, Ibadan. *Ann Trop Med Parasitol* 1971; **65**: 1-20.

Hertzig ME, Birch HG, Richardson SA, Tizard J. Intellectual levels of school children severely malnourished during the first two years of life. *Pediatrics* 1972; **49**: 814-824.

Hill B. The environment and disease: association or causation? *Proc Roy Soc Med* 1965; **58**: 295-300.

Hogh B, Thompson R, Hetzel C, Fleck SL, Kruse NA, Jones I, Dgedge M, Barreto J, Sinden RE. Specific and nonspecific responses to *Plasmodium falciparum* blood-stage parasites and observations on the gametocytemia in schoolchildren living in a malaria-endemic area of Mozambique. *Am J Trop Med Hyg* 1995; **52**: 50-59.

Honigsberger L, Fielding JW, Priestman TJ. Neurological effects of recombinant human interferon. *BMJ* 1983; **286**: 719.

Hoorweg J, Stanfield J. The effect of protein energy malnutrition in early childhood on intellectual; and motor abilities in later childhood and adolescence. *Dev Med Child Neurol* 1976; **18**: 330-350.

Huda SN. Iodine, nutrition, cognition & school development of Bangladeshi school children. Ph.D. thesis. Centre of International Child Health. 1998.

Hudson-Thomas M, Bingham KC, Simmons WK. An evaluation of the HemoCue for measuring haemoglobin in field studies in Jamaica. *Bull World Health Organ* 1994; **72**: 423-426

Hurt N, Smith T, Tanner M, Mwankusye S, Bordmann G, Weiss NA, Teuscher T. Evaluation of C-reactive protein and haptoglobin as malaria episode markers in an area of high transmission in Africa. *Trans R Soc Trop Med Hyg* 1994; **88**: 182-186.

Hurtado EK, Claussen AH, Scott KG. Early childhood anemia and mild or moderate mental retardation. *Am J Clin Nutr* 1999; **69**: 115-119.

Husaini MA, Karyadi L, Husaini YK, Sandjaja, Karyadi D, Pollitt E. Developmental effects of short-term supplementary feeding in nutritionally-at-risk Indonesian infants. *Am J Clin Nutr* 1991; **54**; 799-804.

Hutchinson SE, Powell CA, Walker SP, Chang SM, Grantham-McGregor SM. Nutrition, anaemia, geohelminth infection and school achievement in rural Jamaican primary school children. *Eur J Clin Nutr* 1997; **51**: 729-735.

Jakobsen PH, Bate CA, Taverne J, Playfair JH. Malaria: toxins, cytokines and disease. *Parasite Immunol* 1995; **17**: 223-231.

Jakobsen PH, McKay V, N'Jie R, Olaleye BO, D'Alessandro U, Zhang GH, Eggelte TA, Koch C, Greenwood BM. Decreased antitoxic activities among children with clinical episodes of malaria. *Infect Immun* 1998; **66**: 1654-1659.

James J, Kickbusch I, O'Byrne D. Improving health through schools. *World Health* 1995; **48**: 10-11.

Jasak J, Bijou S. Wide Range achievement Test. Delaware: CL Story Co. Wilmington, 1946.

Johnston FE, Low SM, de Baessa Y, MacVean RB. Interaction of nutritional and socioeconomic status as determinants of cognitive development in disadvantaged urban Guatemalan children. *Am J Phys Anthropol* 1987; **73**: 501-506.

Joos SK, Pollitt E, Mueller WH, Albright DL. The Bacon Chow study: maternal nutritional supplementation and infant behavioral development. *Child Dev* 1983; **54**: 669-676.

Jordan P, Randall K. Bilharziasis in Tanganyika: observations on its effects and the effects of treatment in schoolchildren. *J Trop Med Hyg* 1962; **55**:1-6.

Kandiah M, Lee M, Ng TK, Chong YH. Malnutrition in malaria endemic villages of Bengkoka Peninsula, Sabah. *J Trop Pediatr* 1984; **30**: 23-29.

Karrar AO. A situation analysis of micronutrients deficiencies (Vit A, iodine, iron) in MENA countries. Regional Overview 1. UNICEF/MENA; 1993.

Kaufman AS. Intelligent testing with the WISC-R. John Wiley & Sons, Inc. 1979.

Kendall M, Stuart A, Ord JK. The advanced theory of statistics. Volume 3, Design and analysis, and time-series, fourth edition. Charles Griffin & Company Limited. 1979.

Kimura E, Moji K, Uga S, Kiliku FM, Migwi DK, Mutua WR, Muhoho ND, Aoki Y. Effects of Schistosoma haematobium infection on mental test scores of Kenyan school children. *Trop Med Parasitol* 1992; **43**: 155-158.

Kirkwood BR. Geometric means and measures of dispersion. *Biometrics* 1979; 35: 908-909.

Kirkwood BR. Essentials of medical statistics. Blackweel Scientific Publications. 1991.

Kitua AY, Smith TA, Alonso PL, Urassa H, Masanja H, Kimario J, Tanner M. The role of low level *Plasmodium falciparum* parasitaemia in anaemia among infants living in an area of intense and perennial transmission. *Trop Med Int Health* 1997; **2**: 325- 333.

Kopecky K, Giboda M, Aldova E, Dobahi SS, Radkovsky J. Pilot studies on the occurrence of some infectious diseases in two different areas in south Yemen (Aden). Part I. Parasitology. *J Hyg Epidemiol Microbiol Immunol* 1992; **36**: 253-261.

Koram KA, Bennett S, Adiamah JH, Greenwood BM. Socio-economic determinants are not major risk factors for severe malaria in Gambian children *Trans R Soc Trop Med Hyg* 1995; **89**: 151-154.

Kurtzhals JA, Rodrigues O, Addae M, Commey JO, Nkrumah FK, Hviid L. Reversible suppression of bone marrow response to erythropoietin in *Plasmodium falciparum* malaria. *Br J Haematol* 1997; **97**: 169-174.

Kvalsvig JD. The effects of schistosomiasis on spontaneous play activity in black schoolchildren in the endemic areas. An ethological study. *S Afr Med J* 1981; **60**: 61-64.

Kvalsvig JD, Cooppan RM, Connolly KJ. The effects of parasite infections on cognitive processes in children. *Ann Trop Med Parasitol* 1991; **85**: 551-568.

Kwiatkowski D, Cannon JG, Manogue KR, Cerami A, Dinarello CA, Greenwood BM. Tumour necrosis factor production in Falciparum malaria and its association with schizont rupture. *Clin Exp Immunol* 1989; **77**: 361-366.

Lasky RE, Klein RE, Yarbrough C, Engle PL, Lechtig A, Martorell R. The relationship between physical growth and infant behavioral development in rural Guatemala. *Child Dev* 1981; **52**: 219-226.

Lewis M, Sullivan MW. Infant intelligence and its assessment. In: Wolman BB (Ed.). Handbook of intelligence. New York: Wiley-Interscience. 1985; pp. 505-599.

Licinio J, Kling MA, Hauser P. Cytokines and brain function: relevance to interferon-alpha-induced mood and cognitive changes. *Semin Oncol* 1998; **25** (Suppl): 30-38.

Lockheed ME, Verspoor AM. Improving primary education in developing countries. Oxford: Oxford University Press. 1991.

Lohman TG, Roche AF, Martorell R. Anthropometric standardization Reference Manual. Human Kinetics, Champaign, IL. 1989.

Lopez I, de-Andraca I, Perales CG, Heresi E, Castillo M, Colombo M. Breakfast omission and cognitive performance of normal, wasted and stunted schoolchildren. *Eur J Clin Nutr* 1993; **47**: 533-542.

Loveridge FG, Ross WF, Blair DM. Schistosomiasis : the effect of the disease on educational attainment. *S Afr Med J 1948*; **22**: 260-263.

Lozoff B, Brittenham GM, Viteri FE, Wolf AW, Urrutia JJ. The effects of short-term oral iron therapy on developmental deficits in iron-deficient anemic infants. *J Pediatr* 1982; **100**: 351-357.

Lozoff B, Brittenham GM, Wolf AW, McClish DK, Kuhnert PM, Jimenez E, Jimenez R, Mora LA, Gomez I, Krauskoph D. Iron deficiency anemia and iron therapy effects on infant developmental test performance. *Pediatrics* 1987; **79**: 981-995.

Lozoff B, Jimenez E, Wolf AW. Long term developmental outcome of infant with iron deficiency. *N Engl J Med* 1991; **325**: 687-694.

Lozoff B, Wolf AW, Jimenez E. Iron deficiency anemia and infant development: effects of extended oral iron therapy. *J Pediatr* 1996; **129**: 382-389.

Lozoff B. Explanatory mechansims for poorer development in iron-deficient anaemic infants. In: Grantham-McGregor SM (Ed.). Nutrition, health, and child development. Research advances and policy recommendations. Pan American Health Organisation Scientific Publications, Washington DC, 1998.

Lucas AO, Hendrickse RG, Okubadejo OA, Richards WH, Neal RA, Kofie BA. The suppression of malarial parasitaemia by pyrimethamine in combination with dapsone or sulphormethoxine. *Trans R Soc Trop Med Hyg* 1969; **63**: 216-229.

Luxemburger C, Nosten F, Kyle DE, Kiricharoen L, Chongsuphajaisiddhi T, White NJ. Clinical features cannot predict a diagnosis of malaria or differentiate the infecting species in children living in an area of low transmission. *Trans R Soc Trop Med Hyg.* 1998; **92**: 45-49

Idjradinata P, Pollitt E. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. *Lancet* 1993; **341**: 1-4.

McDonald EM, Mann AH, Thomas HC. Interferons as mediators of psychiatric morbidity. An investigation in a trial of recombinant alpha-interferon in hepatitis-B carriers. *Lancet* 1987; **2**: 1175-1178.

McGregor IA, Gilles HM, Walters JH, Davies AH. Effect of heavy and repeated malarial infections on Gambian infants and children. Effects of erythrocyte parasitization. *BMJ* 1956; **2**: 686-692.

McGregor IA. Malaria: nutritional implications. *Rev Infect Dis* 1982; **4**: 798-804.

McGuire W, D'Alessandro U, Olaleye BO, Thomson MC, Langerock P, Greenwood BM, Kwiatkowski D. C-reactive protein and haptoglobin in the evaluation of a community-based malaria control programme. *Trans R Soc Trop Med Hyg* 1996; **90**: 10-14.

McKay H, Sinesterra L, McKay A, Gomez H, Lioreda P. Improving cognitive ability in chronically deprived children. *Science* 1978; **200**: 270-278.

McKay A, McKay H. Primary school progress after preschool experience: troublesome issues in the conduct of follow-up research and findings from Cali, Colombia, study. In: King K, Meyers R (Ed.) Preventing school failure: the relationship between preschool and primary education. International Development Research Centre, 1983: pp. 32-42.

Marsh K. Malaria- a neglected disease ? *Parasitol* 1992; **104** (Suppl): S35-S69.

Martorell R, Khan LK, Schroeder DG. Reversibility of stunting: epidemiological findings in children from developing countries. *Eur J Clin Nutr* 1994; **48** (Suppl): S45-57.

Menendez C, Kahigwa E, Hirt R, Vounatsou P, Aponte JJ, Font F, Acosta CJ, Schellenberg DM, Galindo CM, Kimario J, Urassa H, Brabin B, Smith TA, Kitua AY, Tanner M, Alonso PL. Randomised placebo controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *Lancet* 1997; **350**: 844-850.

Molineaux L, Gramiccia G. The Garki Project. Geneva: World Health Organization. 1980.

Molyneux ME, Taylor TE, Wirima JJ, Borgstein A. Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. *Q J Med* 1989; **71**: 441-459.

Moock P, Leslie J. Childhood malnutrition and schooling in the Terai region of Nepal. *J Dev Econ*, 1986; **20**: 33-52.

Moodie AD, Bowie MD, Mann MD, Hansen JD. A prospective 15-year follow-up study of kwashiorkor patients. Part II. Social circumstances, educational attainment and social adjustment. *S Afr Med J* 1980; **58**: 677-681.

MOPH. Summary report of the 1994 statistics. Ministry of Public Health. The Yemen Republic, 1994.

Mora JO, Amezquita A, Castro L, Christiansen N, Clement Murphy J, Cobos LF, Cremer HD, Dragastin S, Elias MF, Franklin D, Herrera MG, Ortiz N, Pardo F, de Paredes B, Ramos C, Riley R, Rodriguez H, Vuori-Christiansen L, Wagner M, Stare FJ. Nutrition, health and social factors related to intellectual performance. *World Rev Nutr Diet* 1974; **19**: 205-236.

Muntendam AH, Jaffar S, Bleichrodt N, van-Hensbroek MB. Absence of neuropsycho-logical sequelae following cerebral malaria in Gambian children. *Trans R Soc Trop Med Hyg* 1996; **90**: 391-394.

Murphy JM, Paggano ME, Nachmani J, Sperling P, Kane S, Kleinman RE. The relationship of school breakfast to psychosocial and academic functioning. *Arch Pediatr Adolesc Med* 1998; **152**: 899-907.

Murray MJ, Murray AB, Murray NJ, Murray MB. Diet and cerebral malaria: the effect of famine and refeeding. *Am J Clin Nutr* 1978a; **31**: 57-61.

Murray MJ, Murray AB, Murray MB, Murray CJ. The adverse effect of iron repletion on the course of certain infections. *BMJ* 1978b, **ii**, 1113-1115.

Naik P, Voller A. Serum C-reactive protein levels and falciparum malaria. *Trans R Soc Trop Med Hyg* 1984; **78**: 812-813.

National Malaria Control Program. Annual descriptive report. The Yemen Republic, 1991.

National Malaria Control Program. Malaria control in Republic of Yemen, 1992.

National Malaria Control Program. Malaria Border Meeting, Muscat, Oman, 5-8 September, 1993a.

National Malaria Control Program. Status of resistant malaria in Republic of Yemen, 1993b.

National Malaria Control Program. Plan for acceleration of Malaria Control Program and outbreaks control. 1999

The National Nutrition Survey. Yemen General Grain Corporation and Ministry of Health; 1979.

National Center for Health statistics. NCHS growth curves for children from birth to 18 years. US Department of health, Eduaction and welfare. Washington DC: Goverment Printing Office. 1977.

Neisser. U. changing conceptions of imagery. In: Sheehan PW (Ed.). The Function and nature of imagery. London: Academic Press. 1972.

Nevill CG, Watkins WM, Carter JY, Munafu CG. Comparison of mosquito nets, proguanil hydrochloride, and placebo to prevent malaria. *BMJ* 1988; **297**: 401-403.

Newton CR, Warn PA, Winstanley PA, Peshu N, Snow RW, Pasvol G, Marsh K. Severe anaemia in children living in a malaria endemic area of Kenya. *Trop Med Int Health* 1997; **2**: 165-178.

Newton CR, Taylor TE, Whitten RO. Pathophysiology of fatal falciparum malaria in African children. *Am J Trop Med Hyg* 1998; **58**: 673-683.

Nguyen TH, Day NP, Ly VC, Waller D, Nguyen HP, Bethell DB, Tran TH, White NJ. Post-malaria neurological syndrome. *Lancet* 1996; **348**: 917-921.

Nokes C, Cooper ES, Robinson BA, Bundy DAP. Geohelminth infection and academic assessment in Jamaican children. *Trans R Soc Trop Med Hyg* 1991; **85**: 272-273.

Nokes C, Grantham-McGregor SM, Sawyer AW, Cooper ES, Robinson BA, Bundy DAP. Moderate to heavy infections of *Trichuris trichiuria* affect cognitive function in Jamaican school children. *Parasitology* 1992; **104**: 539-547.

Nokes C, Bundy DAP. Compliance and absenteeism in school children: implications for helminth control. *Trans R Soc Trop Med Hyg* 1993; **87**: 148-152.

Nokes C, Bundy DAP. Does helminth infection affect mental processing and educational achievement? *Parasitol Today* 1994; **10**: 14-18.

Nussler A, Pied S, Pontet M, Miltgen F, Renia L, Gentilini M, Mazier D. Inflammatory status and preerythrocytic stages of malaria: role of the C reactive protein. *Exp Parasitol* 1991; **72**: 1-7.

Nwuga VC. Effect of severe kwashiorkor on intellectual development among Nigerian children. *Am J Clin Nutr* 1977; **30**: 1423-1430.

Oppenheimer SJ, Gibson FD, Macfarlane SB, Moody JB, Harrison C, Spencer A, Bunari O. Iron supplementation increases prevalence and effects of malaria: report on clinical studies in Papua New Guinea. *Trans R Soc Trop Med Hyg* 1986a; **80**: 603-612.

Oppenheimer SJ, Macfarlane SB, Moody JB, Bunari O, Hendrickse RG. Effect of iron prophylaxis on morbidity due to infectious disease: report on clinical studies in Papua New Guinea. *Trans R Soc Trop Med Hyg* 1986b; **80**: 596-602.

Oski-FA, Honig AS. The effects of therapy on the developmental scores of iron-deficient infants. *J Pediatr* 1978; **92**: 21-25.

Oski FA, Honig AS, Helu B, Howanitz P. Effect of iron therapy on behavior performance in nonanemic, iron-deficient infants. *Pediatrics* 1983; **71**: 877-880.

Oski FA. Differential diagnosis of anaemia. In: Nathan DG, Oski FA (Ed.). Hematology of infancy and childhood. Fourth ed., W.B. Saunders Company, 1993.

Palti H, Pevsner B, Adler B. Does anaemia in infancy affect achievement on developmental and intelligence tests? *Hum Biol* 1983; **55**: 183-194.

Palti H, Meijer A, Adler B. Learning achievement and behavior at school of anaemic and non-anaemic infants. *Early Hum Dev* 1985; **10**: 217-223.

Partnership for Child Development . Better health, nutrition and education for the school-aged child. *Trans R Soc Trop Med Hyg* 1997; **91**: 1-2

Partnership for Child Development. The anthropometric status of schoolchildren in five countries in the Partnership for Child Development. *Proc Nutr Soc* 1998; **57**: 149-158.

Pegelow K, Gross R, Pietrzik K, Lukito W, Richards AL, Fryauff DJ. Parasitological and nutritional situation of school children in the Sukaraja district, West Java, Indonesia. *Southeast Asian J Trop Med Public Health* 1997; **28**: 173-190.

Pereira SM, Sundararaj R, Begum A. Physical growth and neurointegrative performance of survivors of protein-energy malnutrition. *Br J Nut* 1979; **42**: 165-171.

Pied S, Nussler A, Pontent M, Miltgen F, Matile H, Lambert PH, Mazier D. C reactive protein protects against preerythrocytic stages of malaria. *Infect Immun* 1989; **57**: 278-282.

Pollitt E, Leibel RL, Greenfield D. Brief fasting, stress, and cognition in children. *Am J Clin Nutr* 1981; **34**: 1526-1233.

Pollitt E, Soemantri AG, Yunis F, Scrimshaw NS. Cognitive effects of iron deficiency anaemia. *Lancet* 1985; **1**: 158.

Pollitt E, Saco-Pollitt C, Leibel RL, Viteri FE. Iron deficiency and behavioral development in infants and preschool children. *Am J Clin Nutr* 1986; **43**: 555-565.

Pollitt E, Hathirat P, Kotchabhakdi NJ, Missell L, Valyasevi A. Iron deficiency and educational achievement in Thailand. *Am J Clin Nutr* 1989; **50** (Suppl): 687-696.

Pollitt E. Malnutrtion and infection in the classroom. Paris: United Nations Educational, Scientific, and Cultural Organization (UNESCO); 1990.

Pollitt E. Does breakfast make a difference in school? *J Am Diet Assoc* 1995; **95**: 1134-1139.

Pollitt E, Gorman KS, Engle PL, Rivera JA, Martorell R. Nutrition in early life and the fulfillment of intellectual potential. *J Nutr* 1995.**125** (Suppl): 1111S-1118S.

Pollitt E, Jacopy E, Cueto S. School breakfast and cognition among nutritionally at-risk children in the Peruvian Andes. *Nutr Rev* 1996; **54**: S22-26.

Pollitt E. Iron deficiency and educational deficiency. *Nutr Rev* 1997; **55**: 133-141.

Pollitt E, Watkins WE, Husaini MA. Three month nutritional supplementation in Indonesian infants and toddlers benefits memory function 8 y later. *Am J Clin Nutr* 1997, **66**: 1357- 1363.

Pollitt E, Mathews R. Breakfast and cognition: an integrative summary. *Am J Clin Nutr* 1998; **67** (Suppl): 804S-813S.

Popkin BM, Lim-Ybanez M. Nutrition and school achievement. *Soc Sci Med* 1982; **16**: 53-61.

Powell C, Grantham-McGregor S, Elston M. An evaluation of giving the Jamaican government school meal to a class of children. *Hum Nutr Clin Nutr* 1983; **37**: 381-388.

Powell CA, Grantham-McGregor S. The ecology of nutritional status and development in young children in Kingston, Jamaica. *Am J Clin Nutr* 1985; **41**: 1322-1331.

Preble OT, Torrey EF. Serum interferon in patients with psychosis. *Am J Psychiatry* 1985; **142**: 1184-1186.

Premji Z, Hamisi Y, Shiff C, Minjas J, Lubega P, Makwaya C. Anaemia and *Plasmodium falciparum* infections among young children in an holoendemic area, Bagamoyo, Tanzania. *Acta Tropica* 1995, **59**: 55-64.

Pringle G, Avery-Jones S. Observations on the early course of untreated falciparum malaria in semi-immune African children following a short period of protection. *Bull World Health Organ* 1966; **34**: 269-272.

Ramalingaswami V. Challenges and opportunities--one vitamin, two minerals. *World Health Forum* 1992; **13**: 222-231.

Reed SC, Wirima JJ, Steketee RW. Risk factors for anemia in young children in rural Malawi. *Am J Trop Med Hyg*. 1994; **51**: 170-174.

Rothman KJ, Greenland S. Causation and causual infernce. In: Detels R, Holland WW, McEwen J, Omenn GS. (Ed.). Oxford Textxtbook of Public Health. Volume 2: The methods of Public Health. Oxford Universty Press, Third ed.; 1997; pp. 617-629.

Rougemont A, Dumbo O, Bouvier M, Soula G, Perrin L, Tamoura B, Yerly S, Dolo A, Brenner E, Kodio B, Srivastava I, Ranque P. Hypohaptoglobinaemia as an epidemiological and clinical indicator for malaria. Results of two studies in a hyperendemic region in West Africa. *Lancet* 1988; **2**: 709-712.

Rowland MG, Cole TJ, Whitehead RG. A quantitative study into the role of infection in determining nutritional status in Gambian village children. *Br J Nutr* 1977; **37**: 441-450.

Salako LA, Ajayi FO, Sowunmi A, Walker O. Malaria in Nigeria: a revisit. *Ann Trop Med Parasitol* 1990; **84**: 435-445.

Schaap HB, Den-Dulk MO, Polderman AM. Schistosomiasis in the Yemen Arab Republic. Prevalence of Schistosoma mansoni and S. haematobium infection among schoolchildren in the central highlands and their relation to altitude. *Trop Geogr Med* 1992; **44**: 19-22.

Schaefer KU, Khan B, Gachihi GS, Kager PA, Muller AS, Verhave JP, McNeill KM Splenomegaly in Baringo District, Kenya, an area endemic for visceral leishmaniasis and malaria. *Trop Geogr Med* 1995; **47**: 111-114.

Schapira A, Beales PF, Halloran ME. Malaria: Living with drug resistance. *Parasitol Today* 1993; **9**: 168-174.

Scrimshaw NS. Frequency, cause, and significance of iron deficiency for the children of Central Asia. *International Child Health* 1998; **IX**: 47-60.

Senanayake N, de-Silva HJ. Delayed cerebellar ataxia complicating falciparum malaria: a clinical study of 74 patients. *J Neurol* 1994; **241**: 456-459.

Seshadri S, Gopaldas T. Impact of iron supplementation on cognitive functions in pre-school and school-aged children : the Indian experience. *Am J Clin Nutr* 1989; **50**: 675-686.

Sexton JD. Impregnated bed nets for malaria control: Biological success and social responsibility. *Am J Trop Med Hyg* 1994, **50** (Suppl): S72-S81.

Sharp PT, Harvey P. Malaria and growth stunting in young children of the highlands of Papua New Guinea. *P N G Med J* 1980; **23**: 132-140.

Shiff C, Checkley W, Winch P, Premji Z, Minjas J, Lubega P. Changes in weight gain and anaemia attributable to malaria in Tanzanian children living under holoendemic conditions. *Trans R Soc Trop Med Hyg* 1996; **90**: 262-265.

Shulman CE, Graham WJ, Jilo H, Lowe BS, New L, Obiero J, Snow RW, Marsh K. Malaria is an important cause of anaemia in primigravidae: evidence from a district hospital in coastal Kenya. *Trans R Soc Trop Med Hyg* 1996; **90**: 535-539.

Sigman M, Neurmann C, Jansen AA, Bwibo N. Cognitive abilities of Kenyan children in relation to nutrition, family characteristics, and education. *Child Dev* 1989; **60**: 1463-1474.

Simeon DT, Grantham-McGregor SM. Effect of missing breakfast on the cognitive function of school children of differing nutritional status. *Am J Clin Nutr* 1989; **49**: 646-653.

Simeon D, Callender J, Wong M, Grantham-McGregor S, Ramdath DD. School performance, nutritional status and trichuriasis in Jamaican schoolchildren. *Acta Paediatr* 1994; **83**: 1188-1193.

Simeon DT, Grantham-McGregor SM, Wong MS. *Trichuris trichiuria* infection and cognition in children: results of a randomized clinical trial. *Parasitology* 1995a; **110**: 457-464.

Simeon DT, Grantham-McGregor SM, Callender JE, Wong MS. Treatment of *Trichuris trichiuria* infections improves growth, spelling scores and school attendance in some children. *J Nutr* 1995b; **125**: 1875-1883.

Smedley H, Katrak M, Sikora K, Wheeler T. Neurological effects of recombinant human interferon. *BMJ* 1983; **286:** 262-264.

Smillie WG, Spencer CR. Mental retardation in school children infested with hookworms. *J Educ Psychol* 1926; **17**: 314-321.

Smith AP, Tyrrell DA, Coyle K, Willman JS. Selective effects of minor illnesses on human performance. *Br J Psychol* 1987a; **78**: 183-188.

Smith AP, Tyrrell DA, Al Nakib W, Coyle KB, Donovan CB, Higgins PG, Willman JS. Effects of experimentally induced respiratory virus infections and illness on psychomotor performance. *Neuropsychobiology* 1987b; **18**: 144-148.

Smith AP, Tyrrell DA, Al Nakib W, Coyle KB, Donovan CB, Higgins PG, Willman JS. The effects of experimentally induced respiratory virus infections on performance. *Psychol Med* 1988; **18**: 65-71.

Smith AW, Hendrickse RG, Harrison C, Hayes RJ, Greenwood BM. The effects on malaria of treatment of iron-deficiency anaemia with oral iron in Gambian children. *Ann Trop Paediatr* 1989a; **9**: 17-23.

Smith AP, Tyrrell DA, al Nakib W, Barrow PG, Higgins PG, Leekam S, Trickett S. Effects and after-effects of the common cold and influenza on human performance. *Neuropsychobiology* 1989b; **21**: 90-93.

Smith AP. Respiratory virus infections and performance. *Philos Trans R Soc Lond B Biol Sci* 1990; **327**: 519-528.

Smith AP, Tyrrell DA, Barrow GI, Coyle KB, Higgins PG, Trickett S, Willman JS. Effects of experimentally induced colds on aspects of memory. *Percept Mot Skills* 1990; **71**: 1207-1215.

Smith AP, Tyrrell DAJ, Coyle KB, Higgins PG. The effects and after-effects of interferon alpha on human performance, mood and physiological functions. *J Psychopharmacol* 1991; **5**: 243-250.

Smith AP. Effects of influenza and the common cold on the Stroop color-word test. *Percept Mot Skills* 1992; **74**: 668-670.

Smith AP, Thomas M, Brockman P, Kent J, Nicholson KG. Effect of influenza B virus infection on human performance. *BMJ* 1993; **306**: 760-761.

Smith T, Genton B, Baea K, Gibson N, Taime J, Narara A, Al-Yaman F, Beck HP, Hii J, Alpers M. Relationships between *Plasmodium falciparum* infection and morbidity in a highly endemic area. *Parasitology* 1994; **109**: 539-549.

Smith T,Hurt N, Teuscher T, Tanner M. Is fever a good sign for clinical malaria in surveys of endemic communities? *Am J Trop Med Hyg* 1995; **52**: 306-310.

Snounou G. Population dynamics of human malaria parasites. *Parassitologia* 1993; **35** (Suppl): 113-116.

Snow RW, Marsh K. New insight into the epidemiology of malaria relevant for disease control. *Br Med Bull* 1998; **54**: 293-309.

Snow RW, Byass P, Shenton FC, Greenwood BM. The relationship between anthropometric measurements and measurements of iron status and susceptibility to malaria in Gambian children. *Trans R Soc Trop Med Hyg* 1991; **85**: 584-589.

Soemantri AG, Pollitt E, Kim I. Iron deficiency anaemia and educational achievement. *Am J Clin Nutr* 1985, **42**: 1221-1228.

Soemantri AG. Preliminary findings on iron supplementation and learning achievement of rural Indonesian children. *AM J Clin Nutr* 1989, **50**: 698-702.

Soewondo S, Husaini M, Pollitt E. Effects of iron deficiency on attention and learning processes in preschool children: Bandung, Indonesia. *Am J Clin Nutr* 1989; **50** (Suppl): 667-673.

SPSS Inc. 6.1. Chicago, IL, 60611. 1994

Sternberg RJ. Human abilities. An information-processing approach. W. H. Freeman and Company. 1985.

Stiles CW. The school grades attained by 2166 white school children (1062 boys, 1104 girls) in the City of X, classified by age, sanitation and intestinal parasites. *United States Public Health Reports* 1915; **20**: 2060-2067.

Stoltzfus RJ. Rethinking anaemia surveillance. *Lancet* 1997a; **349**: 1764-1766.

Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KJ, Albonico M, Savioli L. Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. *Am J Clin Nutr* 1997b; **65**: 153-159.

Stoltzfus RJ, Albonico M, Tielsch JM, Chwaya HM, Savioli L. Linear growth retardation in Zanzibari school children. *J Nutr* 1997c; **127**: 1099-1105.

Stoltzfus RJ, Chwaya HM, Albonico M, Schulze KJ, Savioli L, Tielsch JM. Serum ferritin, erythrocyte protoporphyrin and hemoglobin are valid indicators of iron status of school children in a malaria-holoendemic population. *J Nutr* 1997d ; **127**: 293-298.

Strong EK. Effect of hookworm disease on the mental and physical development of children. New York. The Rockefeller Foundation. International Health Commission; Publication No. 3. 1916.

Sturrock RF, Kariuki HC, Thiongo FW, Gachare JW, Omondi BG, Ouma JH, Mbugua G, Butterworth AE. Schistosomiasis mansoni in Kenya: relationship between infection and anaemia in schoolchildren at the community level. *Trans R Soc Trop Med Hyg* 1996; **90**: 48-54.

Super CM, Herrera MG, Mora JO. Cognitive outcomes of early nutritional intervention in the Bogota study. Abstracts of meeting of the Society for Research in Child Development; 1991.

Taha TT, Broadhead RL. A comparative epidemiological study of malaria between children of Northern and Southern Sudan. *J Trop Pediatr* 1986; **32**: 117-119.

Tatala S, Svanberg U, Mduma B. Low dietary iron availability is a major cause of anemia: a nutrition survey in the Lindi District of Tanzania. *Am J Clin Nutr* 1998; **68**: 171-178.

Thuriaux MC. Notes on the epidemiology of malaria in the Yemen Arab Republic. *Ann Soc Belg Med Trop.* 1971; **51**: 229-237.

Tiwari BD, Godbole MM, Chattopadhyay N, Mandal A, Mithal A. Learning disabilities and poor motivation to achieve due to prolonged iodine deficiency. *Am J Clin Nutr* 1996; **63**: 782-786.

Tomkins A, Watson F. Malnutrition and infection: A review. ACC/SCN State-Of-The-Art Series. United Nations. 1989.

Trape JF, Peelman P, Morault-Peelman B. Criteria for diagnosing clinical malaria among a semi-immune population exposed to intense and perennial transmission. *Trans R Soc Trop Med Hyg* 1985; **79**: 435-442.

Trape JF. Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. *Trans R Soc Trop Med Hyg* 1985; **79**: 181-184.

Trape JF, Zoulani A, Quinet MC. Assessment of the incidence and prevalence of clinical malaria in semi-immune children exposed to intense and perennial transmission. *Am J Epidemiol* 1987; **126**: 193-201.

Trape JF, Fribourg-Blanc A. Ahaptoglobinemia in African populations and its relation to malaria endemicity. *Am J Epidemiol* 1988; **127**: 1282-1288.

Trape JF, Lefebvre-Zante E, Legros F, Druilhe P, Rogier C, Bouganali H, Salem G. Malaria morbidity among children exposed to low seasonal transmission in Dakar, Senegal and its implications for malaria control in tropical Africa. *Am J Trop Med Hyg* 1993; **48**: 748-756.

Trape JF, Rogier C, Konate L, Diagne N, Bouganali H, Canque B, Legros F, Badji A, Ndiaye G, Ndiaye P, Brahimi K, Faye O, Druilhe P, DA-Silva LP. The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. *Am J Trop Med Hyg* 1994; **51**: 123-137.

Trape JF, Rogier C. Combating malaria morbidity and mortality by reducing transmission. *Parasitol Today* 1996; **12**: 236-240.

Ukoli FA, Adams Campbell LL, Ononu J, Nwankwo MU, Chanetsa F. Nutritional status of urban Nigerian school children relative to the NCHS reference population. *East Afr Med J* 1993; **70**: 409-413.

Ulijaszek SJ. Cognitive development. In: Ulijaszek SJ, Johnston FE, Preece MA. The Cambridge encyclopedia of human growth and development. Cambridge University Press. 1998. pp. 245-246.

UNICEF. The state of the world's children. Oxford University Press. 1998.

Upadhyay SK, Agarwal DK, Agarwal KN, Sirvastava KB, Adhikari GS. Brief fasting and cognitive functions in rural school children. *Indian Pediatr* 1988; **25**: 288-289.

van-den-Hombergh J, Dalderop E, Smit Y. Does iron therapy benefit children with severe malaria-associated anaemia? A clinical trial with 12 weeks supplementation of oral iron in young children from the Turiani Division, Tanzania. *J Trop Pediatr* 1996; **42**: 220-227.

van-Hensbroek MB, Morris Jones S, Meisner S, Jaffar S, Bayo L, Dackour R, Phillips C, Greenwood BM. Iron, but not folic acid, combined with effective antimalarial therapy promotes haematological recovery in African children after acute falciparum malaria. *Trans R Soc Trop Med Hyg* 1995; **89**: 672-676.

Valentine AD, Meyers CA, Kling MA, Richelson E, Hauser P. Mood and cognitive side effects of interferon-alpha therapy. *Semin Oncol* 1998; **25**(Suppl): 39-47.

Voller A, Bidwell DE, Bartlett A. Enzyme linked immunosorbent assay. In: Rose N, Friedman H (Ed.). Manual of Clinical Immunology. Washington, DC: American Society for Microbiology, 1980, pp. 359-371.

Vollmer-Conna U, Wakefield D, Lloyd A, Hickie I, Lemon J, Bird KD, Westbrook RF. Cognitive deficits in patients suffering from chronic fatigue syndrome, acute infective illness or depression. *Br J Psychiatry* 1997; **171**: 377-381.

Waber DP, Vuori-Christiansen L, Ortiz N, Clement JR, Christiansen NE, Mora JO, Reed RB, Herrera MG. Nutritional supplementation, maternal education, and cognitive development of infants at risk of malnutrition. *Am J Clin Nutr* 1981; **34** (Suppl): 807-813.

Wachs TD. Relation of mild-to-moderate malnutrtion to human development: correlational studies. *J Nutr* 1995; **125** (Suppl): 2245S-2245S.

Walker AR, Walker BF, Richardson BD. Studies on schistosomiasis in a South African Bantu schoolchild population. *Am J Trop Med Hyg* 1970; **19**: 792-814.

Walker SP, Grantham-McGregor SM, Himes JH, Powell CA, Chang SM. Early childhood supplementation does not benefit the long term growth of stunted children in Jamaica. *J Nutr* 1996; **126**: 3017-3024.

Walter T, Kovalskys J, Stekel A. Effect of mild iron deficiency on infant mental development scores. *J Pediatr* 1983; **102**: 519-522.

Walter T, de-Andraca I, Chadud P, Perales CG. Iron deficiency anemia: adverse effects on infant psychomotor development. *Pediatrics* 1989; **84**: 7-17.

Waite JH, Neilson L. A study of the effect of Hookworm infection upon mental development of North Queensland School Children. *The Medical Journal of Australia* 1919; **1**: 1-7.

World Bank. Yemen situation analysis. Volume I. Situation of women and children in the Republic of Yemen. World Bank, Radda Barnen and UNICEF in partnership with the Government of Yemen. Sana'a, July, 1998.

Warrell DA, Molyneux ME, Beales PF. (Ed.). Severe and complicated malaria. *Trans R Soc Trop Med Hyg* 1990; **84** (Suppl).

Watkins WE. Infection and malnutrition in classroom performance. *Nutrition* 1995; **11**, 59-62.

Watkins WE, Cruz JR, Pollitt E. The effects of deworming on indicators of school performance in Guatemala. *Trans R Soc Trop Med Hyg* 1996; **90**: 156-161.

Weatherall DJ, Abdalla S. The anaemia of *Plasmodium falciparum* malaria. *Br Med Bull* 1982; **38**: 147-151.

Webb TE, Oski FA. Iron deficiency anaemia and scholastic achievement in young adolescents. *J Pediatr* 1973, **82**: 827-830.

Wenlock RW. The epidemiology of tropical parasitic diseases in rural Zambia and the consequences for public health. *J Trop Med Hyg* 1979; **82**: 90-98.

Westhuyzen J, Steyn NP. Haematological nutrition of schoolchildren in the far northwestern cape. *Trop Geogr Med* 1992; **44**: 47-51.

WHO. Prevention and control of intestinal parasitic infections. Geneva. World Health Organization, *World Health Organ Tech Rep Ser* 1987; **749**.

WHO. Vector control for malaria and other mosquito born diseases. Report of WHO study group. *World Health Organ Tech Rep Ser* 1995a; **857**.

WHO. Physical status: the use and interpretation of anthropometry. *World Health Organ Tech Rep Ser* 1995b; **854**.

WHO. Iron deficency anaemia: indicators for assessement and stratigies for prevention. WHO/NUT, 1996a.

WHO. Situation of malaria. *Wkly Epidemiol Rec* 1996b, **5**: 39

Willcox MC, Bjorkman A, Brohult J. The effect of persistent malarial infections on haemoglobin A2 levels in Liberian children. *Trans R Soc Trop Med Hyg* 1985; **79**: 242-244.

Wilson A. Longitudinal analysis of diet, physical growth, verbal development, and school performance. In: Balderston J, Wilson A, Freire M, Simonen M, eds. Malnourished children of the rural poor. Boston, Massachusetts: Auburn House Publishing Company, 1970; pp. 39-81.

Wolde-Gebriel Z, West CE, Gebru H, Tadesse A S, Fisseha T, Gabre P, Aboye C, Ayana G, Hautvast JGAJ. Interrelationship between vitamin A, iodine and iron status in schoolchildren in Shoa Region, Central Ethiopia. *Br J Nutr* 1993; **70**: 593-607.

Wong MS, Simeon DT, Powell CA, Grantham-McGregor SM. Geohelminth infections in school-aged children in Jamaica. *West Indian Med J* 1994; **43**: 121-122.

Zein ZA. Situation analysis of nutrition in Yemen. UNICEF, 1997.

Zucker JR, Lackritz EM, Ruebush TK, Hightower AW, Adungosi JE, Were JB, Metchock B, Patrick E, Campbell CC. Childhood mortality during and after hospitalization in western Kenya: effect of malaria treatment regimens. *Am J Trop Med Hyg* 1996; **55**: 655-660.

12. Appendix

12.1 Responsibilities of the different team members

Although this study was a collaborative work and required assistance from different people, for whom I am indebted, the main team members and their responsibilities are listed below.

12.1.1. Responsibilities of the principal investigator

As principal investigator the tasks I conducted were:

12.1.1.1. In the preparatory phase:

1- Obtained the necessary permission from the Ministry of Education and Ministry of Public Health and from local education and health authorities

2- Translated the cognitive and school achievement tests into Arabic and checked their applicability to Yemeni children through discussions with headmasters and teachers and looking at the children's school work

3- Piloted the cognitive and school achievement tests in 60 schoolchildren and discussed the results with the main supervisor at the Institute of Child Health

4- Wrote an instruction booklet in Arabic for cognitive and school achievement tests

5- Recruited all members of the team: cognitive testers, laboratory technician, health worker and administrator.

6- Selected the sites for training and piloting.

7- Arranged for the cognitive tester trainer to come to the Yemen

8- Assisted the trainer in training the cognitive testers

9- Made the necessary changes to the cognitive tests to make them applicable to Yemeni children.

10- Supervised the cognitive testers during their piloting and assessed their test re-test reliability

11- Supervised the laboratory technician during doing blood sampling, smear preparation and laboratory testing and I checked the interobserver reliability

12- Selected the sites for the study implementation after screening of schools

13- Arranged supply of drug and the placebo

14- Supervised with the administrator all daily logistics

15- Sought the cooperation of community leaders, officials, headmasters and teachers

12.1.1.2. During the implementation:

1- Selected the sample within each school according to the selection criteria

2- Examined each child generally and abdominally, assessed weight and height, did a uniform rating, and assessed the socioeconomic status and history of hungry

3- Gave the treatment to each child and ensured that each child swallowed it in front of me

4- Supervised the cognitive testers and ensured ongoing quality control in 10% of cases

5- Supervised the laboratory technician and ensured ongoing quality control in 10% of cases

6- Supervised the health worker and ensured taking blood samples and storing them properly

7- Supervised the cognitive testers during scoring the results and rechecking independently at least 10 % of them

8- Checked that each set of data was complete before filling

9- Provided the necessary treatment, at the end of the study, for anaemic children and referred those who needed to be followed up to the nearest health center

12.1.1.3. After finishing field work:

1- Supervised data entry with frequent rechecking

4- Did all data analysis

5- Reported the results

12.1.2. Responsibilities of the cognitive testers:

According to the protocol each tester:

- conducted the cognitive tests
- marked the results of tests
- cross-checked the results with the other testers
- copied the results to results book

12.1.3. Responsibilities of the laboratory technician:

According to the protocol he:

- collected malaria smears
- stained smears properly
- examined smears thoroughly
- wrote the results on lab book

12.1.4. Responsibilities of the health worker:

According to the protocol he:

- collected blood samples on filter paper
- measured the haemoglobin by HemoCue
- wrote the results in the lab book

12.1.5. Responsibilities of the administrator:

- Assisted the principal investigator in ensuring that each member of the team is doing what he was supposed to do
- Arranged the children and directed each one to the proper site
- Administered and supervised the school achievement tests
- Ensured that all requirements for the work were available
- Arranged a weekly financial statement for approval by the principal investigator

12.2 The Cognitive

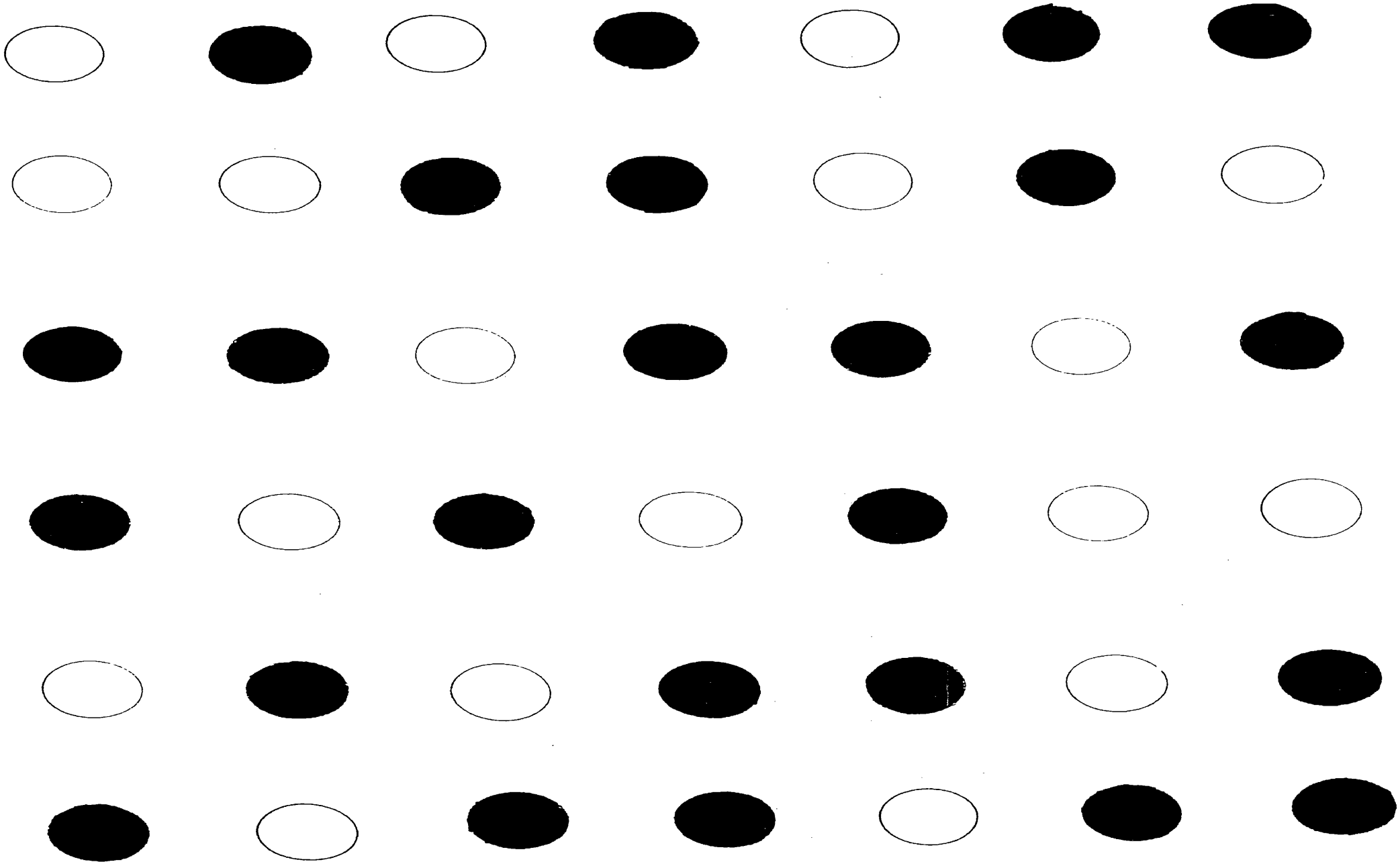
and

school achievement Tests

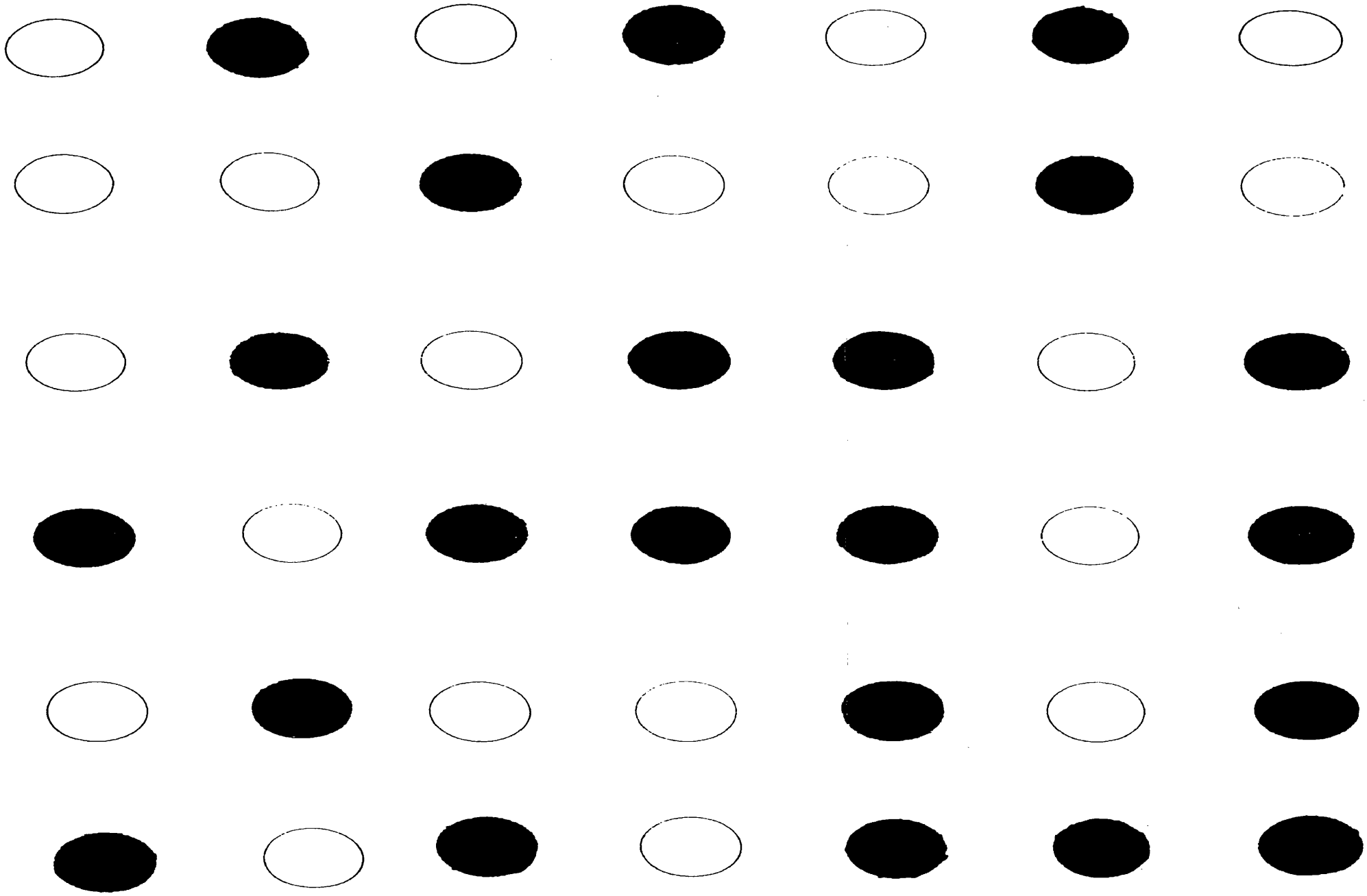
Visual Search (page 1)

Visual Search (page 2)

Stroop test (page 1)



Stroop test (page 2)



Symbol – symbol (page 1)

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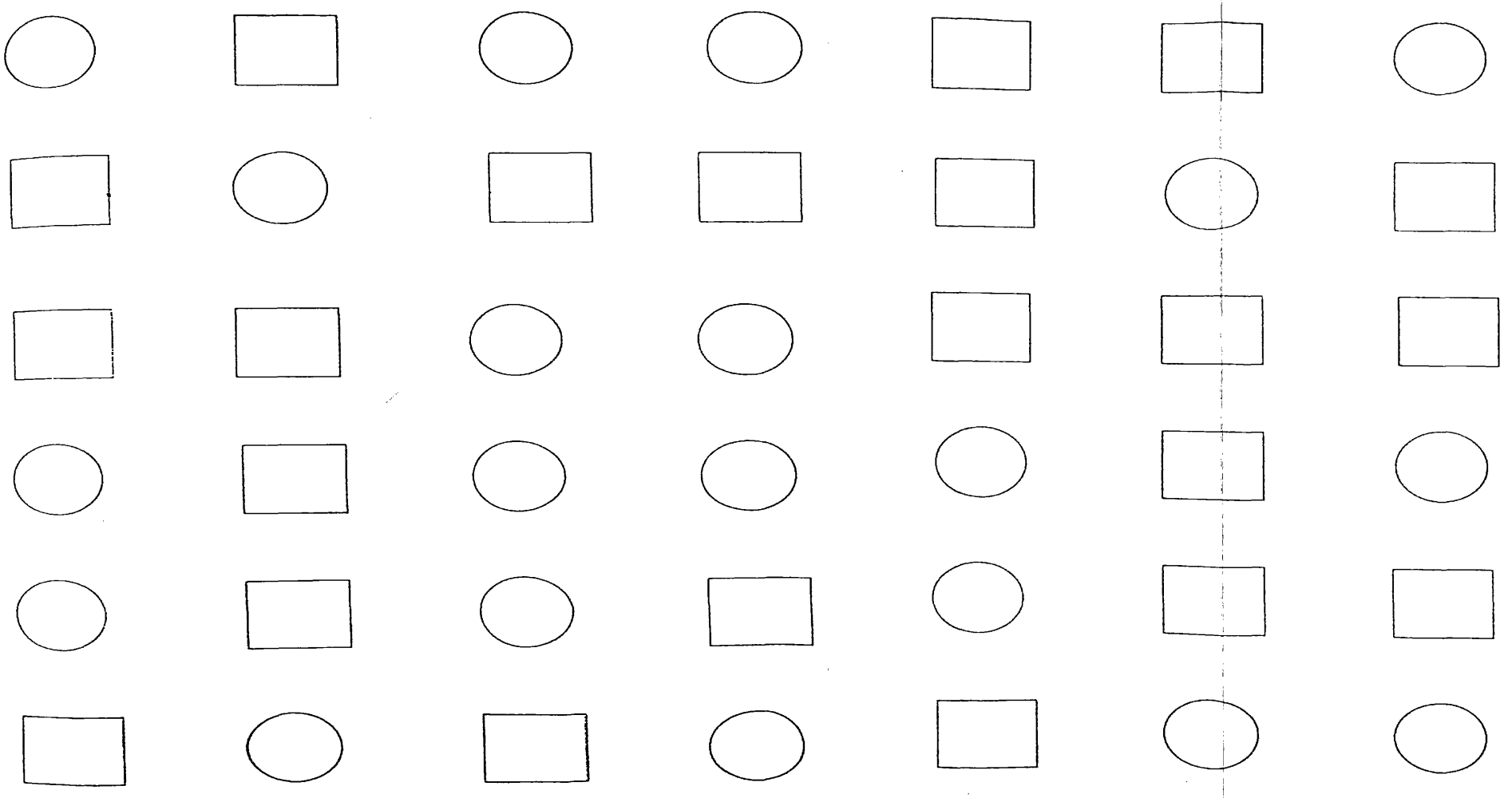
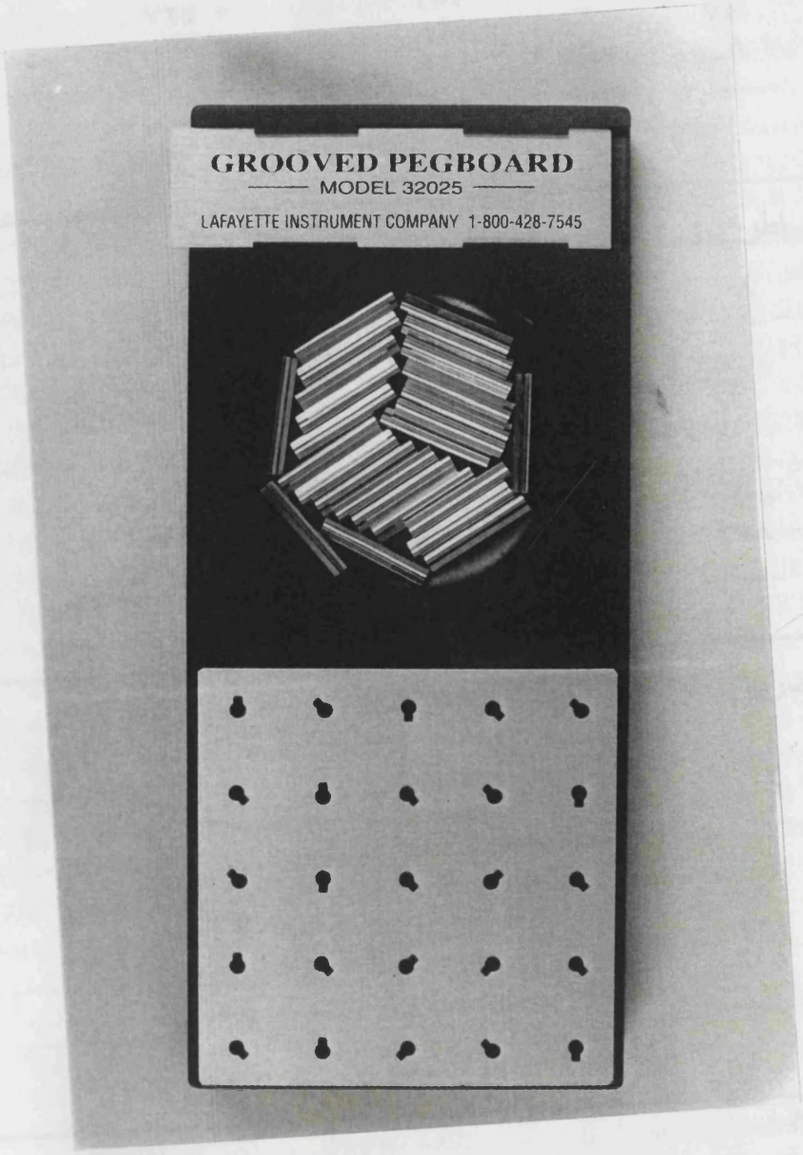


The peg board



بسم الله الرحمن الرحيم

أولاً : اجمع :

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١ +	١ +	٥	٢٤ +
١ +	٢ +	٧ +	٤٠ +

		٤٨
		٢٨٥٣ +
	٤٥٢	٧٢٤ +
٧٥	١٣٧ +	٨٢ +
٨ +	٢٤٥ +	٦٢١٥ +

ثانياً : اطرح :

٨	٥	١٤	٨٤
٠ −	٤ −	٨ −	٣٦ −

		٧٠٠٩٠٤
٧٤٥	٦٢٠٤	٩٠١٨ −
٣٦٨ −	٥٣٠ −	

ثالثاً : اضرب :

٤	٢٣	٤٢٠	٨٣٤
٢ ×	٣ ×	٤ ×	٧ ×

		٧,٩٥٢
٦٣٦	٤٢٠, ٣	٣,٧×
٢٠٨ ×	٢٩ ×	

رابعاً :اقسم :

٨ ÷ ٤ = ٧٢ ÷ ٩ = ٩٦٨ ÷ ٦ =

١٠٥, ٩ ÷ ٣ = ٦٢٦٣ ÷ ٣١ = ٥٧٢ ÷ ٢, ٥ =

٤٧٦٢ ÷ ٥٣٦ =

خامساً : اجمع واوجد الناتج في أبسط صورة :

$\frac{1}{3} + \frac{1}{3} =$

$\frac{1}{2} + \frac{1}{4} + \frac{1}{8} =$

$2\frac{4}{5} + 7\frac{2}{5} =$

$3\frac{2}{5} + 2\frac{1}{4} + 1\frac{5}{6} =$

$6\frac{1}{5} + 4 + \frac{2}{7} + 9\frac{4}{5} =$

سادساً : اطرح واوجد الناتج في أبسط صورة :

$\frac{7}{8} - \frac{3}{8} =$

$4\frac{2}{3} - 2\frac{2}{3} =$

$8\frac{3}{5} =$

$7\frac{1}{6} - \frac{3}{4} =$

$19\frac{1}{5} - 14\frac{2}{3} =$

سابعاً : اضرب واوجد الناتج في أبسط صورة :

$\frac{3}{4} \times \frac{4}{5} =$

$\frac{2}{3} \times 9 =$

$\frac{5}{8} \times \frac{6}{7} =$

$4\frac{3}{8} \times \frac{2}{3} =$

ثامناً : اقسم واوجد الناتج في أبسط صورة :

$\frac{1}{2} \div \frac{3}{4} =$

$\frac{7}{8} \div 3\frac{2}{3} =$

تاسعاً : حول كلاً مما يلي الى كسر عادي :

$3\% =$

$66\frac{2}{3}\% =$

عاشراً : حول كلاً مما يلي الى كسر عشري :

$4\frac{1}{2} =$

$\frac{3}{5} =$

$\frac{9}{100} =$

الطالب :

اسم الطالب : _____

المدرسة : _____

الصف : _____

النتيجة :

الدرجة الكلية : _____

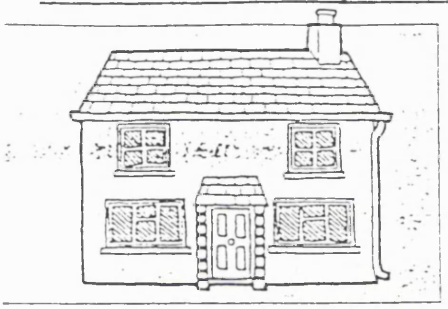
الدرجة المعدلة : _____

ملحوظة : _____

التعليمات :

انظر جيداً إلى الخمس الكلمات التالية . ضع دائرة على الكلمة المناسبة . انظر إلى المثال التالي لتعرف طريقة الحل . إذا أخطأت و اخترت كلمة غير مناسبة فاشطب الكلمة الغير صحيحة كما هو موضح فيما يلي عصان ثم أشر على الكلمة الصحيحة

المثال :



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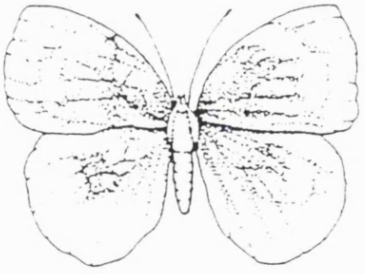

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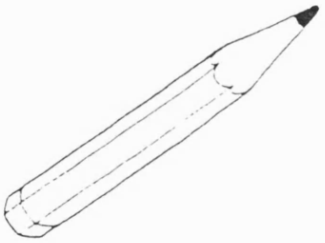

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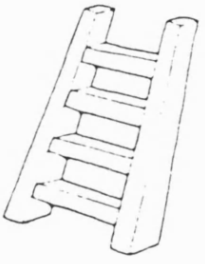

قطة

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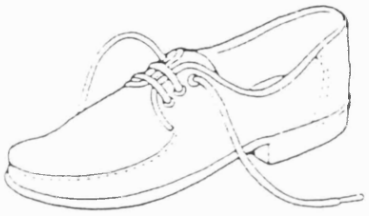

كرة

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شرابه

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إصبع

تعليمات :

أنظر إلى العبارات التالية ثم أملئ الفراغ بالكلمة المناسبة . ضع علامة حوالي الكلمة المناسبة . أنظر إلى المثال التالي . لا تكتب الكلمة في الفراغ

المثال : عضن _____ الرجل

الباب

الكتب

السيارة

القلم

الشنطة

٦- أمها _____ أنها لابد أن تجلس في المنزل

قالت

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لا تستطيع

٧- أنا نظرت إلى نفسي في _____

المرآة

الخفاش

المدرسة

السقف

الوجه

٨- الطالب المجتهد _____ من كتابة دروسه

ينتهي

يقف

يجلس

يمسك

يلعب

٩- يفتح الرجل القفل _____

بالمسطرة

بالمفتاح

بالكتاب

بالقلم

بالباب

١٠- أخت محمد _____

أبن

بنت

ولد

رجل

أخ

١١- أخذ محمد _____ من الشجرة

دفتر

كرسي

ثمرة

ثوب

رجل

١٢- يلعب الأولاد في _____عندما تمطر السماء

تحت

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الخارج

رجل

١٣- قطفت _____ من الورد

حزمة

غذاء

عشاء

جزمة

عشرة

١٤- ___ نوع من الكلاب هذا ؟ سأل الطالب

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١٥- أحمد _____ العقدة بالسكين

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مضغ

١٦- أخي _____ مني بثلاث سنين

أعرض

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صغير

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١٧- ____ الباب بعد أن تخرج

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١٨—عندما ينطفئ الضوء تشعل _____

الكتاب

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القلم

١٩- لا ____ أين وضعت المفتاح

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٢٠- بعد العاصفة نرى _____ في السماء

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الكتاب

المدرسة

٢١- تتسبب النار في كثير من ____

الدمار

الباب

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السماء

القلم

٢٢- _____ أنا ؟ سأل عندما استيقظ

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سوف

أين

هناك

٢٣- الطالبان _____ خلف السيارة

بدأ

يصف

جريا

يريد

أرسل

٢٤-عندما تكون أمها مريضة،سعاد ____ الغذاء

ترسم

تنتهي

تبدأ

تشعر

تطبخ

٢٥- نشتري الدواء من ____

المدرسة

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الباب

٢٦- يسبح السمك في _____

الماء

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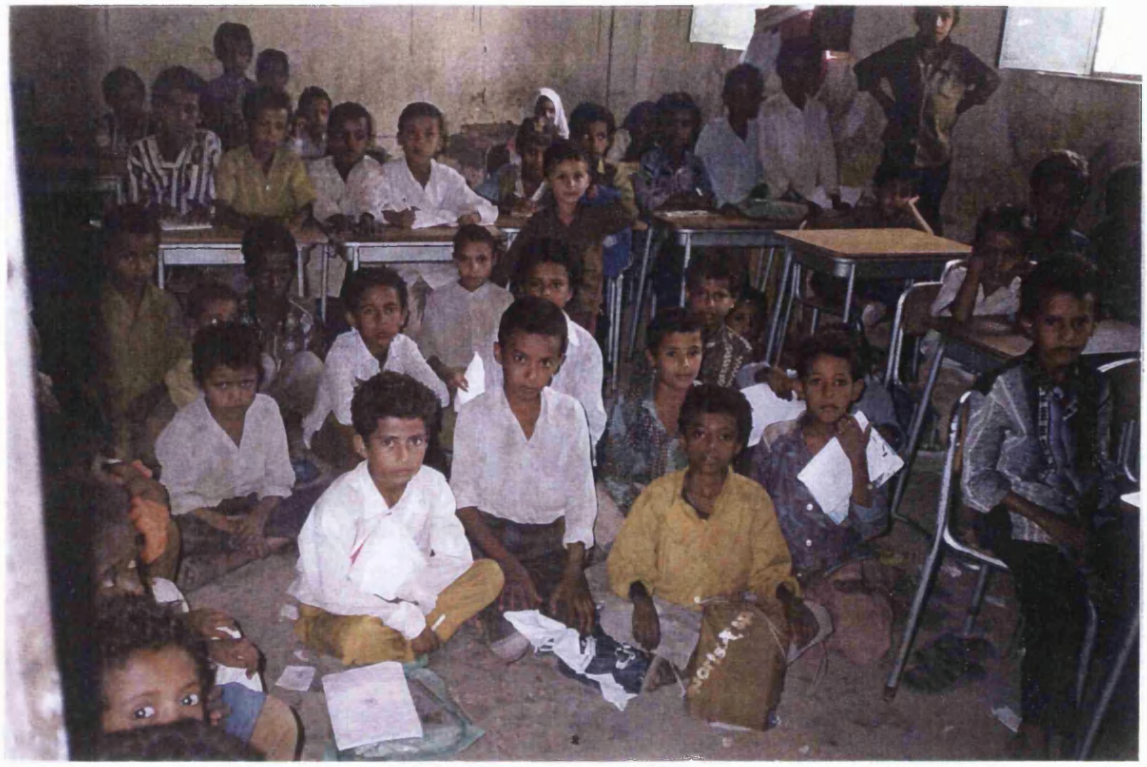
التراب

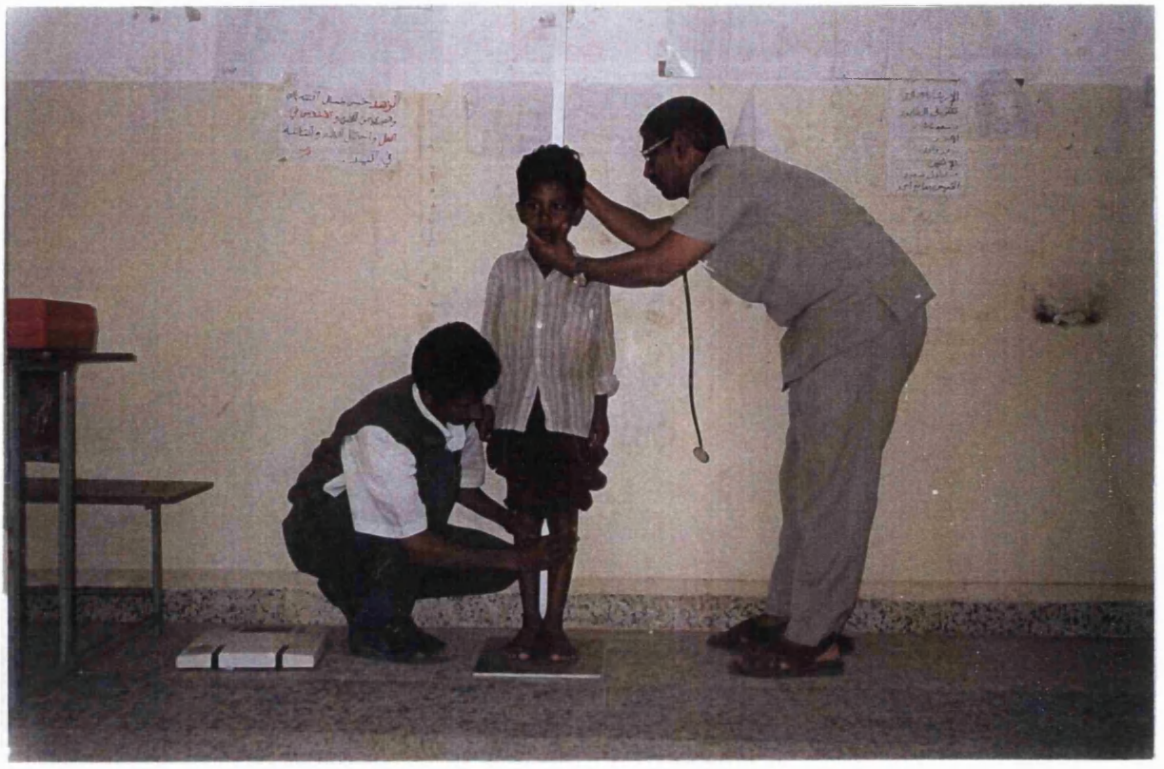
12.3. Some photographs from the field

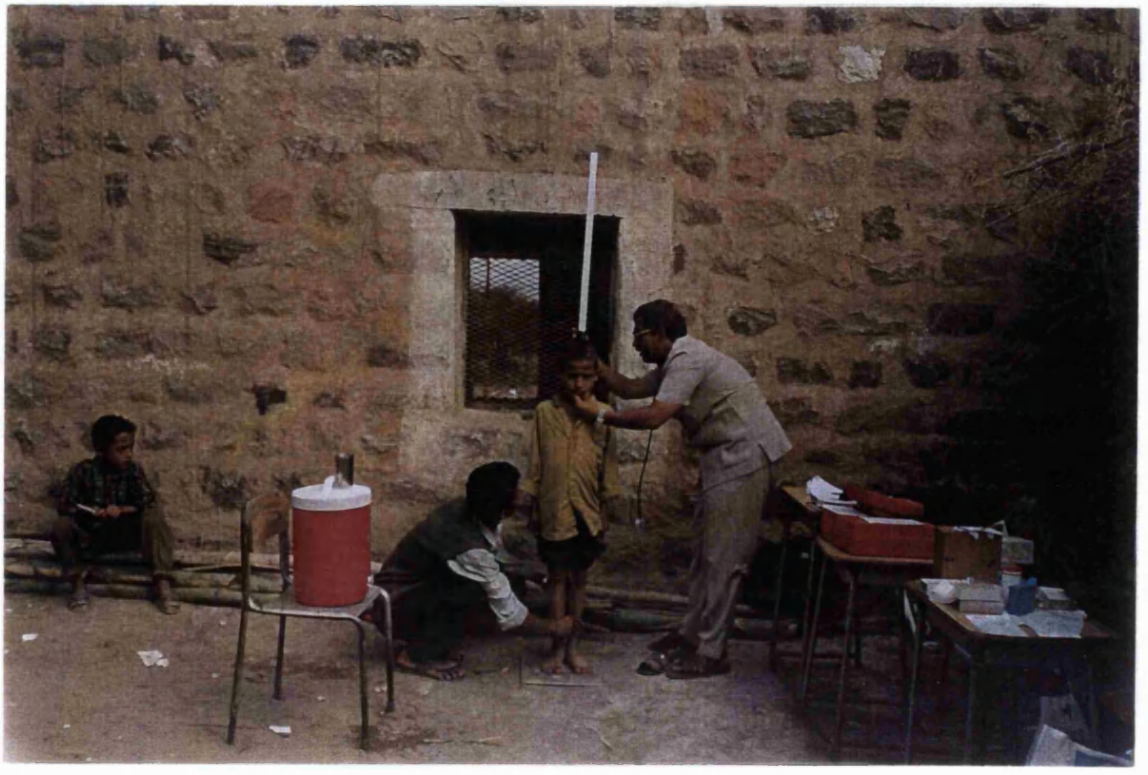
The team

School environment

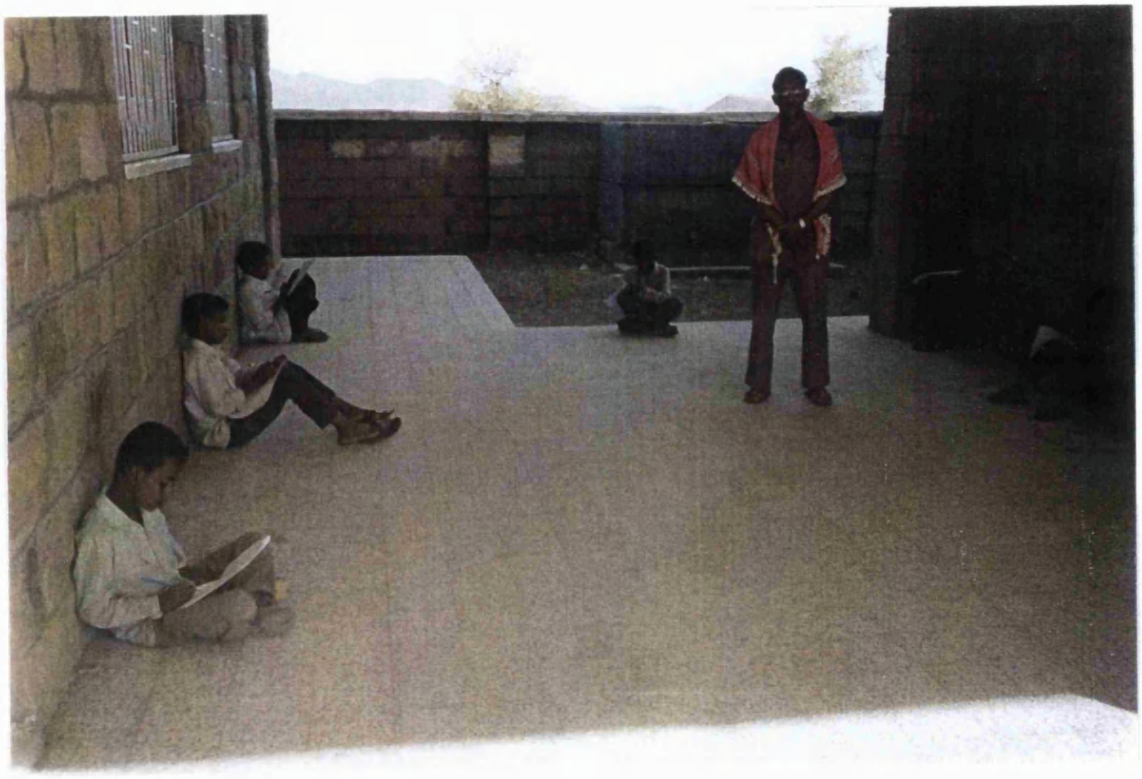


Anthropometric measurements

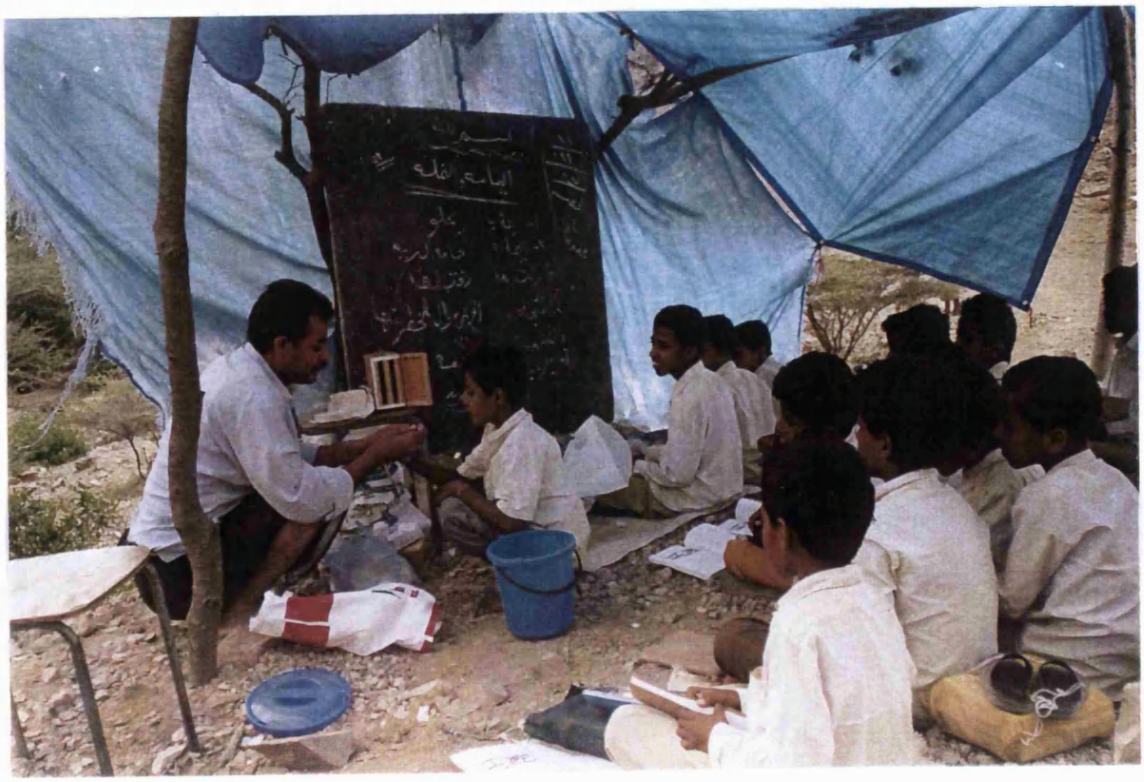




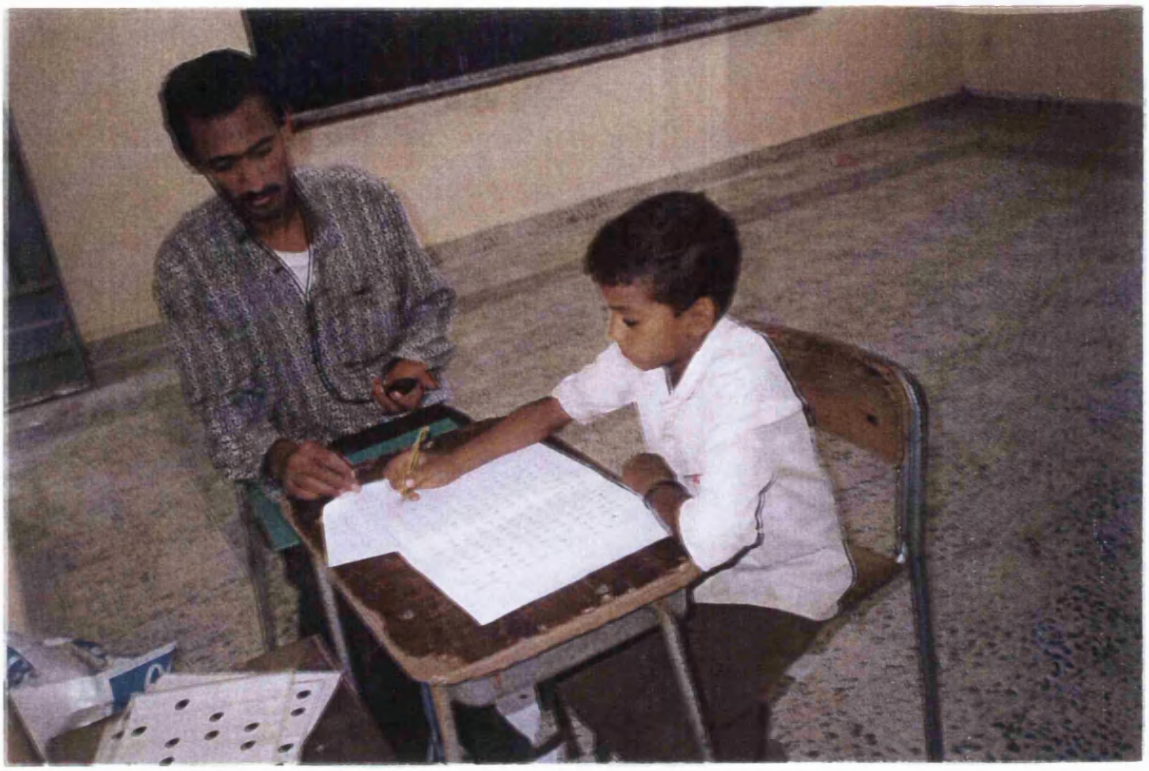
During school achievement tests



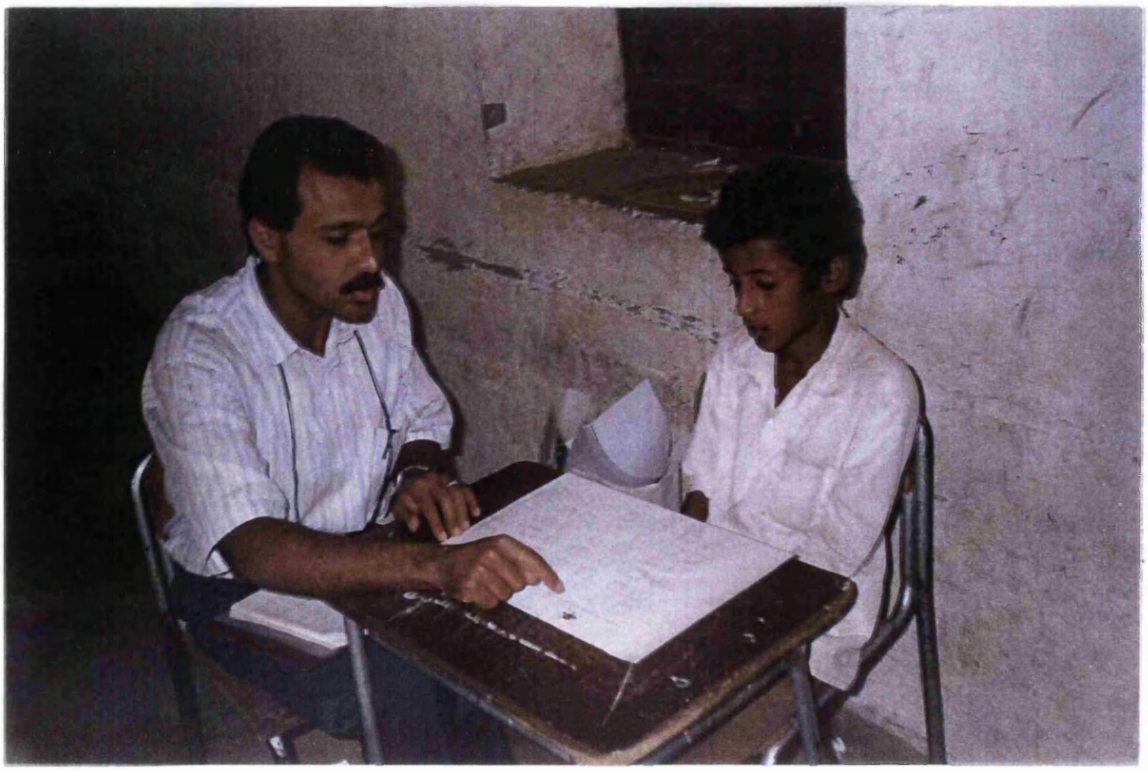
Taking a malaria smear



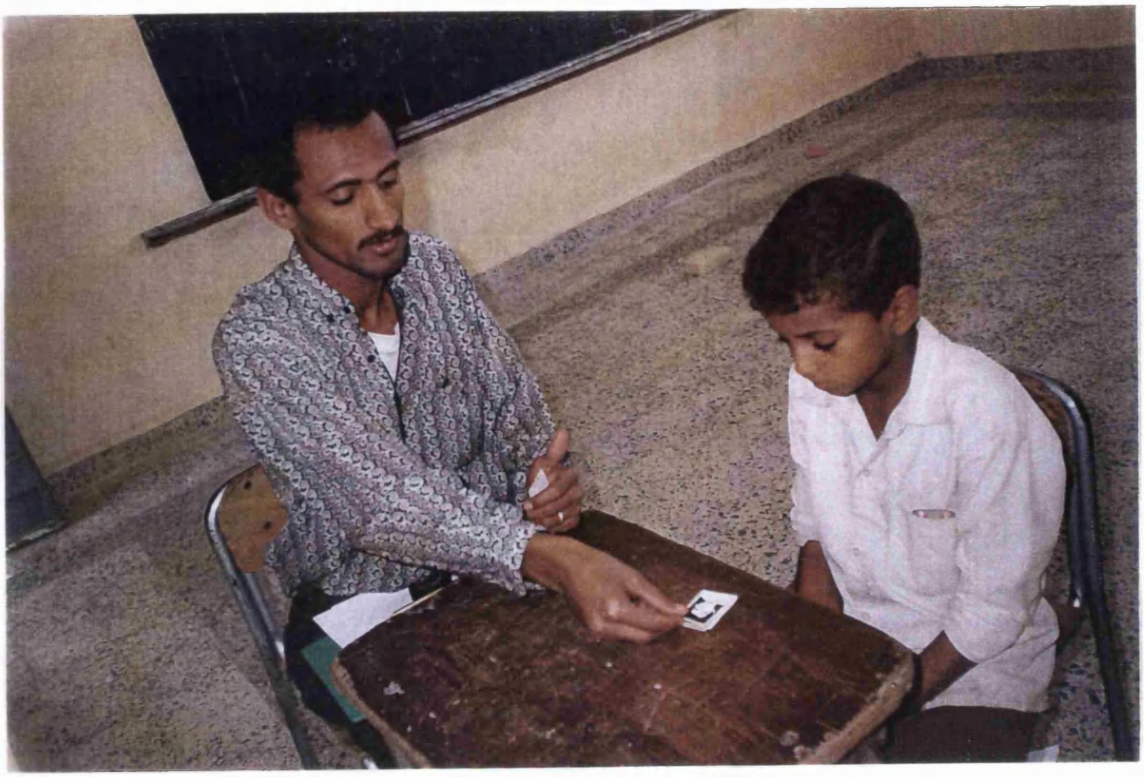
Visual search test



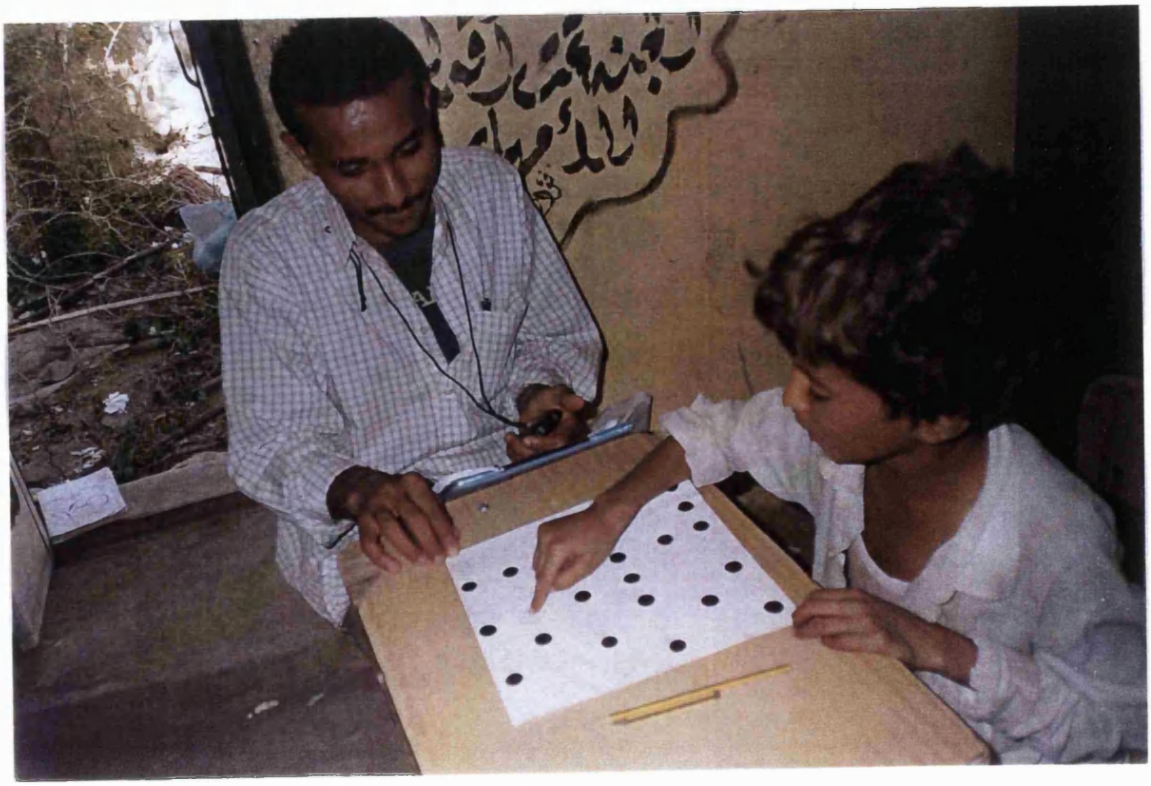
Symbol-symbol test



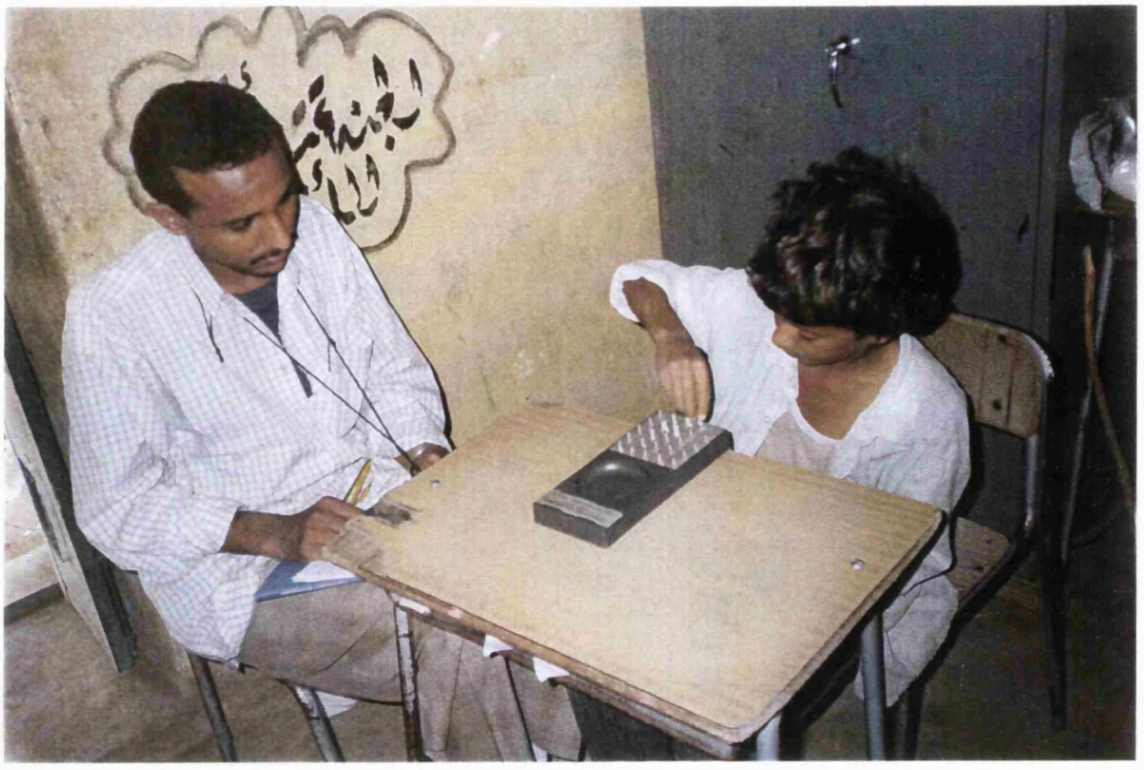
Picture learning test

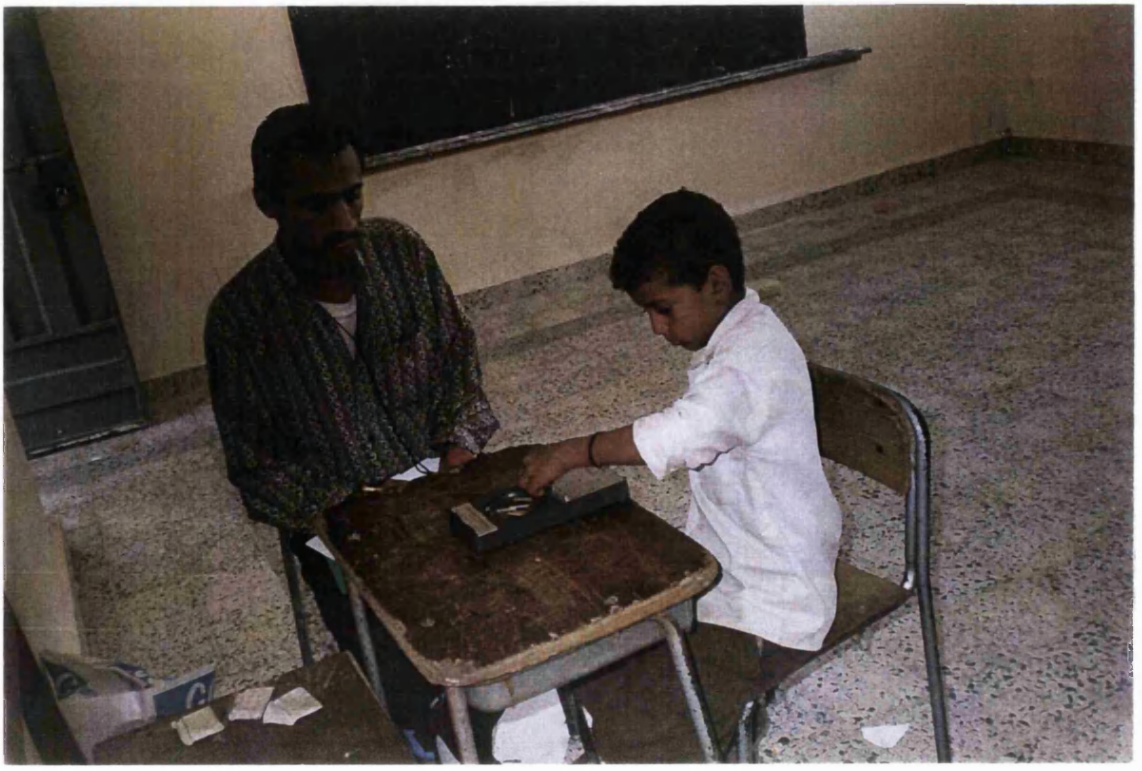


Stroop test



Peg board test





Mosquito breeding sites