SEIZURES IN CHILDHOOD
CEREBRAL MALARIA

A thesis submitted to the University of London for the degree of Doctor of Medicine

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ABSTRACT

Every year, more than one million children in sub-Saharan Africa die or are disabled as a result of cerebral malaria. Seizures complicate a high proportion of cases, and are associated with an increased risk of death and neurological sequelae. This thesis examines the role of seizures in the pathogenesis of childhood cerebral malaria.

The clinical and electrophysiological data presented suggest that seizures may contribute to the pathogenesis of coma in children with cerebral malaria. Approximately one quarter of the patients studied had recovered consciousness within 6 hours of prolonged or multiple seizures, or had seizures with extremely subtle clinical manifestations. EEG recording also demonstrated that electrical seizure activity arose consistently from the posterior temporo-parietal region, a "watershed" area of the brain that is particularly vulnerable to hypoxia. Cerebral computerised tomography (CT) scans from children who had multiple seizures during their clinical course, and who subsequently developed a hemiplegia, revealed infarction in this area.

The thesis discusses the aetiology of seizures in cerebral malaria, and explores the hypotheses that chloroquine may precipitate seizures, and that some of the neurological manifestations of cerebral malaria may be explained by an excitotoxic mechanism.

If anticonvulsant prophylaxis can reduce the incidence of seizures complicating cerebral malaria, this may in turn reduce the risk of neurological sequelae and death. The thesis describes a randomised, controlled intervention study, in which a single intramuscular dose of phenobarbitone 20mg/kg reduced the incidence of seizures by 50% but was associated with an unacceptable doubling of mortality. Post hoc analysis revealed that mortality was highest among those who had been treated with phenobarbitone and multiple doses of diazepam, raising the possibility that respiratory depression was responsible for the increased mortality in the phenobarbitone-treated group. The thesis concludes with a discussion of alternative strategies for anticonvulsant prophylaxis.
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DEDICATION

This thesis is dedicated to the memory of my brother Patrick, who explored so much of Kenya with me.
1. BACKGROUND
Introduction

Almost all of the 1 to 2 million deaths from severe malaria that occur worldwide each year are caused by *Plasmodium falciparum* (WHO 1999a). Cerebral malaria, one of the most serious clinical manifestations, is associated with a mortality of up to 30%, while approximately 10% of survivors are left with permanent neurological sequelae (Molyneux 1989; Bondi 1992; Waller 1995). Seizures complicate a high proportion of cases and, when prolonged and multiple, are associated with an increased risk of death or neurological sequelae (Brewster 1990; Jaffar 1997; van Hensbroek 1997). This thesis examines the role of seizures in the pathogenesis of childhood cerebral malaria.

The introductory chapter will provide a broad overview of the epidemiology and clinical features of *Plasmodium falciparum*, with particular emphasis on cerebral malaria. A review of the classification and neurobiology of seizures will be followed by an outline of the treatment and outcome of status epilepticus. The chapter will end with a description of the prevalence and outcome of seizures in both uncomplicated and severe malaria, and an outline of the central questions that this thesis will address.

Human malaria parasites

Malaria results from infection with one or more of the four species of protozoal plasmodia (*Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*) that infect humans. Transmission follows the bite of a female *Anopheles* mosquito, and the inoculation into the bloodstream of saliva containing infectious sporozoites (Figure 1). Sporozoites circulate in the blood for approximately 30 minutes, before entering hepatocytes or being removed by phagocytic cells. Pre-erythrocytic schizogony in the liver culminates in the release of thousands of merozoites into the bloodstream. Unlike *Plasmodium falciparum* and *P. malariae*, *P. vivax* and *ovale* have the ability to differentiate into hypnozoites, which lie dormant in the liver, and may therefore cause clinical relapses of malaria many years after the initial infection.
Figure 1. Life cycle of the malaria parasite
Once released from the liver, the merozoites invade red blood cells, where they develop into ring forms (trophozoites). Trophozoites are able to metabolise haemoglobin within the red blood cell, producing malaria pigment (haemozoin). Mature trophozoites (schizonts) undergo a second stage of asexual multiplication, producing a species-specific number of merozoites. Although schizonts of *P. vivax*, *P. ovale*, and *P. malariae* may be seen in the peripheral blood, in *P. falciparum* infections they are preferentially sequestered within the microvascular beds of internal organs. Subsequent rupture of the red blood cell causes the release of merozoites into the bloodstream, and the cycle is repeated (approximately every 48 hours for *P. falciparum*) until increasing parasitaemia is inhibited by immunity, chemotherapy, or by the death of the host.

After several cycles of erythrocytic schizogony, some merozoites differentiate into sexual forms (gametocytes), which are ingested by the female *Anopheles* mosquito at the time of a blood meal. The male and female gametocytes fuse to form a zygote, which penetrates the stomach wall of the mosquito. Here, a further stage of asexual reproduction results in the release of approximately 1,000 sporozoites, which migrate through the body cavity of the mosquito to the salivary gland. The entire life cycle is repeated when the mosquito takes a further human blood meal.

**Epidemiology of malaria caused by *Plasmodium falciparum***

**The burden of disease**
Over 40% of the world’s population live where there is a risk of malaria (Figure 2), and approximately 300 million clinical cases occur worldwide each year (WHO 1999b). At least 90% of the 1.2 million deaths caused by severe falciparum malaria occur in sub-Saharan Africa, where malaria accounts for one in five of all deaths in children below the age of five years. The populations most at risk are those for whom poverty, isolation, and inadequate health services mean that there is considerable delay in obtaining appropriate treatment. Malaria is a major impediment to the development and economic advancement
Figure 2. Global distribution of malaria

![Map showing global distribution of malaria risk, with high risk regions in red and low risk regions in green and yellow.](image)
of countries within tropical Africa, and recent research suggests that the economic burden of malaria in Africa is greater than 1% of gross domestic product (GDP) (Nchinda 1998).

The recent resurgence in malaria-related deaths in Africa contrasts dramatically with the global decline in mortality from malaria since 1900. The most important contributory factor is the rapid spread of antimalarial drug resistance (Trape 1998). Resistance to chloroquine is now widespread throughout East and Southern Africa, and is becoming an increasing problem in parts of West Africa. Several countries in East and Southern Africa have been forced to change from chloroquine to sulphadoxine-pyrimethamine (SP) as their first-line treatment for falciparum malaria, yet resistance to SP is increasing fast. Recent data from the East African Network for the Monitoring of Antimalarial Treatment (EANMAT) shows that in Kenya, for example, up to 55% of patients treated with chloroquine and 5-15% of those treated with sulphadoxine-pyrimethamine (SP) have evidence of treatment failure (B. Watkins personal communication). This is extremely worrying, since the cost of any second-line alternatives far exceeds the health budget of most African countries (White 1999b; White 1999d).

But the situation is not hopeless. Malaria is now a major health priority for many countries and international agencies, and considerable amounts of public and private money have been committed to research and control. The World Health Organisation (WHO) has responded to this current crisis with its “Roll Back Malaria” initiative and, at the recent summit in Japan, G8 countries made a commitment to help WHO halve the burden of disease associated with malaria by the year 2010. Insecticide-impregnated bed nets have been shown to reduce all-cause mortality in children below the age of five years by up to 30% (D'Alessandro 1995; Nevill 1996; Lengeler 2000), although it is not clear what the long-term effects of reducing transmission will be (Snow 1997). Over the last decade, advances in molecular technology have resulted in considerable progress towards the development of a malaria vaccine (Riley 1997; Miller 1998).

In response to the drug resistance crisis, the Medicines for Malaria Venture, a joint public-private sector initiative which forms part of the Multilateral Initiative on Malaria
in Africa (Davies 1999), is planning to develop new anti-malarial drugs and drug combinations and to make them available in poor countries. Current research is being directed at the development of new anti-folate combinations, including chlorproguanil-dapsone (Amukoye 1997), which has a shorter elimination half-life than SP and is therefore less likely to induce resistance. There is also considerable interest in the development of combination therapy, using the rapidly acting artemisinin derivatives in combination with other antimalarial drugs (White 1999a; White 1999c).

The natural history of *Plasmodium falciparum* infection: parasitaemia versus disease

In contrast to other infectious diseases, complete immunity to malaria (aparasitaemia plus clinical protection) is probably never achieved, even in settings where individuals have been exposed since birth to continuous, intense malaria transmission (Trape 1994). Clinically “useful” malarial immunity therefore constitutes protection from severe clinical illness, and not the absolute absence of parasites.

Intensity of malaria transmission appears to affect the pattern of both clinical disease (Snow 1994a; Snow 1997) and mortality (Greenwood 1987; Brinkmann 1991; Marsh 1992). In areas of stable transmission, children bear the brunt of malarial morbidity and mortality, with attacks of clinical malaria evident from the age of about four months, and the highest mortality occurring between the ages of one and three years. In areas of very high transmission, the majority of severe cases presenting to hospital are young infants with malarial anaemia, while in lower transmission settings there appears to be an increased proportion of older children with cerebral malaria (Snow 1994a). The subsequent gradual acquisition of acquired immunity means that most individuals above the age of five years will no longer be at risk of death. Infected adults may present with mild symptoms (headache, fever, arthralgia), or remain asymptomatic, and therefore provide a reservoir of infection for subsequent transmission to non-immune individuals. Although non-immune adults do develop life-threatening complications of malaria, the clinical presentation varies in some respects from that of African children (see Table 1).
Table 1. Frequency of severe manifestations of *Plasmodium falciparum* malaria in children and adults

* See Table 3

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<th>Frequency</th>
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<th>SE Asian adults</th>
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<td>Prostration</td>
<td>Inability to sit unassisted, or to drink in the case of children too young to sit</td>
<td>+++</td>
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<tr>
<td>Impaired consciousness</td>
<td>*Blantyre coma score 3 or 4, but able to localise painful stimulus</td>
<td>+++</td>
<td>++</td>
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<tr>
<td>Coma</td>
<td>*Blantyre coma score 2 or less; unable to localise painful stimulus</td>
<td>+++</td>
<td>++</td>
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<tr>
<td>Multiple seizures</td>
<td>2 or more seizures within 24 hours</td>
<td>+++</td>
<td>+</td>
<td></td>
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<tr>
<td>Respiratory distress</td>
<td>Deep breathing (Kussmaul respiration) and indrawing of lower chest wall</td>
<td>+++</td>
<td>+</td>
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<td>Severe anaemia</td>
<td>Haemoglobin &lt;5 g/dl</td>
<td>+++</td>
<td>+</td>
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<td>Hypoglycaemia</td>
<td>Blood glucose &lt;2.2 mmol/l</td>
<td>++</td>
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<tr>
<td>Jaundice</td>
<td>Yellow colouration of sclerae and buccal mucosa</td>
<td>+</td>
<td>+++</td>
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<td>Renal failure</td>
<td>Urine output of &lt;12 ml/kg/24 hours</td>
<td>+</td>
<td>+++</td>
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<td>Pulmonary oedema</td>
<td>Chest x-ray: increased interstitial markings, hazy peri-hilar interstitial shadowing</td>
<td>+/-</td>
<td>++</td>
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<td>Haemoglobinuria</td>
<td>Dark red/black urine, positive for blood on urinanalysis dipstick. Absence of microscopic haematuria</td>
<td>+</td>
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<td>Abnormal bleeding</td>
<td>From gums, nose, venepuncture sites</td>
<td>+/-</td>
<td>+</td>
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<td>Shock</td>
<td>Hypotension, cool peripheries</td>
<td>+</td>
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In situations of high transmission intensity, where a significant proportion of the population may have asymptomatic *P. falciparum* parasitaemia, it becomes difficult to make a definitive diagnosis of malaria. In Kilifi, Kenya, where the studies described in this thesis were carried out, community parasite prevalence rates in children aged one to nine years range from 49% to 74% (Snow 1997). In a setting such as this, therefore, it is not appropriate to base a diagnosis of falciparum malaria solely on the results of a blood smear demonstrating malaria parasites. Although diagnostic specificity may be increased by the use of additional criteria, such as measured fever, this may be at the cost of reducing sensitivity (Armstrong Schellenberg 1994). Diagnostic specificity is, however, dramatically increased by the presence of clinical symptoms and signs known to be associated with severe malaria. It is, therefore, reasonable to diagnose severe malaria in any child with parasitaemia who fulfils the appropriate diagnostic criteria, and in whom other possible diagnoses have been excluded.

The clinical features of falciparum malaria

**Uncomplicated malaria**

The clinical features of uncomplicated malaria are non-specific. Symptoms include fever, vomiting, diarrhoea, and headache, while the presence of persistent vomiting, convulsions, or severe anaemia indicates the need for hospital referral. The key factor is to always consider the diagnosis of malaria in any patient from an endemic area, and to institute prompt treatment with an appropriate dose of an effective antimalarial drug. The danger of delayed or inadequate treatment of uncomplicated malaria is the risk of progression to severe, life-threatening disease.

It is important to remember that, in Africa, the vast majority of patients suffering from febrile illnesses are treated at home with medication bought from a shop or local dispensary. In Kilifi district, a community-based training programme for shopkeepers significantly increased the number of transactions involving the sale of an appropriate dose of chloroquine for the treatment of childhood fevers (Marsh 1999). This approach clearly has considerable potential as an innovative malaria control strategy. Presumptive treatment for
malaria also occurs in health clinics throughout Africa, since the lack of equipment and personnel precludes the use of microscopy.

Severe malaria
Any patient with malaria who is unable to tolerate oral medication, has evidence of vital organ dysfunction, or a high parasite count, is at an increased risk of dying (WHO 2000). The exact risk depends upon the age and background immunity of the patient, and on access to appropriate treatment. Since over 50% of hospital deaths from severe malaria occur within the first six hours of admission, immediate assessment and treatment are mandatory.

Table 1 lists the clinical features that are indicative of severe malaria in patients with *P. falciparum* asexual parasitaemia and no other clearly defined cause for their symptoms. It is clear that the clinical manifestations of severe malaria vary considerably between adults and children. Jaundice and renal failure are prominent features of severe malaria among Southeast Asian adults (Trang 1992; Wilairatana 1994), while convulsions, respiratory distress, and severe anaemia are characteristic of severe disease in children (Molyneux 1989; Marsh 1995; Waller 1995; Allen 1996). Since African children account for over 90% of all deaths from severe malaria, it is useful to classify this group further according to prognosis, in order to identify those at highest risk of dying. Recent research has identified two clinical features, namely respiratory distress (deep breathing, shown to be strongly associated with metabolic acidosis (English 1996a)) and impaired consciousness, that are associated with a greatly increased risk of death (Marsh 1995). Table 2 (WHO 2000) incorporates these findings into a practical classification, likely to be of particular value in situations where limited resources need to be directed at the most severely ill children.
Table 2. Practical classification of severe malaria in children

* See Table 3

Group 1

Children at increased risk of dying who require immediate parenteral antimalarial drugs and supportive therapy

a. Prostrated children: Three subgroups of severity should be distinguished
   - Prostrate but fully conscious
   - Prostrate with impaired consciousness but not in coma (*Blantyre coma score 3 or 4)
   - Coma (the inability to localise a painful stimulus)

b. Respiratory distress (acidotic breathing)
   - Mild: sustained nasal flaring and mild intercostal indrawing (recession)
   - Severe: the presence of either marked indrawing (recession) of the lower chest wall or deep (acidotic) breathing

Group 2

Children who show none of the above features, but who require supervised management because of the risk of clinical deterioration

a. Children with a haemoglobin of <5 g/dl

b. Children with 2 or more seizures within a 24-hour period

Group 3

Children who require parenteral treatment because of persistent vomiting, but who lack any specific clinical or laboratory features of groups 1 or 2 (above)
Impaired consciousness
The conscious level of children with severe malaria is most conveniently assessed using the Blantyre Coma Scale (BCS) (Molyneux 1989). This scale (Table 3), a modified version of the Glasgow Coma Scale used for assessing level of consciousness in adults (Teasdale 1974), is based on the verbal, motor, and ocular responses to a painful stimulus. The BCS is simple, easy to learn, does not depend upon a child’s ability to speak, and gives a good overall assessment of disease severity (Newton 1997a). It is, however, of limited use in children below the age of 8 months, who have not yet acquired the developmental skills necessary to localise a painful stimulus (Newton 1997a). Furthermore, parts of the assessment (such as the decision as to whether a cry is “appropriate” or not) are essentially subjective, and the BCS requires local standardisation in order to minimise the effect of inter-observer variation.

Children who score 4 or less on the BCS are said to have impaired consciousness, while those who are unable to localise a painful stimulus (in whom the BCS will generally be 3 or less) fulfil the definition of unrousable coma. Children in unrousable coma who have *P. falciparum* parasitaemia and no other apparent cause for their encephalopathy are considered to have cerebral malaria. It is important to appreciate, however, that “impaired consciousness” and “cerebral malaria” merely describe the spectrum of a clinical syndrome that may have many causes. Consciousness is usually impaired after a convulsion or following the administration of anticonvulsant drugs, and in these situations it is conventional to allow one hour to elapse before assessing the BCS. Similarly, it is not appropriate to apply the term cerebral malaria to children in whom normal consciousness is restored by the administration of glucose.

Cerebral malaria
African children with cerebral malaria typically present with a short (1-3 day) history of fever, accompanied in approximately 50% of cases by vomiting, and in up to 80% of cases by seizures (Molyneux 1989; Steele 1995; Waller 1995; Crawley 1996; Olumese 1999). In these series, children were unconscious for a median of 8 hours (range 1-72
Table 3. A coma scale for children (Blantyre coma score)
(Molyneux et al. Quarterly Journal of Medicine (1989); 71 (265): 441-459)

<table>
<thead>
<tr>
<th>Clinical response</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Best motor response</strong></td>
<td></td>
</tr>
<tr>
<td>Localises painful stimulus*</td>
<td>2</td>
</tr>
<tr>
<td>Withdraws limb from pain**</td>
<td>1</td>
</tr>
<tr>
<td>Non-specific or absent response</td>
<td>0</td>
</tr>
<tr>
<td><strong>Verbal response</strong></td>
<td></td>
</tr>
<tr>
<td>Appropriate cry</td>
<td>2</td>
</tr>
<tr>
<td>Moan or inappropriate cry</td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td><strong>Eye movements</strong></td>
<td></td>
</tr>
<tr>
<td>Directed (follows mother's face)</td>
<td>1</td>
</tr>
<tr>
<td>Not directed</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
</tr>
<tr>
<td>Maximum 5 (fully conscious)</td>
<td></td>
</tr>
<tr>
<td>Minimum 0 (deep coma)</td>
<td></td>
</tr>
</tbody>
</table>

* Painful stimulus: rub knuckles on patient's sternum

** Painful stimulus: firm pressure on thumbnail bed with horizontal pencil
hours) prior to hospital admission, and in over 50% of cases the onset of coma followed one or more seizures (Crawley 1996).

Clinical examination reveals the presence of brain stem signs (dysconjugate eye movements, decerebrate posturing) in approximately one third of cases (Molyneux 1989; Newton 1991; Newton 1997b; Olumese 1999). The studies described in this thesis have also led to the realisation that children with “cerebral malaria” who present with conjugate eye deviation, nystagmus, salivation, and hypoventilation may be in subtle status epilepticus. Treatment with anticonvulsant drugs may restore consciousness (Crawley 1996; Crawley 1998).

Abnormalities of the ocular fundus have been demonstrated by direct and indirect ophthalmoscopy in over 50% of patients with cerebral malaria (Lewallen 1993; Lewallen 1999). Haemorrhages are the commonest finding, and occur in over one third of cases. These may range in number from 1 to more than 100 and in children, unlike adults, (Looareesuwan 1983a) are not associated with a poor outcome (Lewallen 1996). Papilloedema, found in only 6% of cases (Lewallen 1999), is associated with a relative risk of an adverse outcome (death or neurological sequelae) that is 5.2 times that of children without this finding (Lewallen 1993).

Most children with cerebral malaria recover consciousness within 24 hours of the start of treatment, and the majority go on to make a complete recovery (Molyneux 1989; Steele 1995; Waller 1995). Mortality in different series ranges from between 15-27% (Molyneux 1989; Marsh 1995; Waller 1995; van Hensbroek 1996a). Clinical features consistently shown to be associated with an increased risk of death include deep coma, respiratory distress, and multiple convulsions (Molyneux 1989; Marsh 1995; Jaffar 1997). Laboratory findings of poor prognostic significance include hypoglycaemia (blood glucose below 2.2 mmol/l), acidosis (capillary pH < 7.300), raised plasma lactate (venous lactate 5 mmol/l or more), hyperparasitaemia (parasite density greater than 1 million/µl), and 20% or more of the total parasite count comprising mature trophozoites and schizonts (Taylor 1988; Krishna 1994; Marsh 1995; Waller 1995; Jaffar 1997).
Neurological sequelae occur in a median of 14% (range 11-23%) of survivors at the time of discharge from hospital (Molyneux 1989; Brewster 1990; Bondi 1992; van Hensbroek 1997). Many of these children improve over the ensuing weeks, however, and 7-9% have sequelae that are still present one month after discharge. The commonest problems are hemiplegia, spastic quadriplegia, ataxia, blindness, and speech problems. Factors consistently associated with an increased risk of neurological sequelae are prolonged coma, multiple or prolonged convulsions, and young age. Two recent studies have addressed the possibility that children surviving cerebral malaria may have cognitive problems that could adversely affect their subsequent educational attainments (Muntendam 1996; Holding 1999). Both studies tested a group of children approximately 42 months after an episode of cerebral malaria, and compared their performance with a group of age-matched controls. One study (Holding 1999) demonstrated significant impairment in the ability to initiate, plan, and carry out tasks (the executive functions) among children who had previously had cerebral malaria, while the other study (Muntendam 1996) found no significant difference between the two groups. Further studies are needed to clarify this issue.

The pathophysiology of cerebral malaria

Although the pathological features of cerebral malaria have been described in numerous studies (Edington 1967; MacPherson 1985; Pongponratn 1991; Patnaik 1994), marked heterogeneity between these studies has made their interpretation difficult. The introduction of standardised WHO clinical diagnostic criteria, and recent developments in electron microscopy (EM), immunohistochemistry, and molecular biology have now made it possible to address specific questions regarding the pathogenesis of cerebral malaria (Turner 1997; Newton 1998a). One should note, however, that although the pathological hallmark of cerebral malaria is sequestration of parasitised erythrocytes in the deep microvasculature of the brain (see below), the cause of coma remains unknown. In addition, any pathogenic mechanism needs to explain how, in the vast majority of cases, coma resolves spontaneously, without residual neurological sequelae.
Sequestration of parasitised erythrocytes

Post-mortem histology and electron microscopy of brain tissue from patients with cerebral malaria shows that the cerebral capillaries and post-capillary venules are distended by numerous parasitised erythrocytes, attached by electron dense "knob proteins" to cerebral endothelial cells. This process, sequestration, is mediated through cytoadherence of parasitised erythrocytes to the endothelial lining of the cerebral microvasculature (Berendt 1994b). A number of host molecules, including thrombospondin, intracellular adhesion molecule-1 (ICAM-1), CD36, and E-selectin, are able to bind parasitised erythrocytes, and therefore act as endothelial ligands. Immunohistochemical studies on brain tissue from adults dying of cerebral malaria have demonstrated increased expression of ICAM-1 and E-selectin, with significant co-localisation of sequestered parasitised erythrocytes (Turner 1994). In a recent post-mortem study on 50 Vietnamese adults, evidence of cerebral sequestration was observed in all patients with cerebral malaria (G Turner personal communication).

Adhesion of infected erythrocytes to endothelial ligands is mediated by parasite derived antigens expressed on the red cell surface, of which one of the most important is Pfemp1 (*P. falciparum* erythrocyte membrane protein 1) (Newbold 1997b). These antigens are encoded by a large family of *Var* genes, which have the ability to undergo clonal antigenic variation (Kyes 1997). It is hoped that future studies of ligand-binding *var* gene sequences will help elucidate the mechanisms underlying the variation in cytoadherence phenotype between parasite strains. The increased capacity of parasites causing cerebral malaria to bind to ICAM-1 (Newbold 1997a) supports the hypothesis that severity of clinical disease is associated with specific parasite cytoadherence phenotypes. Further evidence comes from the recently demonstrated association between severe malaria and specific receptor-binding phenotypes, namely those capable of binding immunoglobulin, heparin, and soluble CD31 (K Marsh personal communication), and those involved in rosette formation (the adherence of parasitised erythrocytes to two or more uninfected erythrocytes *in vitro*) (Carlson 1990; Treutiger 1992; Rowe 1995).
Although sequestration appears to be a central event in the pathogenesis of cerebral malaria, the clinical picture cannot be explained satisfactorily by a single mechanism (Berendt 1994a; Clark 1994). Some authors propose that sequestration is merely an epiphenomenon, and that the rapidly reversible symptoms of cerebral malaria may be mediated by soluble circulating factors such as cytokines or nitric oxide (Clark 1991; Clark 1992). It seems more likely that sequestration of parasitised erythrocytes initiates a chain of events that includes the release of soluble mediators from parasitised erythrocytes or host cells in the brain, leading ultimately to coma.

Cytokines

The concept that severe malaria may be mediated by the products of activated macrophages (Clark 1981) arose from the observation that clinical similarities exist between severe malaria and endotoxaemia. There is now a considerable body of clinical and laboratory evidence to suggest that cytokines play an important role in the pathogenesis of severe malaria. Soluble antigens of *P. falciparum*, which share many of the properties of bacterial lipopolysaccharide, are potent inducers of TNF release from monocytes *in vitro* (Kwiatkowski 1989; Taverne 1990). Several clinical studies have now confirmed the positive association between plasma concentrations of TNF and mortality from severe malaria (Grau 1989; Kern 1989; Kwiatkowski 1990; Krishna 1994). More recently, a large case-control study demonstrated that Gambian children with a genetic polymorphism in the TNF promoter region had a seven-fold increase in the risk of death or neurological sequelae from cerebral malaria (McGuire 1994). Plasma concentrations of the pro-inflammatory cytokines interleukin (IL)-1 (Kwiatkowski 1990), IL-6 (Kern 1989), and IL-8 (Friedland 1993) have been shown to be correlated with disease severity, although there is considerable overlap between different patient groups. Severe malaria may also be associated with an inadequate negative feedback response to IL-10, which has been shown to counter-regulate the pro-inflammatory response to *P. falciparum* malaria (Ho 1995; Ho 1998).

There are a number of limitations to the hypothesis that cytokines *per se* play a major role in the pathogenesis of severe malaria. Firstly, plasma concentrations of pro-inflammatory
cytokines are very high in *P. vivax* malaria, particularly following schizogony in synchronous infections (Karunaweera 1992), yet *P. vivax* is not lethal and does not cause coma. If the pro-inflammatory cytokines do play a central role in severe malaria, this is likely to be at a local level, and may not be reflected in systemic cytokine measurements. Finally, although anti-TNF antibody has been shown to be anti-pyretic (Kwiatkowski 1993), there was no evidence of clinical benefit when it was administered to Gambian children with severe malaria in a prospective, randomised trial (van Hensbroek 1996b).

**Nitric oxide**

TNF is a potent inducer of nitric oxide synthase type 2 (NOS2), and investigators have hypothesised that, in cerebral malaria, nitric oxide (NO) may play a central role in the pathogenesis of coma (Clark 1992). High local concentrations of TNF may induce excessive synthesis of nitric oxide (NO) within cerebrovascular endothelial cells. NO diffuses across the blood-brain barrier, and may inhibit the glutamate-induced entry of calcium into post-synaptic neurones, so reducing the activity of calcium-dependent constitutive NO synthase. The consequent reduction in the level of NO within post-synaptic neurones could result in profound but reversible coma. The same mechanism has been postulated to underlie coma induced by general anaesthesia or ethanol (Clark 1992).

The testing of this hypothesis has centred round the measurement in plasma of the stable NO metabolites nitrate and nitrite (collectively termed reactive nitrogen intermediates, RNI). Results from studies adopting this approach have been contradictory. Some studies have found a positive correlation between plasma RNI and disease severity (Cot 1994; Al Yaman 1996; Kremsner 1996), while others have shown either no association (Agbenyega 1997; Taylor 1998) or an inverse relationship (Anstey 1996). Discussion has focussed on the role of two potential confounding factors, namely dietary nitrogen and reduced clearance of RNI due to renal impairment. Anstey, who controlled for both in his carefully designed study, also measured NOS2, and found that levels were undetectable in children with cerebral malaria (Anstey 1996). He went on to demonstrate an age-related pattern of NO production among asymptomatic, malaria-exposed Tanzanian children that was the reverse of the age-related pattern of mortality from cerebral malaria.
among that group (Anstey 1999). The elevation of NO production that was observed among infants and older children suggests that this may be a mediator of anti-disease immunity.

**Raised intracranial pressure**

Raised intracranial pressure (ICP), which is a feature of many paediatric encephalopathies (Minns 1991), appears to play a more significant role in the pathogenesis of cerebral malaria in children compared to adults. At lumbar puncture, opening cerebrospinal fluid (CSF) pressures were found to be normal in non-immune adults with cerebral malaria (Warrell 1986), while cerebral computerised tomography (CT) (Looareesuwan 1983b) and magnetic resonance imaging (Looareesuwan 1995) suggested that, in this group of patients, cerebral oedema is a rare, agonal event. In a controlled study on adult patients with cerebral malaria, dexamethasone was associated with prolongation of coma and an increased complication rate (Warrell 1982). These findings suggest that, in adults, raised ICP does not appear to play an important role in the pathogenesis of cerebral malaria.

But the situation appears different in children. Cerebrospinal fluid opening pressure at lumbar puncture was raised in two studies on African children with cerebral malaria (Newton 1991; Waller 1991). In a subsequent study on 23 Kenyan children, ICP was directly measured by means of a subarachnoid catheter (Newton 1997b). Concurrent measurement of intra-arterial pressure meant that it was also possible to calculate cerebral perfusion pressure (CPP). All children had raised ICP, though in 10 cases this was mild (maximum ICP 10-20 mm Hg, minimum CPP > 50 mm Hg). Thirteen children had intracranial hypertension of a degree that would normally merit specific intervention. Of these, 9 had intermediate intracranial hypertension (maximum ICP > 20 mm Hg, minimum CPP 40 – 50 mm Hg for >15 minutes continuously), and in 4 cases this was severe (maximum ICP >40 mm Hg, minimum CPP < 40 mm Hg for >15 minutes continuously). Although intravenous mannitol controlled ICP in children with intermediate intracranial hypertension, it failed to prevent progression to an intractable state in children with severe intracranial hypertension, of whom two died (one with
clinical signs compatible with the medullary stage of transtentorial herniation), and two were left with severe neurological sequelae. Ten of these children were included in a study that compared the findings from transcranial Doppler ultrasonography on 50 children with cerebral malaria with those from 115 conscious Kenyan children (Newton 1996). In children with severe intracranial hypertension, a linear relationship was found between CPP and blood flow velocity, suggesting that cerebral autoregulation may have been impaired. Of four children who died, three had sonographic features compatible with progressive intracranial hypertension.

Intracranial pressure rises if there is an increase in the volume of cerebrospinal fluid, blood, or brain tissue. CT scans from both adults and children with cerebral malaria showed no evidence of acute hydrocephalus (Looareesuwan 1983b; Newton 1994). Brain swelling (defined as the loss of cerebrospinal fluid spaces) was, however, documented on cerebral CT scans from 6/14 Kenyan children recovering from cerebral malaria, while conspicuously small ventricles were seen in two further patients (Newton 1994). In two cases this appearance resolved completely, and the children went on to make a full recovery. In the remaining four cases, diffuse brain swelling was associated with the presence of hypodense lesions and loss of grey/white differentiation, appearances consistent with the presence of cytotoxic oedema secondary to cellular damage. Severe intracranial hypertension had been documented in two of these children, and all four developed severe neurological sequelae. These findings suggest that, for the majority of children with cerebral malaria in whom intracranial hypertension is only mild or moderate, increased cerebral blood volume is likely to account for the brain swelling. Increased blood volume may result from impaired venous drainage (secondary to sequestration), vasodilatation (secondary to acidosis or nitric oxide), reduced blood viscosity (secondary to anaemia) or increased cerebral blood flow (secondary to seizures). In the few children who develop severe intracranial hypertension, cellular damage and secondary cytotoxic oedema may result from reduced cerebral perfusion, hypoglycaemia, or uncontrolled seizure activity.
Seizures and status epilepticus: definitions and classification

Definitions
The following definitions, formulated in 1993 by the International League against Epilepsy (ILAE), will be used throughout this thesis:

Epileptic seizure: A transitory clinical manifestation of sudden onset, presumed to result from an abnormal and excessive discharge of a set of neurons in the brain. Clinical features, perceived by either the patient or an observer, can include an alteration of consciousness, and motor, sensory, autonomic, or psychic events.

Status epilepticus: A single epileptic seizure of more than 30 minutes duration, or a series of epileptic seizures over a period of more than 30 minutes, with failure to regain normal function between each seizure.

Classification
In 1993, the ILAE produced the following classification of epileptic seizures, which is based predominantly on clinical criteria:

1. Classification by type:
   
   Generalised: A seizure is considered generalised when there is no evidence of a focal onset, and clinical symptomatology provides no information on anatomical localization. Generalised seizures may be subdivided into convulsive seizures with predominantly tonic, clonic, or tonic-clonic features, non-convulsive (absence) seizures, and myclonic seizures.

   Partial: A seizure is considered partial when there is clinical evidence of a focal onset. The initial clinical features are of great localising value, since they arise from neuronal activation of part of one cerebral hemisphere. Seizures are described as simple partial when alertness and the ability to interact appropriately with the environment are maintained. When impairment of consciousness, amnesia, or
confusion during or after a seizure is reported, the seizure is classified as complex partial. Partial seizures may become secondarily generalised.

2. Classification by cause:

Provoked (acute symptomatic): These seizures arise from known or suspected cerebral dysfunction. They occur in association with an acute systemic toxic or metabolic insult, or in association with an acute insult (infectious, metabolic, toxic, or traumatic) to the central nervous system. Seizures complicating malaria clearly fall within this category.

Unprovoked (seizures of unknown aetiology): include idiopathic epilepsy syndromes with specific clinical and electroencephalographic characteristics, and cryptogenic seizures for which no risk factors have been identified.

Neurobiology of seizures and status epilepticus

Neurochemistry

Seizures reflect an imbalance between excitation and inhibition in the brain (Mody 1992), and computer models of neuronal networks suggest that only a small reduction in inhibition will cause the system to discharge excessively (Traub 1982). In the brain, the most important excitatory process is the excitatory postsynaptic potential (EPSP), while inhibition is mediated through the inhibitory postsynaptic potential (IPSP) (Fisher 1995). Over 100 neurotransmitters or neuromodulators are involved in neuronal transmission, of which the amino acid glutamate is probably the most important excitatory neurotransmitter, and gamma-aminobutyric acid (GABA) the most important inhibitory neurotransmitter (Fisher 1991).

The glutamate receptor has a complex macromolecular structure, and to date at least five subtypes have been demonstrated (Wasterlain 1993). Of these, the most studied is the N-methyl-aspartic acid (NMDA) receptor (Dingledine 1983). While other glutamate receptor subtypes open sodium channels and generate depolarizing EPSPs, the NMDA
receptor comes into play once the neuron has been partially depolarized, and amplifies the response. Depolarization of the neuron causes magnesium ions to be extruded from the ion channel within the NMDA receptor, so opening the channel to an influx of calcium ions. These depolarize the neuron further and initiate a multiplicity of changes that are critical to learning and memory (Collingridge 1987; Worley 1987). Excessive calcium within the neuron can, however, lead to cell injury and death (Sloviter 1991). Because it is inactivated at normal resting potentials, and becomes active in neurons that are already depolarized, the NMDA receptor is believed to play a significant role in the generation of epileptiform discharges. NMDA antagonists and channel blockers are now under study as potential anticonvulsant drugs, and several are being assessed for their possible neuroprotective action after stroke. The special role of magnesium as a "guardian" of the NMDA channel has led to its use in the treatment of seizures, and in a multicentre, randomised study, magnesium sulphate was shown to be highly effective at controlling eclamptic seizures (Duley 1995).

The GABA receptor is also a macromolecular complex, comprising sites for GABA binding, regulatory sites, and ionic channels (Costa 1981). When GABA, or a pharmacological GABA agonist, binds to the receptor it initiates a change in its protein configuration. This opens a channel for the influx of chloride and, in the mature brain, produces the hyperpolarization of the IPSP. In the first week of postnatal life, however, when excitatory inputs are still poorly developed, stimulation of GABA receptors results in depolarisation (Ben-Ari 1994). During this period, therefore, GABA receptors provide the excitatory drive necessary for the outgrowth of pyramidal cells. Benzodiazepines and barbiturates, which interact with regulatory sites on the GABA complex and augment the inhibitory activity of GABA, are effective anticonvulsants (MacDonald 1979).

Neurophysiology of status epilepticus
Since ethical constraints prevent detailed recording from the cerebral neurons of living human subjects, much of our current knowledge of the neurophysiology of seizures and status epilepticus has come from animal models. Unfortunately, no ideal animal model exists, and since physiological changes at a systems (as opposed to a cellular) level show
marked inter-species variation, caution is needed before extrapolating experimental findings from animals to the human condition (Loscher 1988; Fisher 1989).

Although the electroclinical features of isolated seizures are similar to those seen in the initial stages of status epilepticus, there is evidence to suggest that the precipitating factors are not the same (Shorvon 1994b). In animal experimentation, the intensity of an epileptic stimulus (duration of electrical stimulation or dose of a chemical convulsant) determines whether isolated seizures or status occur. In humans, there is an increased propensity for status following drug withdrawal, fever, or metabolic disturbance. Seizure initiation requires the presence of cells with intrinsic burst-generating properties (pacemaker cells), loss of postsynaptic inhibitory control around these cells, and synchronisation of the epileptic discharges (Schwartzkroin 1986). Cells with burst potential are particularly evident in layers CA1 and CA3 of the hippocampus, and in layers IV and V of the neocortex. Inhibition is largely mediated by GABA receptors, as described above, while synchrony is thought to result from both glutamate-mediated excitatory neurotransmission and non-synaptic mechanisms. Epileptogenesis may result from a number of processes, including alteration in the properties of neuronal membranes leading to hyperexcitability, selective impairment of inhibitory processes, and gliosis with the resultant accumulation of ionic potassium (Prince 1985). The activity of individual cells forms the basis for subsequent synchronised discharges from large populations of neurones (hypersynchrony) (Wong 1986).

Propagation of epileptic discharges occurs largely along existing pathways, but may also utilise non-synaptic mechanisms (Meldrum 1988). It is now clear that, as status progresses, specific physiological and chemical changes occur which perpetuate the seizure activity. Experimental induction of limbic status epilepticus in the rat, a useful model of focal status, suggests that seizure activity is maintained within a feedback loop linking hippocampal and parahippocampal structures (Lothman 1989). Hippocampal slices from these animals show a decrease in GABA-mediated inhibition and an altered sensitivity to extracellular ions, suggesting that these are the biochemical changes that might underlie chronic focal epilepsy (Lothman 1990). Cycling of electrographic activity
is also a common feature of complex partial status in humans, and the identification of a similar underlying mechanism may provide a target for future therapy.

The increased incidence of status epilepticus in children compared to adults is probably due to a combination of increased seizure susceptibility and decreased ability to mount an adequate inhibitory response. The seizure threshold in the immature brain appears to be lower than in the mature brain, although the mechanisms underlying this susceptibility remain unclear. Excitatory synapses mature earlier than inhibitory synapses, increasing the likelihood that excitation-inhibition imbalance may occur. In addition, the immature cerebral cortex has a high synaptic density, and this may contribute to the development of hypersynchrony among neural groups (Huttenlocher 1987; Schwartzkroin 1993).

Detailed neurophysiological data on generalised status epilepticus has come from the experiments of Meldrum and Horton on bicuculline-induced seizures in the adolescent baboon (Meldrum 1973a). At the onset of status, myoclonic jerks are associated with irregular widespread cortical spike-wave activity on electroencephalogram (EEG). A tonic spasm, lasting for 10-30 seconds, is then followed by continuous clonic activity, with rhythmic poly-spikes and waves on EEG. This clinical and electrographic pattern can be maintained for approximately one hour, after which the limb jerking starts to wax and wane and to become asymmetrical, and EEG activity becomes irregular in both rhythm and distribution. At the end of status, postictal electrographic silence is followed by the appearance of isolated biphasic waves, which gradually become more rhythmic and generalised, evolving into diffuse high-voltage delta activity. A similar sequence of electrographic events has been observed during convulsive status in both humans and in three different rat models of status (Treiman 1990), and presumably reflects evolving neurochemical changes or possible neuronal damage secondary to prolonged seizure activity.

Meldrum and Horton were the first to point out that prolonged status results in physiological decompensation, the shift from phase I (compensation) to phase II (decompensation) occurring after about 20-30 minutes of continuous seizure activity in
baboons, and after approximately 30-60 minutes in humans. During phase 1 cerebral
blood flow is greatly increased, and is sufficient to compensate for the great increase in
cerebral metabolic activity. Blood pressure and cardiac output both rise, and autonomic
over-activity results in sweating, hyperpyrexia, bronchial secretion, and salivation.
Arteriovenous differences for oxygen and carbon dioxide both fall, and glucose and lactate levels rise. In phase II, the physiological mechanisms compensating for the
increase in cerebral metabolism begin to fail. Cerebral autoregulation breaks down progressively, and cerebral blood flow becomes increasingly dependent on systemic
blood pressure. Hypotension develops and, in the terminal stages, may be profound.
Falling blood pressure reduces cerebral blood flow and metabolism. The high metabolic
demands of the epileptic cerebral tissue now cannot be met, and there is a risk of ischaemic or metabolic damage. Blood glucose falls and severe hypoglycaemia can
develop. Hypokalaemia and acidosis may induce cardiac arrythmias, which are a
common cause of death. Intracranial pressure can rise precipitously during the late stages
of status, and the combined effects of systemic hypotension and intracranial hypertension
can compromise the cerebral circulation and cause cerebral oedema. Other late
complications are acute tubular necrosis secondary to myoglobinuria or dehydration,
acute hepatic failure, rhabdomyolysis, and disseminated intravascular coagulation.

Neuropathology of status epilepticus
Assessment of the neuropathological features of status epilepticus is complicated by the
fact that pathological changes within the brain may represent the cause or the result of
either status or its systemic complications. Whilst there is no doubt that status can induce
cerebral damage, the degree to which this occurs in humans, and the nature of the
changes, are both still highly controversial (Shorvon 1994a). The situation has been
complicated by differing definitions of status epilepticus, and by the fact that propensity
to cerebral damage varies with the age of the patient and with the cortical site, duration,
and type of status.
**Human pathological studies**

Gross postmortem examination of the brain of patients dying in status epilepticus is often normal, although cerebral swelling, congestion, and scattered haemorrhages are observed in some patients. The hippocampus may be swollen during the acute phase of status, especially in small children, and atrophic changes may subsequently develop in both the hippocampus and the cerebellum. Hippocampal damage was first described in a series of patients with chronic epilepsy (Sommer 1880). The gliosis and pyramidal cell loss, occurring predominantly in the CA1 region of the hippocampus, is now referred to as Ammon's horn sclerosis, hippocampal sclerosis, or mesial temporal sclerosis. Further studies of the acute cerebral changes in status epilepticus have confirmed these findings, and have also documented damage in the CA3 region of the hippocampus, certain regions of the cerebral neocortex, and in the cerebellum (Norman 1964; Margerison 1966; Ounsted 1966). The relative vulnerability of the CA1 and CA3 regions to damage in human status has recently been confirmed by the quantitative assessment of neuronal density (DeGiorgio 1992), and may be due to the high concentration of NMDA and kainate receptors in this region. Sano and Malamud (Sano 1953) originally described the association between hippocampal sclerosis and anterior temporal spikes-wave discharges in patients with epilepsy. Demonstration of the increased vulnerability of the childhood brain to status-induced ischaemic damage (Margerison 1966; Ounsted 1966; Corsellis 1983) subsequently led to the hypothesis that status epilepticus in early childhood may cause ischaemic brain damage and hippocampal sclerosis, which in turn may cause temporal lobe epilepsy.

The mechanism of the cerebral changes in status remains speculative. A number of hypotheses have been proposed in the past, including cerebral vascular spasm, hypotension, systemic hypoxia, and cerebral oedema causing mesial temporal tentorial herniation and anterior choroidal and posterior cerebral artery compression (Shorvon 1994b). The absence of either oedema or tentorial herniation in pathological specimens makes the latter theory unlikely. Other workers have suggested that the seizure discharges themselves may damage the brain through ischaemic or metabolic injury (Falconer 1964; Ounsted 1966). There is now a considerable body of evidence to suggest
that excitotoxic amino acids play an important role in the development of structural brain damage secondary to status epilepticus. Direct application of glutamate onto hippocampal cultures causes neuronal death, similar to that seen in experimental animal models of status epilepticus (see below) (Vornov 1995). Neuronal death appears to be mediated by co-activation of NMDA and AMPA (alpha-amino-3-hydroxy-5-methylisoxazole) receptors, since blockade of either receptor type prevents neuronal injury. Binding of glutamate to the NMDA receptor causes an influx of calcium, and high intracellular concentrations of calcium initiate a large number of processes that may damage the cell (Choi 1994). These include the activation of protein C kinase which damages the cell wall, the formation of nitric oxide which inhibits mitochondrial respiration both directly and indirectly through the formation of cytotoxic peroxynitrite free radicals, and the activation of phospholipase A$_2$, which breaks down membrane lipids (Dawson 1992; Bruno 1993). Glutamate receptor stimulation also regulates the expression of a number of effector genes, some of which activate brain repair mechanisms and are therefore neuroprotective, while others induce cell death (Akins 1996).

**Animal pathological studies**

The clearest evidence that status epilepticus can result in cerebral damage has come from animal experimentation, of which the classic studies were on adolescent baboons (Meldrum1973a; Meldrum 1973c). Meldrum and Brierley used bicuculline, a GABA-antagonist, to induce generalized seizures, lasting for between 82 and 299 minutes, in 10 baboons. The systemic manifestations, namely hypertension, acidosis and hyperglycaemia followed by increasing hyperpyrexia, hypotension and hypoglycaemia, were strikingly similar to those observed in human status. Neuronal damage was incurred from about 25 minutes, and consisted of ischaemic changes in the CA1 region of the hippocampus, layers III, V, and VI of the neocortex, the arterial watershed zone of the cerebellum, and portions of the basal ganglia. This pattern corresponded to the selective pattern of vulnerability seen in the human brain after status epilepticus. The authors considered that, had the animal survived, the dead nerve cells would have been removed by phagocytosis and replaced by glial proliferation, resulting in cerebral and cerebellar atrophy and sclerosis of the hippocampus.
The experiments were then repeated in eight paralysed and artificially ventilated baboons, in whom hypotension, acidosis, hypoxia, and hypoglycaemia were prevented (Meldrum 1973c). Neuropathological examination revealed a similar pattern of neocortical and hippocampal damage, although the pathological changes were generally less severe. Only the cerebellar damage was totally prevented by paralysis, suggesting that was related to hyperpyrexia and hypotension. It was concluded that the cerebral changes seen in status were not of vascular origin, but resulted from impaired cellular metabolism.

Work on other animal models of generalised convulsive status has largely confirmed the findings of Meldrum and colleagues, although more recent research has concentrated on focal status, especially that involving the limbic or mesial temporal lobe structures. Systemic or intra-amygdaloid injection of kainic acid, a glutamic acid analogue, will induce limbic status epilepticus in the rat. This results in a disseminated pattern of acute brain damage, particularly affecting the CA3 and CA4 regions of the hippocampus. The extent of damage correlates with the severity and duration of seizure activity (Olney 1974; Olney 1979). Other convincing experimental models are those that show “remote” damage resulting from seizure spread, so differentiating “epileptic” damage from intrinsic damage produced by the experimental lesion (Sloviter 1983). Differences in the distribution, degree, and timing of cerebral damage caused by seizure activity, hypoglycaemia, and hypoxia have also been the subject of intensive study (Siesjo 1986).

**The emergency treatment of generalised tonic-clonic status epilepticus**

Generalised tonic-clonic status epilepticus is a medical emergency. Immediate treatment is required because outcome is directly related to the duration of status. The three main aims of treatment are to stop seizure activity as quickly as possible, to maintain adequate cardiovascular and respiratory function (with particular emphasis on the avoidance of hypotension and hypoxia), and to minimise other metabolic, systemic, and medical complications. The following modified treatment regimen is based on the facilities and
drugs that were available at the Wellcome Trust-KEMRI Clinical Research Unit at the time the studies described in this thesis were carried out (Crawley 1996; Crawley 2001):

**General measures**

0 - 10 minutes
- Assess cardiorespiratory function, secure airway, give oxygen
- Establish intravenous access
- Emergency anticonvulsant drug therapy (see below)

10 - 30 minutes
- Monitor neurological status, vital signs, electrocardiogram (ECG), oximetry
- Measurement of blood gases, electrolytes, glucose, full blood count
- Emergency treatment of hypoglycaemia if indicated (glucose 0.5 g/kg i/v over 15 minutes)

30 - 90 minutes
- Specialized monitoring, as indicated: intra-arterial blood pressure, central venous pressure, intracranial pressure (ICP) monitoring, electroencephalography (EEG) or cerebral function monitor
- Pressor therapy, mannitol as required: there were no facilities for mechanical ventilation at the Clinical Research Unit, but patients could be intubated and hand ventilated in an emergency
- Active treatment of any additional complications: hyperpyrexia, hypoglycaemia, electrolyte disturbance, lactic acidosis, acute renal failure

**Emergency anticonvulsant drug therapy**

0 - 30 minutes
- Emergency anticonvulsant therapy is started at 5 minutes
- Diazepam: 0.3 mg/kg i/v over 2 minutes or 0.5 mg/kg of intravenous solution per rectum. If seizure activity continues, a maximum of 3 further doses of diazepam can be given at 10 to 15-minute intervals
Then

Paraldehyde: 0.2 ml/kg i/m or per rectum. The dose can be repeated after 10 minutes, and the risk of accumulation is low. Paraldehyde has a longer duration of action than diazepam, and blood levels are virtually constant between 20 minutes (time of peak concentration) and 120 minutes of a single i/m injection. In contrast, blood levels of diazepam fall from high peak to subtherapeutic levels (< 200ng/ml) within 20 minutes of i/v injection (Shorvon 1994b))

30 – 90 minutes

Phenytoin: 15 – 20 mg/kg i/v over 30 minutes

Or

Phenobarbitone: 15 – 20 mg/kg i/m or i/v over 30 minutes

Refractory status (> 90 minutes)

Thiopentone: 3 – 5 mg/kg i/v over 30 seconds, followed by 1 – 5 mg/kg/hour

Maintenance of long-term anti-epileptic therapy in tandem with emergency treatment

Outcome of status epilepticus in children

There are several points that should be remembered when considering the large number of studies describing the outcome of status epilepticus in children. Most studies are confined to convulsive status, are retrospective, and are based on patients within a hospital setting. This introduces an obvious source of bias, since milder cases are more likely to have been missed, thereby magnifying the apparent overall mortality and morbidity from status (Verity 1998). In retrospective series, it is often difficult to determine whether deaths should be attributed to status or to a wide variety of underlying conditions. Changing definitions of status pose additional obstacles to accurate risk assessment.

In children, there are considerable differences between the outcome of febrile status epilepticus (status epilepticus associated with fever in a neurologically normal child
between the ages of 6 months and 5 years) and non-febrile convulsive status. The outcome from non-febrile status is primarily dependent on the underlying aetiology, which in turn is dependent on the age of the child (Shorvon 1994a). The prognosis of febrile status is generally good, with a very low risk of neurological or cognitive impairment, but a significant risk of subsequent epilepsy (21%, compared to 0.5-1% for the population as a whole) if seizures are prolonged (Verity 1993). About half of these children will go on to have complex partial seizures, of whom many will have mesial temporal sclerosis. Although the relationship between convulsive status epilepticus and mesial temporal sclerosis is presumed to be causative, this has not been proved. Approximately 50% of adults with temporal lobe epilepsy secondary to mesial temporal sclerosis have a history of prolonged febrile convulsions in childhood (Shorvon 1994b).

There has been an apparent decrease in mortality and morbidity from convulsive status epilepticus since 1970, when Aicardi and Chevrie published their retrospective review of 239 hospitalised cases (Aicardi 1970). In this series, mortality was 11%, while 53% of patients had a poor neurological or cognitive outcome. The hemiconvulsion, hemiplegia, epilepsy syndrome documented in this series is now a rare complication of convulsive status, occurring only in children with seizures lasting for more than one hour. By 1997, mortality had decreased to between 0% and 6% (Maytal 1989; Lacroix 1994; Eriksson 1997), while between 15% and 33% had neurological sequelae. There are a number of possible explanations for this observation. Benzodiazepines had not been introduced into clinical practice in 1970, and the incidence of prolonged seizures was consequently likely to be higher. Aicardi and Chevrie also required that seizures lasted for at least one hour to fulfil their definition of status epilepticus, while recent studies have used a cut-off of only 30 minutes. The longer a seizure lasts, the more difficult it becomes to treat, increasing the likelihood of a poor outcome (Shorvon 1994a).

Prospective, community-based epidemiological studies that attempt to define the incidence and outcome of convulsive status epilepticus avoid the bias that is inevitably introduced by the study of hospital-based populations. Unfortunately, such studies are difficult to perform, since they are time-consuming and depend upon the existence of a
network between all hospitals in a delineated area. Using this approach, DeLorenzo and colleagues in Richmond, Virginia, found a total incidence of convulsive status epilepticus of 41/100,000 residents, this figure rising to 147/100,000 in infants aged 1 month to 1 year (DeLorenzo 1995). The mortality in children was only 2.5%, with all the deaths being caused by non-central nervous system infections. Among a cohort of 14,676 children who were born during one week in 1970 and followed for 10 years as part of the Child Health and Education Survey, 32 had one or more episodes of convulsive status epilepticus (Verity 1993). Two of these children died, both of whom had presented with non-febrile status epilepticus. One child developed neurological sequelae, while non-febrile seizures developed in 21% of children following prolonged febrile convulsions.

Seizures as a complication of childhood malaria

Local perception of seizures

Seizures are well recognised by the rural community of Mijikenda farmers living in the Kilifi district of Kenya. (Further details of this population are given in Chapter 2). Seizures, or nyuni, are perceived as a serious childhood illness, requiring immediate attention (Mwenesi 1995a; Molyneux 1999b). The recognised symptoms include high fever, rolling up of the eyes, “looking terrified”, “chewing the teeth”, unconsciousness, jerking of the arms and legs, and frothing at the mouth. Within the local community, seizures are interpreted from both a biocultural and a biomedical standpoint (Molyneux 1999a). The biocultural view emphasises the importance of spirits or witchcraft, the local words nyama and ndege being used to describe the animal or bird-like spirits that are thought to have entered the child’s brain and caused the seizures. In this instance, help is usually obtained from a local healer, or mganga. In contrast, the biomedical view is that seizures are caused by fever, usually secondary to malaria, and that appropriate treatment should obtained from a clinic or hospital. Consequently, a child with seizures may be subjected to a wide variety of possible treatments, ranging from the administration of antimalarial and/or antipyretic drugs, to local herbs, being washed with urine, or hung in a pit latrine (since the pungent smell is thought to drive out evil spirits). The choice of biomedical or biocultural treatment is affected by a mother’s previous experience of each
health system, the views of her husband and family elders, and financial and logistic considerations. In many instances a mother will try both approaches: “It’s like betting, because you cannot be sure where the problem can be solved. You can start with the hospital first, and in case the hospital fails you can visit the healer” (interview with Mijikenda mother in Kilifi District. (Molyneux 1999a)). It is clear that, to be effective, any malaria control strategy must take into account the prevailing beliefs and behavioural patterns of the local community.

Seizures in uncomplicated malaria
The true prevalence of seizures in African children is largely unknown, due to the absence of accurate community-based data (Snow 1994b). A recent community survey of childhood fevers in Kilifi district found that between 2% and 5% of fever episodes in children under the age of 5 years were associated with seizures (V. Marsh, personal communication). Seizures, however, account for a large proportion of the children presenting to hospitals and clinics in sub-Saharan Africa, of which malaria is the single most important reported cause (Hendrickse 1971; Asindi 1993).

Recent inpatient studies from Kenya (Waruiru, 1996; J Berkley personal communication) have reported seizures in at least 25% of children admitted with non-cerebral malaria. Seizures were commonest in children below the age of 3 years and, in a study from Thailand, were significantly associated with infections caused by *P. falciparum* compared to *P. vivax* (Wattanagoon 1994).

Seizures and cerebral malaria
Seizures complicate the clinical course of cerebral malaria in approximately 60% to 80% of cases (Lemercier 1969; Molyneux 1989; Marsh 1995; Steele 1995; Waller 1995; Olumese 1999). When prolonged or multiple, they are associated with an increased risk of death (Jaffar 1997), gross neurological sequelae (Molyneux 1989; Brewster 1990; Bondi 1992; van Hensbroek 1997), and subsequent cognitive difficulties (Holding 1999). It is therefore surprising that, despite their high prevalence and potential pathogenic
importance, there have been few detailed studies of seizures in childhood cerebral malaria.

**Aims of this thesis**

1. To provide a detailed description of the clinical and electrophysiological features of seizures in African children with cerebral malaria.

2. To explore two hypotheses regarding the aetiology of seizures in cerebral malaria, namely that:
   a. That the ingestion of chloroquine may be a precipitating factor
   and
   b. They may be caused by the release of the endogenous excitotoxin quinolinic acid.

3. To conduct a randomised, controlled intervention study to assess whether a single intramuscular dose of phenobarbital 20 mg/kg given on admission can reduce the frequency of seizures complicating the clinical course of childhood cerebral malaria.
2. METHODS
Introduction

The studies described in this thesis form part of a collaborative research programme, run jointly by the Kenyan Institute of Medical Research (KEMRI) and the University of Oxford, and funded by the Wellcome Trust. The studies were carried out between January 1994 and January 1998 at the KEMRI Clinical Research Unit, based at Kilifi District Hospital. The first part of this chapter contains background information on the demography, geography, and health services of the study site and surrounding area. A review of the routine clinical management of children admitted to the paediatric ward, with particular reference to the management of severe malaria, is then followed by a description of the methods used for the collection, management, and statistical analysis of clinical data.

Study site

The town of Kilifi is located 60 kilometres north of Mombasa, on the coast of Kenya (Figure 3). The area, situated 4° south of the Equator, has mean daily temperatures of between 22°C and 30°C, with relative humidity of about 70%. Approximately 1000 mm of rain falls each year, mostly between the months of April to July and October to November. Malaria transmission (of which over 95% is *Plasmodium falciparum*) follows the seasonal pattern of rainfall, with peak periods occurring between June to August and December to January. The principal malaria vectors are of the *Anopheles gambiae* complex (Mbogo 1993). There is marked local heterogeneity in entomological inoculation rate (EIR), with rates varying between approximately 2 and 200 infected bites per person per year. However, most residents of Kilifi district are exposed to an average of 4 infective bites per person per year (Mbogo 1995).

Kilifi town has a population of approximately 13,000, and is the administrative centre for Kilifi district. This predominantly rural area has an estimated population of 500,000, with most individuals belonging to the Mijikenda ethnic group (Snow 1993a). The Mijikenda family system is patriarchal and polygamous (Parkin 1991). Subsistence
Figure 3. Map of Kenya showing Kilifi district
farming is widespread, the main cash crops being coconuts and cashews, while maize is
grown for home consumption. Household incomes are frequently supplemented by
remittances from family members working in Kilifi, Mombasa, and Malindi (Molyneux
1999a). Part of this rural population, living in a geographically defined study area to the
north and south of the town, has been the subject of extensive demographic surveillance
(Snow 1993a). The 1998 Kenya Demographic Health Survey revealed an infant mortality
rate of approximately 45 per 1000 live births (compared to 6 per 1000 live births in the
UK).

Formal healthcare services for this population are provided by Kilifi District Hospital,
and by 12 government dispensaries and private health clinics. Dispensaries operate on a
cost-retrieval basis, charging approximately 20 to 40 KSH (20 to 40 pence) to treat a
child with fever. Private clinics, charging approximately 150 KSH to treat a febrile child,
are relatively expensive (Molyneux 1999a). There are in addition approximately 22
Community Health Workers (CHW), trained to supply a limited range of subsidised
drugs from their homes, and to refer patients to other facilities when necessary. Access to
these services is, however, a problem for the majority of rural dwellers. One third of all
rural households are located more than 2 kilometres from a bus-stage, which makes
access to the district hospital and clinics in Kilifi both difficult and time-consuming. In
addition, over 60% of rural households are more than 2 kilometres from the nearest clinic
or dispensary, and approximately half are more than 2 kilometres from a CHW
(Molyneux 1999a). Consequently, it is not surprising that the majority of people turn to
the informal sector for first-line healthcare. Over 80% of rural households are within one
kilometre of a shop, of which the majority stock antipyretics and antimalarials.
Approximately 80% of mothers in rural areas initially purchase drugs from a local shop
when their child develops fever (Mwenesi 1995b; Marsh 1999; Molyneux 1999b). The
other source of informal healthcare to which mothers have easy access is from waganga,
or traditional healers. There is considerable variation in the reputation, “speciality”, and
charges of different waganga (Molyneux 1999a).
Kilifi District Hospital has a forty-bed paediatric ward, to which approximately 5000 children (about 10% of those seen in the paediatric outpatient department) are admitted each year. Malaria, acute respiratory tract infections, and gastroenteritis account for the majority of paediatric admissions, while malaria contributes to most of the deaths (Snow 1994c). The services offered by Kilifi District Hospital are typical of many government hospitals in sub-Saharan Africa. During periods of intense malaria transmission, more than 120 children may be inpatients on the paediatric ward at any one time, and may be cared for by as few as 2 to 3 nurses. At the same time of year, individual clinical officers in the outpatient department may see up to 100 patients each day. Drugs are often in short supply, and facilities for laboratory investigation are limited to microscopy (of blood smears, urine, and faeces), determination of packed cell volume, and a crossmatch service. Biochemical analyses and measurement of blood gases are not available, while radiological services are intermittent. Consequently, the Clinical Research Unit operates its own laboratory service, which offers a full range of haematological, biochemical, and microbiological investigations.

The work presented in this thesis was performed in a six-bedded high dependency research ward, which was supported by the KEMRI Clinical Research Unit, and situated adjacent to the main paediatric ward.

Patients

Children were admitted to hospital through the outpatient department, which was staffed by Ministry of Health clinical officers. These health workers, who were answerable to the Medical Superintendent for the hospital, cared for all acute medical and surgical emergencies. The staff of the Research Unit did not, therefore, determine hospital admission policy, and consequently the pattern of paediatric admissions reflected that of other district hospitals in coastal Kenya.

Shortly after admission, all children were reviewed by a clinician from the Research Unit, and a fingerprick blood sample was taken for measurement of haemoglobin and
preparation of a malaria parasite slide. Quantitative parasite counts were performed on thick and thin blood films that had been stained in 10% Giemsa for 10 minutes. The number of asexual forms of *Plasmodium falciparum* were then counted per 100 white blood cells (thick film) or per 500 red blood cells (thin film). Children with pre-defined signs of severe malaria (see Table 2) (Marsh 1995) were then transferred to the high dependency research ward. Use of this definition made it possible to accurately identify more than 90% of the patients who had a high risk of dying from malaria in hospital.

All children recruited to the studies described in this thesis were aged 9 months and above, and had a diagnosis of cerebral malaria. Cerebral malaria was defined as unrousable coma (inability to localise a painful stimulus, equivalent to a Blantyre coma score of 3 or less, see Table 3 (Molyneux 1989)), in the presence of asexual *Plasmodium falciparum* parasitaemia. Children with seizures were assessed a minimum of 30 minutes after cessation of clinical seizure activity. Children who were hypoglycaemic (blood glucose below 2.2 mmol/l) at the time of admission, and who recovered consciousness following the administration of intravenous glucose, were also excluded from the studies.

**Clinical assessment**

A full history and examination were performed on admission to the research ward, and results recorded onto a standard proforma (See Appendix). Children subsequently underwent frequent clinical review, with 4-hourly monitoring of vital signs (temperature, pulse, respiratory rate), Blantyre coma score (Molyneux 1989), transcutaneous oxygen saturation (N180 pulse oximeter, Nellcor, USA), and blood glucose (using indicator sticks (BM 1-44 test strips) and Refloflux II reflectance meters (Boehringer-Mannheim, UK)). All children underwent neurological examination at the time of discharge from hospital, and all were asked to return after one month (following the clinical and electrophysiological studies) or after 3 months (following the phenobarbitone prophylaxis study) for neurodevelopmental assessment.
Laboratory investigations

On admission to the research ward, venous blood was taken for full blood count (M 530 Coulter Counter, Luton UK), glucose (glucose oxidase method, Analox GM6), sodium and potassium (Corning 614 Na+/K+ autoanalyser, Corning Diagnostics Ltd, Halstead, UK), urea (Beckman BUN analyzer 2, Beckman, Fullerton, USA), and creatinine (Beckman creatinine analyzer 2, Beckman, USA). Blood gases were measured with a Corning 178 pH/blood gas analyser (Corning, Halstead, UK), with all measurements being individually corrected for the patient’s haemoglobin and temperature. Blood cultures were performed on all children, while those that were unconscious underwent lumbar puncture. Since intracranial pressure is a feature of cerebral malaria (Newton 1997b), lumbar puncture was delayed until the neurological status of the child had improved, or was done post-mortem for those who died. Cerebrospinal fluid was assessed by microscopy and culture, and all samples with a white cell count of above 10 cells per μl were screened for antigens of Haemophilus influenzae and Streptococcus pneumoniae.

Antimalarial and antibiotic chemotherapy

Children were treated with a loading dose of intravenous quinine dihydrochloride (15 mg/kg), with subsequent doses of 10 mg/kg every 12 hours (Winstanley 1994). During 1994, children were randomised to receive intravenous quinine or intramuscular artemether, 3.2 mg/kg loading dose and 1.6 mg/kg every 24 hours, as part of a multicentre study comparing the efficacy of the two treatment regimes (Murphy 1996). Parenteral therapy was replaced by a single oral dose of sulphadoxine-pyrimethamine (SP) once the child had received at least 3 doses of parenteral treatment and was able to tolerate oral drugs.

All children were treated with intravenous benzylpenicillin (60 mg/kg every 6 hours) and chloramphenicol (25 mg/kg every 6 hours) until the results of lumbar puncture and blood culture were available.
Supportive care

Intravenous fluids and blood were given as clinically indicated (Crawley 1999). Hypoglycaemia (blood glucose below 2.2 mmol/l) was corrected with a slow intravenous bolus of glucose 0.5 g/kg. Children with rectal temperatures of above 38.5°C were treated with paracetamol (15 mg/kg per rectum or via nasogastric tube every 6 hours). Anticonvulsant treatment was described in Chapter 1. Oxygen was available if required, but there were no facilities for artificial ventilation.

Ethical permission

Approval for all studies was obtained from the Kilifi Scientific Coordinating Committee, the KEMRI Scientific Coordinating Committee, and the Kenyan National Ethical Review Committee. Informed, written consent was also obtained from the parent or guardian of each child enrolled into a study. An explanation, written in the caretaker’s first language (KiSwahili or KiGiriama), was read aloud by a trained fieldworker, and caretakers were encouraged to ask questions. It was stressed that failure to grant permission would not in any way compromise the clinical care given to the child, and that consent could be withdrawn at any time without penalty.

Data collection and management

All clinical and laboratory data were double entered in dBase IV (Ashton Tate, USA) onto IBM personal computers. Range checking and verification were carried out using Foxpro 2 software (Fox Holdings, USA). Each patient was given a unique study number.

Statistical analyses

Data were analysed with SPSS version 5.0 (SPSS Corporation, Gorinchem, The Netherlands) and Stata version 5.0 (Stata Corporation, College Station, Texas, USA).
Descriptive statistics are presented as means with 95% confidence intervals, or medians with interquartile ranges.

Univariate analysis
Analysis of variance (ANOVA) and Student’s $t$ test were used for comparisons of normally distributed quantitative data, with logarithmic transformation if the distribution was skewed. The Mann-Whitney test and Spearman’s rank correlation coefficient were used for data without a normal distribution. Categorical data were compared with Fisher’s exact test.

Multivariate analysis
Multivariate logistic regression was used to examine the relationship between admission characteristics and subsequent outcome.

Survival analysis
In the randomised controlled trial of phenobarbitone prophylaxis, recovery times in the two groups were compared by survival analysis, using the log-rank (proportional hazards) and Peto-Peto-Wilcoxon (non-proportional hazards) (Peto 1972) tests for equality of survivor function.
3. CLINICAL and ELECTROPHYSIOLOGICAL FINDINGS
Introduction

Cerebral malaria is a major cause of death and disability in sub-Saharan Africa (WHO 1999b), yet its pathophysiology remains poorly understood (Marsh 1996; English 1997a). Prolonged, multiple seizures are a prominent clinical feature (Molyneux 1989; Marsh 1995; Waller 1995), and are associated with an increased risk of death (Jaffar 1997) and neurological sequelae (Brewster 1990; Jaffar 1997; van Hensbroek 1997). Despite their importance, and in common with many other causes of childhood coma, there have been few descriptions of the clinical and electroencephalographic features of seizures in cerebral malaria (Lemercier 1969). Electroencephalography (EEG) can supply the clinician with information on the origin and distribution of seizure activity, detect subtle or sub-clinical seizures, and may reveal abnormal electrical activity of prognostic significance (Hughes 1994). It is therefore of potential value in the clinical management of cerebral malaria, and may improve understanding of the underlying pathophysiology. This chapter describes the clinical and EEG findings from a detailed study of 65 children with cerebral malaria.

Methods

Principles of management are described in Chapter 2. Children were eligible for this study if they were aged 9 months or above, and fulfilled the WHO criteria for cerebral malaria (see Chapter 1). During the study period of January to September 1994, 110 inpatient children fulfilled these entry criteria. Of these 65 were recruited since, for technical reasons, it was only possible to study 2 children at any one time.

Electrophysiology

Electroencephalographic (EEG) recordings were made on a 14-channel Medelec 1A94 EEG machine. Silver/silver chloride electrodes were fixed with Elefix and tape to the child's shaved head, and the International 10-20 system used for electrode placement. Recordings were taken within 6 hours of admission, and at 12 hourly intervals until recovery of consciousness. Continuous recordings using a CFAM (cerebral function
analysing monitor, Medaid Ltd, UK) were obtained from selected children who had been unconscious for more than 24 hours or who had prolonged or subtle seizure activity. All EEGs were subsequently analysed by a consultant neurophysiologist, who knew the age of each child and what drugs they had been given, but was blind to any other clinical information.

**Intracranial pressure monitoring**

As part of a separate study, which aimed to document the pattern of intracranial pressure (ICP) in children with cerebral malaria, and to determine the efficacy of mannitol (Newton 1997b), ICP was monitored in selected deeply comatose children using a subarachnoid catheter (Camino 110-4B).

**Anticonvulsant treatment**

All clinical seizures were timed and recorded by medical or nursing staff, and classified as generalised tonic-clonic, partial motor, or partial with secondary generalisation (Hopkins et al. 1995). Seizures lasting for more than 5 minutes were treated with diazepam 0.3 mg/kg, given as a slow intravenous injection over 2 minutes. Children with recurrent (more than 3) seizures or status epilepticus (continuous clinical seizure activity lasting for 30 minutes or more) were treated with a loading dose of intravenous phenytoin 18 mg/kg and, if that failed, with intramuscular phenobarbitone 18 mg/kg. One child with intractable generalised status epilepticus required treatment with intravenous thiopentone 4 mg/kg.

**Follow up**

All survivors were seen one month after discharge for neurological examination and repeat EEG. Cerebral computerised tomography (CT) was performed on children with neurological sequelae.
Table 4. Admission clinical and laboratory features by outcome

<table>
<thead>
<tr>
<th>Clinical or laboratory feature</th>
<th>Normal recovery (1) N = 50</th>
<th>Sequelae (2) N = 8</th>
<th>Died (3) N = 7</th>
<th>P values‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)*</td>
<td>33.1 (27.6 - 39.7)</td>
<td>16.1 (9.1 - 28.5)</td>
<td>38.7 (22.2 - 67.5)</td>
<td>0.02 for 2 v 1 0.04 for 2 v 3</td>
</tr>
<tr>
<td>Duration of coma before admission (hours)*</td>
<td>6.3 (4.7 - 8.3)</td>
<td>11.5 (5.0 - 26.4)</td>
<td>13.3 (6.9 - 25.8)</td>
<td>0.07</td>
</tr>
<tr>
<td>Status epilepticus† before or after admission</td>
<td>28/50 (56%)</td>
<td>6/8 (75%)</td>
<td>2/7 (29%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Blantyre score+ 2 or less</td>
<td>35/50 (76%)</td>
<td>5/8 (63%)</td>
<td>7/7 (100%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Opisthotonic posturing</td>
<td>2/50 (4%)</td>
<td>1/8 (12%)</td>
<td>4/7 (57%)</td>
<td>0.003 for 3 v 1</td>
</tr>
<tr>
<td>Median (IQR) parasitaemia (per μl)</td>
<td>85200 (990 - 1017600)</td>
<td>11854 (1264 - 220000)</td>
<td>81600 (420 - 352800)</td>
<td>0.27</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>6.5 (5.8 - 7.2)</td>
<td>8.0 (5.4 - 10.6)</td>
<td>7.8 (5.0 - 10.6)</td>
<td>0.20</td>
</tr>
<tr>
<td>Median (IQR) glucose (mmol/l)</td>
<td>5.9 (0.7 - 11.3)</td>
<td>5.0 (0.7 - 8.3)</td>
<td>3.4 (0.4 - 7.8)</td>
<td>0.17</td>
</tr>
<tr>
<td>Median (IQR) sodium (mmol/l)</td>
<td>134 (127 - 142)</td>
<td>139 (128 - 168)</td>
<td>139 (127 - 147)</td>
<td>0.12</td>
</tr>
<tr>
<td>Potassium (mmol/l)*</td>
<td>4.3 (4.0 - 4.5)</td>
<td>4.7 (4.2 - 5.3)</td>
<td>4.3 (3.2 - 5.9)</td>
<td>0.5</td>
</tr>
<tr>
<td>Urea (mmol/l)*</td>
<td>4.1 (3.3 - 5.2)</td>
<td>8.6 (3.0 - 24.7)</td>
<td>6.5 (3.6 - 11.8)</td>
<td>0.06</td>
</tr>
<tr>
<td>Creatinine (μmol/l)*</td>
<td>52 (45 - 61)</td>
<td>104 (55 - 197)</td>
<td>72 (38 - 137)</td>
<td>0.009 for 2 v 1</td>
</tr>
<tr>
<td>Base excess</td>
<td>-5 (-8 to -3)</td>
<td>-11 (-17 to -5)</td>
<td>-13 (-21 to -5)</td>
<td>0.04 for 3 v 1</td>
</tr>
</tbody>
</table>

Data are mean (95% CI) or number (%) unless stated; * Geometric mean
† Any clinical seizure reported (before admission) or observed (after admission) to last for 30 minutes or more
+ 0 = deep coma 5 = full consciousness
‡ Where p< 0.05, results of multiple comparison tests (Scheffe or Bonferroni) are given
Results

Admission characteristics
Sixty-five children, of age range 9 months to 11 years (median 30 months), were recruited to the study. All children had previous normal development. Prior to hospital admission, 68% (44/65) had seizures which, in 27 cases, were reported to have lasted for at least 30 minutes. Thirteen children (20%) had received diazepam on or up to 6 hours prior to admission, in doses of between 0.2 – 1 mg/kg. In 5 cases, diazepam had been administered by intramuscular injection. Children had been comatose for between 1 and 72 hours (median 7 hours) prior to admission and, in over 50% of cases, the onset of coma had been heralded by seizure activity. At the time of admission, 75% of the children were deeply unconscious with a Blantyre coma score of 2 or less. Admission clinical and laboratory findings are shown in Table 4.

Clinical course
Of the 65 children studied, 14/65 (22%) recovered consciousness within six hours of multiple or prolonged seizures that had occurred on or immediately prior to hospital admission. Following admission, 62% (40/65) had at least one seizure, while 19 children had more than 5 seizures. Seventy five percent of all seizures occurred within 24 hours of admission. Twenty-eight percent (18/65) of the children had an episode of clinical status epilepticus (continuous clinical seizure activity lasting for 30 minutes or more), while 3 further children had continuous electrical discharges lasting for 30 minutes or more with no apparent clinical correlates (electrographic status epilepticus). Twenty three percent of the children (15/65) had seizures that were either clinically subtle or electrographic (see Subtle seizures, below). Fifty-two percent of all seizures were partial motor, 14% partial with secondary generalization, and 34% generalized tonic-clonic. Children with generalised seizures were significantly older than those with partial motor seizures (mean age 64.0 months (95% CI 44.7 - 83.5) versus 26.7 months (95% CI 20.6 - 32.9) for partial motor; p < 0.001). Median duration of seizure activity was 3 minutes (range 0.5 to 600
minutes). Of 310 seizures documented, only 130 (42%) were associated with a rectal temperature of 38 °C or above, and 2 with hypoglycaemia (blood glucose <2.2 mmol/l).

**Subtle seizures**

Fifteen children had continuous electrical seizure discharges on EEG, but clinical features that were either extremely subtle (subtle seizures) or not discernible (electrographic seizures). Children with subtle seizures typically presented in coma, with a history of a prolonged seizure that had either terminated spontaneously, or as a result of treatment with diazepam. Tonic eye deviation, nystagmus, salivation, and hypoventilation were the most distinctive clinical features. The nystagmus was of large amplitude, with a slow phase of movement that crossed the midline. Respiration was shallow and irregular, and the children were hypoxaemic (arterial oxygen saturation below 80%) and hypercarbic (pCO₂ above 6.5 kPa). EEG showed continuous spike-wave discharges over the posterior temporo-parietal region contralateral to the direction of eye deviation (Figure 4). These children recovered consciousness, as judged by their ability to localise a painful stimulus, a median of 5 hours (range 1 to 8) after anticonvulsant treatment. The clinical features of subtle seizures are illustrated in the enclosed video.

Clinical features also became increasingly subtle during prolonged generalised seizures. One child was admitted following a generalised seizure at home that had lasted for six hours. Tonic-clonic movements had ceased one hour before admission, and the child was deeply comatose, with irregular respiration, salivation, and priapism. Despite the paucity of clinical features, EEG revealed continuous, generalised seizure activity.
Figure 4. Left posterior temporo-parietal discharge
Electrophysiology

Background activity

Admission EEGs were dominated by high amplitude (>100 μv) slow wave activity with frequencies of between 0.5-7 Hz (Figure 5). Admission recordings of very slow frequency (0.5-3 Hz) were obtained from 17/65 (26%) children, and were associated with an increased risk of death (odds ratio 25.6 (95% CI 2.8 to 235; p=0.004) (Table 5). This association remained significant despite adjustment for the possible confounding effects of age, glucose, and base excess. Recordings of very slow frequency were not significantly associated with duration of coma, administration of diazepam in the 12 hours prior to admission EEG, age, parasitaemia, glucose, or base excess (p >0.1 for all comparisons). Ten children had admission recordings that were asymmetric (Figure 6), with increased slow wave activity over all or part of one hemisphere. Nine of these children had a history of multiple partial motor seizures or status epilepticus in the six-hour period prior to admission, and 3 developed neurological sequelae.

Ictal activity

A striking feature of the EEGs in this series was the consistent localisation of ictal discharges over the posterior temporo-parietal regions (Figure 4). Over 75% of ictal or interictal discharges occurred in this region, sometimes spreading to involve both temporo-parietal regions, or one or both cerebral hemispheres. In 8 cases, electrical seizure activity persisted for between 2 and 140 minutes (median 45 minutes) after successful treatment of a clinical seizure with diazepam.

The onset of generalised electrical and clinical seizure activity usually followed an increased frequency of bilateral, predominantly posterior, interictal spike-wave discharges (Figure 7). Runs of up to 40 short, generalised seizures, each lasting for 0.5 to 1 minute (Figure 8), were observed in four children and were followed by marked post-ictal flattening.

Inter ictal epileptic activity

Interictal spikes and sharp waves were observed on admission EEGs from 3 children, all with a history of prolonged (seizure activity lasting for more than one hour) partial motor
status epilepticus. The discharges were bilateral and multifocal, and located predominantly in the temporo-parietal regions (Table 6). All of these children developed neurological sequelae.

**Episodic low amplitude events (ELAE)**
Periods of relative attenuation of background activity lasting 3-5 seconds were seen on admission EEGs from 5 children. In 2 cases, these followed the administration of intravenous diazepam 0.3 mg/kg for the treatment of seizures. Four of these children recovered normally, while one child died 7 hours after admission.

**Burst suppression**
A burst suppression pattern, consisting of bursts of polymorphous complexes occurring synchronously over both hemispheres, and alternating with periods of relative quiescence, was observed on the initial EEGs from 2 children (Figure 9). One child had been hypotensive and hypoglycaemic on admission, and the other had a history of multiple, prolonged generalised seizures. Both children died.
Figure 5a. Background slow-wave activity in cerebral malaria

Figure 5b. Normal EEG (age 12 months) for comparison
Figure 6. Postictal EEG asymmetry
Figure 7a. Recruiting rhythm: increased frequency of bilateral interictal spike wave discharges

Figure 7b. Onset of generalised seizure
Figure 8. CFAM tracing showing multiple short generalised seizures

Figure 9. Burst suppression
Intracranial pressure monitoring

Ten children had simultaneous EEG and intracranial pressure (ICP) monitoring. Four children had clinical seizures during ICP monitoring. Intracranial pressure rose by a median of 164% (range 108-285) during the 18 generalised seizures, and by 50% (range 0-186) during the 14 partial seizures recorded. Concomitant rise in mean arterial pressure meant that cerebral perfusion pressure was maintained above 50 mmHg during clinical seizures. There was no significant rise in ICP during two 90-minute periods of electrographic seizure activity confined to the posterior temporo-parietal regions. At intracranial pressures of below 20 mmHg, fluctuations in ICP appeared to have no effect on the background frequency of the EEG. EEG recording from one child with severe intracranial hypertension, however, showed widespread slow wave activity (frequencies of 0.5 – 1 Hz) in association with episodes of opisthotonic posturing, during which ICP rose to peaks of 30 to 40 mmHg (Figure 10a). Within 10 hours, EEG showed attenuation of activity in the right temporo-parietal region (Figure 10b), and the right pupil became dilated with sluggish reaction to light. Over the next five hours, despite regular doses of mannitol 0.5 g/kg, the ICP rose to above 80 mmHg, the cerebral perfusion pressure fell below 30 mmHg, and the entire EEG became flat and featureless. ICP rose to a peak of 125 mmHg, and the child died following a respiratory arrest.

A further child of 3 years was admitted with a 12-hour history of opisthotonic posturing. The initial EEG was reactive, with a background frequency of 2-6 Hz. ICP monitoring was commenced at 7 hours, and the child also started continuous CFAM recording. Initial intracranial pressures ranged from 25-30 mmHg, with peaks of 40 mmHg during episodes of opisthotonus. Over the next 4 hours, despite inotropic support, the mean arterial pressure remained below 75 mmHg, and rising intracranial pressure consequently caused a progressive decline in cerebral perfusion pressure. Subsequent infusion of mannitol was accompanied by a marked deterioration in clinical condition, and CFAM recording showed a sharp drop in background frequency. The child died shortly afterwards following a cardio-respiratory arrest.
Figure 10a. Slow wave activity associated with onset of opisthotonic posturing

Figure 10b. EEG 10 hours later
(Note marked attenuation of electrical activity in right temporo-parietal region: T4-C4, C4-Cz, T6-P4, P4-Pz)
Outcome

Seventy-seven percent (50/65) of the children made a full recovery, 7/65 (11%) died, and 8/65 (12%) were left with neurological sequelae (4 hemiplegia, 2 spastic quadriplegia, 1 cognitive and speech problems, 1 grand mal epilepsy) that were still present one month after discharge. Among survivors, median time to recovery of consciousness was 20 hours (range 4-112 hours). Serial EEGs showed a gradual increase in background frequency over the period from admission to recovery of consciousness. The fifty children who made a full recovery had normal EEGs one month after discharge. Details of the children who died or developed neurological sequelae are shown in Tables 4, 5, and 6. Children with sequelae were significantly younger (p = 0.02) and had a significantly higher creatinine (p = 0.009) compared to those who recovered fully. Admission base excess was significantly greater (p = 0.04) in children who died compared to those who recovered fully, while four of the seven children who died had sustained opisthotonic posturing during their clinical course in hospital. There were otherwise no significant differences between the three outcome groups in the admission variables, but numbers are small. Follow-up EEGs from children with hemiplegia showed decreased activity in the contralateral parieto-temporal region, and cerebral computerised tomography (CT) scans showed infarction in the same region (Figure 11). Follow-up EEGs from two children with spastic quadriplegia and one child with severe cognitive and speech problems were of low amplitude, with a paucity of normal cerebral rhythms, while CT scans from these children showed global cerebral atrophy.
<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Any status epilepticus*</th>
<th>Number of seizures after admission</th>
<th>Background EEG activity</th>
<th>Localisation of ictal or inter-ictal discharges</th>
<th>Time of death (hours from admission)</th>
<th>Mode of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>No</td>
<td>2</td>
<td>2Hz Unreactive, low amplitude</td>
<td>None</td>
<td>15</td>
<td>Cardio-respiratory arrest</td>
</tr>
<tr>
<td>50</td>
<td>No</td>
<td>0</td>
<td>0.5-2Hz End-stage electrographic status</td>
<td>Continuous ictal discharges L hemisphere</td>
<td>4</td>
<td>Cardio-respiratory arrest</td>
</tr>
<tr>
<td>32</td>
<td>No</td>
<td>0</td>
<td>3-7Hz Widespread slowing to 1Hz with opisthotonus</td>
<td>None</td>
<td>25</td>
<td>Respiratory arrest</td>
</tr>
<tr>
<td>132</td>
<td>No</td>
<td>7</td>
<td>0.5-3Hz Low amplitude</td>
<td>None</td>
<td>63</td>
<td>Respiratory arrest</td>
</tr>
<tr>
<td>31</td>
<td>No</td>
<td>0</td>
<td>0.5-2Hz Episodic low amplitude events</td>
<td>None</td>
<td>8</td>
<td>Respiratory arrest</td>
</tr>
<tr>
<td>35</td>
<td>Yes</td>
<td>0</td>
<td>2-6Hz Burst suppression</td>
<td>None</td>
<td>11</td>
<td>Respiratory arrest</td>
</tr>
<tr>
<td>27</td>
<td>Yes</td>
<td>15</td>
<td>Burst suppression</td>
<td>None</td>
<td>16</td>
<td>Respiratory arrest</td>
</tr>
</tbody>
</table>

Table 5. EEG findings from children who died

* Clinical seizure reported (before admission) or observed (after admission) to last for 30 minutes or more
<table>
<thead>
<tr>
<th>Age (months)</th>
<th>18</th>
<th>9</th>
<th>11</th>
<th>11</th>
<th>9</th>
<th>13</th>
<th>56</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any status epilepticus*</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Number of clinical seizures after admission</td>
<td>2</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt;20</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Background EEG activity</td>
<td>3-5 Hz R 2 Hz L</td>
<td>2-4 Hz</td>
<td>2-5 Hz</td>
<td>1-4 Hz L 0.5-2 Hz R</td>
<td>2-6 Hz</td>
<td>3-7Hz R 2 Hz L</td>
<td>3-7 Hz</td>
<td>1-4 Hz</td>
</tr>
<tr>
<td>Localisation of ictal or interictal discharges</td>
<td>L temporo-parietal interictal sharp slow waves</td>
<td>L temporal ictal discharges</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>L parieto-occipital</td>
<td>R mid temporal Ictal and interictal discharges</td>
<td>Bilateral parieto-occipital interictal discharges</td>
</tr>
<tr>
<td>Type of sequelae</td>
<td>R hemiplegia</td>
<td>R hemiplegia</td>
<td>R hemiplegia</td>
<td>L hemiplegia</td>
<td>Spastic quadriplegia</td>
<td>Spastic quadriplegia</td>
<td>Attention and speech deficits</td>
<td>Grand mal epilepsy</td>
</tr>
<tr>
<td>Follow-up EEG one month post discharge</td>
<td>Asymmetric Increased slow activity on L</td>
<td>Asymmetry of sleep spindles Low amplitude, featureless over L posterior quadrant</td>
<td>Asymmetry of sleep spindles Increased slow activity L posterior quadrant</td>
<td>Asymmetry of sleep spindles Increased slow activity R posterior quadrant</td>
<td>Low amplitude, featureless</td>
<td>Movement artifact</td>
<td>Abnormal sleep activity</td>
<td>Normal EEG with hyperventilation</td>
</tr>
<tr>
<td>CT scan one month post discharge</td>
<td>Infarction L parieto-occipital region</td>
<td>Infarction L parieto-occipital region</td>
<td>Failed to attend</td>
<td>Infarction R parieto-occipital region</td>
<td>Generalised cerebral atrophy</td>
<td>Generalised cerebral atrophy</td>
<td>Generalised cerebral atrophy</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Table 6. EEG and CT scan results from children with neurological sequelae

* Clinical seizure reported (before admission) or observed (after admission) to last for 30 minutes or more
Figure 11. Cerebral CT scan in child with right hemiplegia
(Shows extensive area of infarction in left temporo-occipital region (1 and 2) plus associated cerebral atrophy)
Discussion

Traditionally, it has been assumed that sequestration of parasitised red blood cells occurs within the cerebral microvasculature of all comatose patients with falciparum malaria (MacPherson 1985; WHO 2000). Clinical and experimental work has shown, however, that the pathophysiology of cerebral malaria is highly complex, and that a number of different pathological mechanisms may cause coma (Marsh 1996). Reduced cerebral perfusion (secondary to raised intracranial pressure and systemic hypotension), severe anaemia, hypoglycaemia, and the local release of both cytokines and nitric oxide, may all be contributory factors (White 1987; Clark 1991; Marsh 1996; English 1997a; Newton 1997b). Our study suggests that an additional pathophysiological factor, seizures, may play an important role in the pathogenesis of coma.

Over one third of the children in this study had more than 5 seizures or an episode of status epilepticus during their clinical course in hospital. Twenty-two percent recovered consciousness within six hours of prolonged or multiple seizures, suggesting that their coma may have been a postictal phenomenon, directly related to seizures. Following a prolonged seizure, it is thought that the brain enters a phase of “cortical exhaustion”, with depletion of adenosine triphosphate, glucose, oxygen, and the accumulation of lactic acid (Shorvon 1994b). The EEG may be flattened or show diffuse slow wave activity during this postictal period, the duration of which may be prolonged after prolonged or multiple seizures. Some children in this study received large (up to 1 mg/kg) doses of diazepam before admission, which may have depressed consciousness further. In several cases, diazepam was administered by intramuscular injection and, since the absorption of diazepam from this site is extremely variable, this may have led to high blood concentrations and secondary sensorineural and respiratory depression (Gamble 1975; Langslet 1978).

This study has also shown that, in a proportion of children with cerebral malaria, coma is due to continuing subtle seizure activity, which is likely to go undetected, but which is responsive to anticonvulsant drugs. EEG was essential for identifying the fifteen children in whom seizures were either clinically subtle (tonic eye deviation, nystagmus,
hypoventilation) or electrographic. Although tonic deviation of the head and eyes commonly occurs during epileptic seizures, nystagmoid eye movements are an unusual ictal manifestation. The clinical characteristics of the nystagmus in these patients were consistent with epileptic nystagmus type two (EN-2), which typically has a seizure focus in the temporo-parietal-occipital cortex, contralateral to the direction of the nystagmus beats (Kaplan 1993; Harris 1997; Newton 1998a). This area overlaps the putative cortical region of visual motor sensitivity (V5), which is important for the generation of ipsiversive smooth pursuit (Lekwuwa 1996). Subtle seizures have not been described in previous studies of childhood cerebral malaria but are of great clinical importance, since several of these children recovered consciousness within a few hours of anticonvulsant treatment. In addition, epileptic inhibition of respiration may prevent some of these children from compensating for a profound metabolic acidosis (English 1996a; Crawley 1998). In a busy, understaffed hospital in the developing world such seizures are easily missed, yet their clinical features are sufficiently distinct for them to be recognised by medical or nursing staff with appropriate training, without the need for EEG.

The pathogenesis of seizures in childhood cerebral malaria is likely to be complex and multifactorial, and is discussed in more detail in Chapter 4. Although fever is known to precipitate seizures in young children (Wallace 1988) it is of note that, in this series, over 50% of all seizures occurred when the rectal temperature was below 38 °C. In addition, only two seizures were associated with blood glucose of below 2.2 mmol/l. Instead, this study suggests that cerebral hypoxia may be one of the factors predisposing to seizures. EEG recordings demonstrated that electrical seizure activity consistently arose from the posterior temporo-parietal region, which is a "watershed" area, lying between territories supplied by the middle cerebral (carotid circulation) and posterior cerebral (vertebro-basilar circulation) arteries. Consequently, it is particularly vulnerable to hypoxia when oxygen delivery to the brain is compromised as a result of sequestration, severe anaemia, or inadequate cerebral perfusion due to hypotension or raised intracranial pressure (MacPherson 1985; Newton 1994; English 1997a; Newton 1997b). Because of their anatomical position close to the temporal lobes in the tentorial notch, the posterior cerebral arteries may be particularly vulnerable to compromise in those patients who have acute intracranial hypertension and
incipient transtentorial herniation, as illustrated by the EEGs in Figure 10. Raised intracranial pressure, reduced cerebral perfusion pressure, and transtentorial herniation have been documented in cerebral malaria (Newton 1994; Newton 1997b), and in other paediatric encephalopathies (Minns 1991). By initiating the release of excitotoxic mediators such as glutamate or quinolinic acid (Dobbie 2000), local hypoxia may precipitate seizures, which, by raising intracranial pressure and increasing the demand for oxygen and glucose, may exacerbate the situation further. Although there is no evidence for failure of cerebral autoregulation in the majority of these patients, relatively poor collateral circulation in some patients may mean that cerebral blood flow in the border-zone between the carotid and vertebro-basilar territories is insufficient to compensate for increased local demand. In these patients, a vicious cycle might then be generated, leading to intractable partial seizures and eventually to focal infarction (Figure 11).

Diffuse background slow wave activity on EEG occurs in many conditions (metabolic, toxic, and infectious) that have a generalised effect on the brain (Plum 1982; Hughes 1994). Background slow wave activity in cerebral malaria has been described in adults (Stotka 1973; Chen 1991; Thumasupapong 1995), and in one previous study on children (Lemercier 1969). The mechanism for the increased slow wave activity seen during episodes of intracranial hypertension and opisthotonus is unknown, but may have been due to concurrent hyperventilation, leading to hypocapnia and cerebral vasoconstriction (Kiloh 1981). Intracranial pressure rose acutely during seizures, and was accompanied by a concurrent rise in mean arterial pressure, suggesting that cerebral autoregulation was intact. In one case, however, mean arterial pressure was insufficient to maintain adequate cerebral perfusion, despite treatment with pressor agents. Infusion of mannitol was followed by clinical and electrophysiological deterioration, suggesting that the integrity of the blood-brain barrier had been breached.

EEGs have been used to predict prognosis in a number of childhood encephalopathies (Pampiglione 1968; Kuroiwa 1980; Tasker 1988). In this study, burst suppression or background activity of very slow frequency was associated with an increased risk of death. Of five children with episodic low amplitude events (ELAE) on initial EEG, four made a
full recovery. In two of these cases, ELAE were associated with the administration of diazepam, which may partly explain why their prognosis appears better here than in other types of encephalopathy (Brenner 1975; Rae-Grant 1991). Children with a history of status epilepticus prior to admission, and an initial EEG showing asymmetric background activity or continuous inter ictal multifocal spike waves, had an increased incidence of neurological sequelae. Overall, almost 70% of the children with an adverse outcome (death or neurological sequelae) had one or more poor prognostic features on initial EEG. Although this suggests that in some cases significant cerebral pathology must have had occurred prior to hospital admission, anticonvulsant prophylaxis given at the time of admission may prevent further cerebral damage. There is an urgent need to find a prophylactic anticonvulsant drug that is both safe and effective in children with cerebral malaria. This will be discussed further in Chapter 6.

Electrophysiological monitoring using serial EEG and CFAM has made it possible to identify a wide variety of seizures (clinical, subtle, and electrographic) in children with cerebral malaria, and has emphasised their importance in the pathogenesis of coma. We have been able to correlate the electroencephalographic and clinical findings from this group of unconscious children, something that is not possible in most parts of the developed world where the majority of unconscious children are ventilated. The information obtained from this study may, therefore, provide some valuable insights into a wide variety of other paediatric encephalopathies.
4. AETIOLOGY

Is chloroquine a risk factor?
Introduction

What accounts for the high prevalence of seizures in childhood cerebral malaria? This chapter discusses various aetiological hypotheses, and then considers in further detail the possible role of chloroquine and its active metabolite desethylchloroquine.

Since fever is known to precipitate seizures in young children (Verity 1985; Wallace 1988), and the majority of children with cerebral malaria have fever as a presenting feature, it is not unreasonable to consider that the seizures of cerebral malaria may be "febrile seizures". However, among the 65 children with cerebral malaria described in Chapter 3, and for 582 children with malaria whose case notes were reviewed retrospectively (Waruiru 1996), more than 50% of all seizures occurred at rectal temperatures of below 38°C. In addition, "febrile seizures" are usually generalised and of short duration (Wallace 1988), while the majority of seizures associated with malaria are partial motor, with a high proportion being prolonged (lasting for more than 5 minutes), or multiple (2 or more seizures) (Molyneux 1989; Akpede 1993).

These factors, plus the observation that, despite similar peak temperatures, seizures complicate twice as many cases of malaria caused by *Plasmodium falciparum* compared to *Plasmodium vivax* (Wattanagoon 1994), suggest that the pathological processes induced by *Plasmodium falciparum* may be specifically epileptogenic. *Plasmodium falciparum* is the only species of malaria parasite to undergo sequestration in deep capillary beds, and the characteristic histopathological feature of cerebral malaria is intense sequestration of parasitized red blood cells in the cerebral microvasculature (MacPherson 1985). This suggests that local interference with blood flow could lead to seizures, either directly as a result of hypoxia, as discussed in Chapter 3, or by initiating the release of excitotoxic mediators such as glutamate or quinolonic acid (see Chapter 5).

Seizures can also result from metabolic disturbances, such as hypoglycaemia or electrolyte imbalance. However, no association between hypoglycaemia and seizures was found during a study of severe malaria in Gambian children (White 1987), or in the study
described in Chapter 3. Mild hyponatraemia occurred in a small proportion of children in this study, but was not significantly associated with the occurrence of seizures.

Despite the relentless spread of drug-resistant *Plasmodium falciparum*, chloroquine continues to be widely used throughout Africa for the treatment and prevention of malaria. In addition, the drug is used in many parts of the world as second-line treatment for hepatic amoebiasis and connective tissue disease. A number of case reports in the medical literature have described an association between seizures and the ingestion of chloroquine, both at therapeutic concentrations (Torrey, 1968; Fish 1988; Luijckx 1992) and following overdose (Cann 1961; Kiel 1964; Jaffe 1988; Riou 1988). A large proportion of patients admitted to hospital in Kenya with malaria have taken chloroquine, obtained from shops or local dispensaries, prior to attending hospital (Snow 1992; Marsh 1999). The purpose of the following study, therefore, was to investigate the possibility that seizures in childhood cerebral malaria may be caused or exacerbated by chloroquine or its active metabolite desethylchloroquine.

**Methods**

The study formed part of a randomised, double-blind, placebo-controlled trial of phenobarbitone prophylaxis in childhood cerebral malaria, which will be described in detail in Chapter 6. Children aged 9 months to 13 years were recruited if they had cerebral malaria (see Chapter 2). As soon as possible after admission, children were randomised to receive a single intramuscular dose of phenobarbitone 20 mg/kg or identical placebo. Blood was taken at baseline, 2, 4, 8, 12 and 24 hours for measurement of phenobarbitone, chloroquine (CQ), and desethylchloroquine (DCQ). Analysis of the relationship between blood CQ and DCQ concentrations and subsequent seizure activity was confined to the placebo group.

**Treatment**

Children received standard treatment for cerebral malaria (Crawley 1999). The number and duration of all seizures were recorded using timers pre-set to alarm at 5, 15, and 30
minutes. Seizures lasting for 5 minutes or more were treated in a standardised manner, with intravenous diazepam 0.3 mg/kg and, as second-line therapy, intramuscular paraldehyde 0.2 ml/kg.

**Chloroquine assay**

Chloroquine (CQ) and desethylchloroquine (DCQ) concentrations were measured in whole blood using a modification of the method of Patchen et al (Patchen 1983). Blood samples (100μl) were extracted with a combination of methyl-tert-butyl-ether and hexane (1:1), centrifuged (1000 x g; 10 minutes), and the organic layer separated and evaporated in a water bath (37 °C) under a gentle stream of nitrogen. The residue was reconstituted in mobile phase (100μl) and analyzed by high-performance liquid chromatography, using ultraviolet detection at 340nm. The lower limit of detection for both chloroquine and desethylchloroquine was 5μg/l.
Table 7. Comparison of baseline admission characteristics in children with cerebral malaria

(CQ: chloroquine; DCQ: desethylchloroquine; values are median (interquartile range) unless stated)

<table>
<thead>
<tr>
<th>SEIZURES AFTER ADMISSION</th>
<th>No ((n = 50))</th>
<th>Yes ((n = 59))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td></td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td>Geometric mean (95% CI)</td>
<td>32.7 (28.0 - 38.1)</td>
<td>28.7 (25.1 - 32.7)</td>
<td></td>
</tr>
<tr>
<td>Seizures before admission</td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>No. (%)</td>
<td>39/50 (78%)</td>
<td>56/59 (95%)</td>
<td></td>
</tr>
<tr>
<td>Anticonvulsants before admission</td>
<td>7/50 (14%)</td>
<td>17/59 (29%)</td>
<td>0.07</td>
</tr>
<tr>
<td>No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline CQ (µg/l)</td>
<td>227.5 (79.4 - 430.2)</td>
<td>169.4 (75.1 - 374.9)</td>
<td>0.53</td>
</tr>
<tr>
<td>Baseline DCQ (µg/l)</td>
<td>364.0 (131.3 - 709.4)</td>
<td>352.3 (81.9 - 580.1)</td>
<td>0.53</td>
</tr>
<tr>
<td>AUC (_{0-12}) CQ (µg.hr/l) ((n = 43))</td>
<td>2058.9 (1014.1 - 5469.4)</td>
<td>2057.6 (802.8 - 2536.8)</td>
<td>0.37</td>
</tr>
<tr>
<td>AUC (_{0-12}) DCQ (µg.hr/l) ((n = 39))</td>
<td>4534.8 (2420.3 - 6416.6)</td>
<td>4285.2 (1985.0 - 5740.6)</td>
<td>0.53</td>
</tr>
<tr>
<td>Parasitaemia (µl/l)</td>
<td>64805 (29987 - 140049)</td>
<td>42293 (22473 - 79598)</td>
<td>0.39</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.0 (3.0 - 5.3)</td>
<td>4.1 (3.3 - 5.3)</td>
<td>0.83</td>
</tr>
<tr>
<td>Base excess</td>
<td>-7 (-12 to -4)</td>
<td>-8 (-13 to -3)</td>
<td>0.90</td>
</tr>
</tbody>
</table>
Table 8. Baseline drug levels for children with and without status epilepticus

<table>
<thead>
<tr>
<th>STATUS EPILEPTICUS AFTER ADMISSION</th>
<th>No</th>
<th>Yes</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 100)</td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>Baseline CQ (µg/l)</td>
<td>227.5 (85.6–441.2)</td>
<td>75.1 (7.4–116.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Baseline DCQ (µg/l)</td>
<td>375.9 (134.8–701.2)</td>
<td>81.9 (0–322.9)</td>
<td>0.07</td>
</tr>
<tr>
<td>AUC 0–12 CQ (µg.hr/l) (n = 43)</td>
<td>2170.1 (1089.5–4053.6)</td>
<td>470.3 (211.3–659.7)</td>
<td>0.009</td>
</tr>
<tr>
<td>AUC 0–12 DCQ (µg.hr/l) (n = 39)</td>
<td>4769.4 (2420.3–6416.6)</td>
<td>1188.7 (392.5–1985.0)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Chloroquine (CQ); Desethylchloroquine (DCQ); Area under the concentration curve for 0-12 hours (AUC 0-12)

Values expressed as median (interquartile range)
Results

Samples
Of 340 patients recruited to the main phenobarbitone prophylaxis study, 170 received placebo. Sufficient blood was available for measurement of CQ and DCQ on 109 (64%) of these patients. There was no difference in the frequency or duration of seizures between patients who had levels measured and those who did not (p > 0.1 for all comparisons). Chloroquine was detected in 100/109 (92%) of the baseline blood samples. Median (interquartile range) baseline concentrations were 214.9 μg/l (79.4 – 427.3) for CQ and 349.3 μg/l (90.3 – 594.7) for DCQ. Of 80 children who had 3 or more CQ levels taken during their hospital course, 29 (36%) were thought to have received CQ in the period immediately (< 24 hours) before hospital admission, since the concentration of CQ in their blood fell by more than 50% during the first 24 hours of admission (White 1988b). These children had median (interquartile range) baseline levels of CQ and DCQ that were significantly higher than those given chloroquine more than 24 hours prior to admission (CQ 374.9 μg/l (278.6 – 888.3) versus 114.8 μg/l (41.0 – 185.8), p = 0.0001; DCQ 580.1 μg/l (312.3 – 1156.2) versus 284.1 μg/l (52.6 – 518.9), p = 0.001).

Seizures
Table 7 compares the baseline characteristics of those who had seizures with those who did not. Overall, fifty four percent (59/109) of the children had one or more witnessed seizures after admission. Of the children with undetectable baseline blood levels of chloroquine, 6/9 (67%) had between 1 and 27 (median 5) seizures. Forty one percent (45/109) had a seizure lasting for 5 minutes or more, while 8% (9/109) had an episode of status epilepticus (seizure lasting for 30 minutes or more). Children who had received chloroquine less than 24 hours prior to admission had a similar frequency and duration of seizures to those who had received CQ earlier (p > 0.4 for all comparisons). There was no significant correlation between the total number of seizures after admission and median baseline concentrations of CQ (Spearman’s rho –0.089, p = 0.36) or DCQ (Spearman’s
rho -0.039, p = 0.69). Baseline levels of CQ and DCQ and the corresponding area under the concentration curves (AUC0-12) were not significantly associated with the occurrence of seizures lasting for 5 minutes or more (p > 0.5 for CQ, DCQ, and AUC0-12). Baseline CQ levels and AUC0-12 were, however, significantly reduced in the nine children who had an episode of status epilepticus (Table 8). Multivariate logistic regression analysis, taking into account factors likely to affect the risk of seizures in hospital (Table 7) failed to change the significance of these results.
Discussion

There are many possible explanations for the high incidence of seizures in childhood cerebral malaria. Fever, known to precipitate seizures in young children (Wallace 1988), is an almost universal feature of cerebral malaria, although many of the seizures in cerebral malaria occur at temperatures of below 38 degrees Centigrade (Crawley 1996). Hypoglycaemia (White, 1987) and hyponatraemia (English 1996b), both complications of severe malaria, may also induce seizures. Sequestration of parasitised red blood cells in the cerebral microvasculature, the histopathological hallmark of cerebral malaria, may cause seizures through hypoxia, or by initiating the release of excitotoxic mediators, such as quinolinic acid (Dobbie 2000).

Throughout Africa, chloroquine continues to be widely used for the treatment of febrile illnesses at the community level, as illustrated by this and by previous studies (Snow 1992; Marsh 1999). Baseline chloroquine concentrations in this study were generally at the low end of the therapeutic range, and were well below concentrations (2000µg/l and above) considered toxic (Riou 1988). The association between chloroquine and seizures has been described in both adults and children, at therapeutic concentrations (Torrey 1968; Fish 1988; Luijckx 1992) and following overdose (Cann 1961; Kiel 1964; Jaffe 1988; Riou 1988). Seizures may occur within a few hours of overdose (Cann 1961; Kiel 1964; Jaffe 1988), or between one day and several weeks after the start of treatment with therapeutic doses of chloroquine (Luijckx 1992). The concentration of chloroquine within the brain is approximately four times that of plasma (Titus 1989), and experimental evidence suggests that chloroquine may precipitate seizures by attenuation of gamma aminobutyric acid (GABA) pathways (Amabeoku 1992), and by the enhancement of dopaminergic neurotransmission (Amabeoku 1993).

In this study, however, there was no association between baseline levels, area under the concentration curve (which reflects both absorption and subsequent elimination) of chloroquine and its active metabolite desethylchloroquine, and the number of subsequent seizures in children with cerebral malaria. In the small sub-group of children who had
one or more episodes of status epilepticus, baseline levels of chloroquine and desethylchloroquine were reduced.

There are a number of limitations to this study. The dose and route of chloroquine administration were not known, and it was only possible to obtain a rough estimate of the time of administration in 73% of the patients. Information on seizures that occurred prior to admission was based on clinical history, which can be unreliable, and analysis was therefore confined to seizures that were witnessed by clinical staff in hospital. In addition, it is not known whether the reported association between chloroquine ingestion and seizures is a dose-related or idiosyncratic phenomenon. Although this study suggests that the seizures of childhood cerebral malaria are unlikely to be caused by chloroquine alone, more definitive evidence would come from prospective longitudinal studies. Such studies could assess the prevalence of seizures complicating cerebral malaria in relation to changing patterns of chloroquine usage. The rapid spread of chloroquine resistance, and the urgent need to change to alternative antimalarial drugs, is likely to provide such an opportunity in the near future.
5. AN EXCITOTOXIC MECHANISM
for
CHILDHOOD CEREBRAL MALARIA?
Introduction

Despite many years of clinical and experimental investigation, the pathogenesis of cerebral malaria remains poorly understood. This chapter explores the possibility that some of the clinical features may be explained by an excitotoxic mechanism.

The adherence of parasitised erythrocytes to endothelial cells of the cerebral vasculature initiates a cascade of events that cause intense endothelial activation on the luminal side of the blood-brain barrier (Turner 1994). Much less is known about events occurring on the abluminal side of the blood-brain barrier. Experimental stimulation of cells of the macrophage/monocyte lineage by cytokines causes the parallel induction of indoleamine-pyrrole 2,3-dioxygenase, GTP cyclohydrolase I, and nitric oxide synthase (Werner 1989; Sakai 1995). These enzymes catalyse the first step of pathways leading to the formation of quinolinic acid, neopterin, tetrahydrobiopterin, and nitric oxide (see Figure 12). In the brain, microglia constitute the resident macrophage/monocyte cells. Experimental stimulation of microglia with cytokines also causes induction of these enzymes (Sakai 1993; Heyes 1996).

Quinolinic acid is an endogenous excitotoxin that is a selective agonist of N-methyl-D-aspartate (NMDA) glutamate receptors (Stone 1993). When applied to the central nervous system of experimental animals or to cerebral tissue culture, it causes seizures, sodium influx with reversible neuronal swelling, and calcium influx with delayed neuronal disintegration (Lapin 1982; Choi 1992; Giulian 1993). Thus, the toxic action of quinolinic acid could explain the neurological complications of childhood cerebral malaria, namely seizures, reversible cerebral oedema, and permanent neurological damage.

Nitric oxide synthases catalyse the formation of the gas nitric oxide from arginine (Figure 12) (Nathan 1994). NMDA receptors are modulated by endogenous nitric oxide, with increasing concentrations of nitric oxide causing receptor blockade, and decreasing concentrations potentiating NMDA-induced calcium influx (Manzoni, 1992; Manzoni,
Nitric oxide synthases require tetrahydrobiopterin as an essential co-factor. GTP-cyclohydrolase catalyses the first step in the tetrahydrobiopterin synthesis pathway (Figure 12), in which dihydronopterin acts as an intermediary. Activation of human macrophage/monocyte cells results in the accumulation of the breakdown product neopterin, and the concentration of neopterin in biological fluids is widely used as a marker of immune activation (Wachter 1989). 5-hydroxyindoleacetic acid (5-HIAA) is a metabolite of serotonin, whose concentration in cerebrospinal fluid (CSF) reflects serotonin turnover.

We hypothesised that intense cerebrovascular endothelial activation in cerebral malaria causes activation of microglia within the brain. These activated cells then produce quinolinic acid, which may be a cause of the cerebral symptoms. In order to test this hypothesis, we have measured CSF concentrations of quinolinic acid, its precursor tryptophan, 5-HIAA, individual pterin species, and nitrate plus nitrite (stable breakdown products of nitric oxide) in CSF from children recovering from cerebral malaria.
Figure 12. Metabolic inter-relationships between quinolinic acid, neopterin, tetrahydrobiopterin, and nitric oxide

Quinolinic acid $\xrightarrow{1}$ Tryptophan $\xrightarrow{2}$ Serotonin $\rightarrow$ 5-HIAA

NMDA $\xrightarrow{4}$ NO $\rightarrow$ Arginine $\rightarrow$ Citrulline

BH2 $\xrightarrow{4}$ NO $\rightarrow$ Arginine $\rightarrow$ Citrulline

1: Indoleamine-2,3-dioxygenase
2: Tryptophan monooxygenase
3: GTP-cyclohydrolase I
4: Nitric oxide synthase
5-HIAA: 5-hydroxyindolacetic acid
BH2: Dihydrobipterin  BH4: Tetrahydrobipterin
NEO: Dihydroneopterin triphosphate  GTP: Guanosine triphosphate
NO: Nitric oxide
NMDA: N-methyl-D-aspartate glutamate receptor
Methods

Children aged 8 months and above were recruited if they fulfilled the definition for:

- Cerebral malaria, namely failure to localise pain in the presence of *Plasmodium falciparum* parasitaemia.

  Or

- Malaria with impaired consciousness, defined as a Blantyre coma score (Table 3) of 4 or less in the presence of *Plasmodium falciparum* parasitaemia

Children received standard treatment for cerebral malaria, as described in Chapter 2. CSF was obtained by lumbar puncture once conscious level had started to improve. In 11 children who died, CSF was obtained within 15 minutes of death. The first 1ml aliquot of CSF was used for diagnostic purposes, while the second and third 1ml aliquots were used for this study. The third 1ml was collected into a bottle containing 1ml each of dithioerythritol (DTE) and diethylenetriaminopentaacetic acid (DETAPAC). The second and third 1ml aliquots were frozen at the bedside on dry ice, and stored at -70 °C until analysis. All CSF analysed had a normal cell count.

The concentrations of the following compounds were measured in CSF: quinolinic acid, tryptophan, 5-HIAA, total neopterin, tetrahydrobiopterin, dihydrobiopterin, and nitrate plus nitrite. Quinolinic acid was measured by gas chromatography electron impact mass spectrometry after tert-butyldimethylsilyl derivitization (Dobbie 1997). Tryptophan was measured by high-performance liquid chromatography (HPLC) with fluorometric detection after precolumn derivatization with *o*-phthalaldehyde-2-mercaptoethanol (Dobbie 1997). 5HIAA was measured by HPLC with electrochemical detection (Hyland 1993). Total neopterin, tetrahydrobiopterin, and dihydrobiopterin were measured by HPLC with dual electrochemical and fluorometric detection (Hyland 1993). Nitrate plus nitrite were measured by spectrophotometry (Clelland 1996). Reference ranges for the metabolite concentrations (n = 24 to 78 depending on the metabolite) were constructed from children and young adults with a variety of neurological or metabolic diseases in
whom no disturbance of the biochemical pathways was expected, who were living in the United Kingdom. In these children, CSF was sampled after a 4-hour fast. There was an age-related decrement in CSF concentration for all metabolites except neopterin, and therefore all children with cerebral malaria or malaria with impaired consciousness were exactly age-matched to the reference group. We were also able to collect an age-matched local reference group of 9 children who were being investigated for seizures, or were undergoing staging for Burkitt’s lymphoma. CSF from these children was used for comparison of nitrate plus nitrite concentrations, but was not suitable for measurement of quinolinic acid or the pterin species, because the majority of these patients were febrile.
Table 9. Cerebrospinal fluid metabolite concentrations in cerebral malaria

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Geometric mean (95% CI)</th>
<th>Cerebral malaria</th>
<th>Geometric mean ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinolinic acid (nmol/l)</td>
<td>16.2 (12.3 - 21.5)</td>
<td>229 (188 - 278)</td>
<td>14.1 (9.8 - 20.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Nitrate plus nitrite (µmol/l)</td>
<td>12.6 (10.5 - 15.1)</td>
<td>5.7 (5.1 - 6.5)</td>
<td>0.45* (0.35 - 0.59)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total neopterin (nmol/l)</td>
<td>17.0 (15.2 - 19.1)</td>
<td>186 (162 - 213)</td>
<td>10.9 (9.1 - 13.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tetrahydrobiopterin (nmol/l)</td>
<td>33.9 (30.2 - 38.0)</td>
<td>119 (106 - 134)</td>
<td>3.5 (3.0 - 4.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dihydrobiopterin (nmol/l)</td>
<td>3.8 (2.9 - 4.9)</td>
<td>12.7 (11.2 - 14.4)</td>
<td>3.3 (2.6 - 4.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>5-Hydroxyindoleacetic acid (nmol/l)</td>
<td>194 (172 - 218)</td>
<td>233 (210 - 259)</td>
<td>1.2 (1.0 - 1.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Tryptophan (µmol/l)</td>
<td>2.7 (2.4 - 3.0)</td>
<td>2.5 (2.1 - 3.0)</td>
<td>0.93 (0.77 - 1.1)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

* Geometric mean ratio (95% CI) for local reference range: 0.55 (0.45 - 0.68), p < 0.001
Table 10. Cerebrospinal fluid metabolite concentrations in the different outcome groups

<table>
<thead>
<tr>
<th></th>
<th>Normal recovery (n = 74)</th>
<th>Neurological sequelae (n = 11)</th>
<th>Died (n = 12)</th>
<th>Analysis of variance statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quinolinic acid</strong></td>
<td>194 (161 - 234)</td>
<td>359* (170 - 759)</td>
<td>429* (188 - 978)</td>
<td>$F_{2,95} = 5.35$ $P = 0.006$</td>
</tr>
<tr>
<td>(nmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nitrate plus nitrite</strong></td>
<td>5.4 (4.7 - 6.1)</td>
<td>6.3 (4.3 - 9.2)</td>
<td>8.1 (5.1 - 12.9)</td>
<td>$F_{2,93} = 2.64$ $P = 0.076$</td>
</tr>
<tr>
<td>(µmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total neopterin</strong></td>
<td>180 (154- 251)</td>
<td>169 (114 - 251)</td>
<td>243 (141 - 419)</td>
<td>$F_{2,95} = 1.12$ $P = 0.33$</td>
</tr>
<tr>
<td>(nmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tetrahydrobiopterin</strong></td>
<td>130 (116 - 144)</td>
<td>101 (74.8 - 134)</td>
<td>114 (62.1 - 210)</td>
<td>$F_{2,93} = 1.67$ $P = 0.19$</td>
</tr>
<tr>
<td>(nmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dihydrobiopterin</strong></td>
<td>11.0 (9.8 - 12.3)</td>
<td>14.5 (9.9 - 21.2)</td>
<td>28.2* (16.9 - 47.1)</td>
<td>$F_{2,95} = 15.7$ $P &lt; 0.001$</td>
</tr>
<tr>
<td>(nmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5-Hydroxyindoleacetic</strong></td>
<td>216 (194 - 241)</td>
<td>242 (173 - 338)</td>
<td>335* (220 - 511)</td>
<td>$F_{2,93} = 3.94$ $P = 0.023$</td>
</tr>
<tr>
<td>acid (nmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tryptophan</strong></td>
<td>2.4 (2.0 - 2.8)</td>
<td>2.4 (1.6 - 3.7)</td>
<td>3.5 (1.6 - 7.8)</td>
<td>$F_{2,93} = 1.18$ $P = 0.31$</td>
</tr>
<tr>
<td>(µmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significantly increased (analysis of variance with Duncan's new multiple range test)
Results

Ninety-seven children were studied, of whom 79 had cerebral malaria, and 18 had malaria with impaired consciousness. Median age was 2.2 years (95% CI 0.7 to 6.9 years). Eighty-two children (85%) had CSF taken within 48 hours of admission. There were no differences in any of the metabolite concentrations between CSF taken within 48 hours of admission (n = 82) compared with that taken after 48 hours (n = 15). There were also no significant differences in metabolite concentrations between the children with cerebral malaria and those with impaired consciousness. Geometric mean ratios (cerebral malaria/malaria with impaired consciousness) for each metabolite were as follows. Quinolinic acid 1.1 (95% CI 0.5-1.9), neopterin 0.9 (95% CI 0.6 – 1.2), tetrahydrobiopterin 0.8 (95% CI 0.6 – 1.1), dihydrobiopterin 1.1 (95% CI 0.8 – 1.5), 5HIAA 1.0 (95% CI 0.8 – 1.4), tryptophan 1.2 (95% CI 0.8 – 1.8), nitrate plus nitrite 1.2 (95% CI 0.9 – 1.5). Therefore, for subsequent analysis, children with cerebral malaria and those with malaria and impaired consciousness were grouped together as “cerebral malaria" for simplicity (Table 9).

In comparison with the reference range, children with cerebral malaria had highly significant increases in the CSF concentrations of quinolinic acid, neopterin, tetrahydrobiopterin, and dihydrobiopterin (Table 9). There was a significant but small increase in 5HIAA, and no change in tryptophan concentration. There was no significant difference or obvious trend in metabolite concentrations between children who had seizures or status epilepticus and those who did not. In contrast, CSF nitrate plus nitrite concentrations were significantly reduced in cerebral malaria compared to both the United Kingdom reference range (Table 9) and the local reference range. There was no difference in nitrate plus nitrite concentrations between the United Kingdom and local reference ranges (geometric mean ratio 0.82 (95% CI 0.60 – 1.11), p = 0.19).

In children with cerebral malaria, concentrations of CSF quinolinic acid, dihydrobiopterin, and 5HIAA were significantly different across the three outcome groups (Table 10). CSF quinolinic acid was significantly increased in survivors with
neurological sequelae and those who died compared with those who recovered normally.
CSF dihydrobiopterin and 5HIAA concentrations were significantly increased in children
who died compared to those who survived.

Discussion

Our results show that Kenyan children with cerebral malaria have a characteristic
neurochemical profile, with increased CSF concentrations of quinolinic acid and
neopterin, and a reduced concentration of nitrate plus nitrite. Increased concentrations of
quinolinic acid (Brouwers 1993; Hagberg 1993; Heyes 1995) and neopterin (Weiss 1998)
within the central nervous system (CNS) have been demonstrated in a number of CNS
infections, and in a murine model of cerebral malaria (Sanni 1998). There is evidence that
this results from induction of indoleamine 2,3-dioxygenase and GTP cyclohydrolase I,
the first enzymes in the pathways leading to quinolinic acid and neopterin synthesis
respectively (Wachter 1989; Heyes 1993; Nathan 1994).

Experimental support for our findings comes from the work of Sanni and others, who
demonstrated a 3-fold increase in brain quinolinic acid concentrations in mice with fatal
cerebral malaria due to experimental infection with *Plasmodium berghei* ANKA (Sanni
1998). In a previous study of children with cerebral malaria, Weiss and co-workers found
CSF concentrations of total neopterin and biopterin that were considerably lower than
those detected in this study (Weiss 1998). The clinical and epidemiological
characteristics of cerebral malaria are similar in Zambia and Kenya, and methodological
differences may account for the variation between the two studies.

Conditions that cause induction of indoleamine 2,3-dioxygenase and GTP cyclohydrolase
I have also been shown experimentally to cause induction of nitric oxide synthase,
leading to increased synthesis of nitric oxide (Sakai 1995). Nitrate and nitrite are stable
breakdown products of nitric oxide, and clinical and experimental evidence suggests that
concentrations of nitrate plus nitrite in lumbar CSF reflect nitric oxide synthase activity
within the brain (Salter 1996; Dobbie 1997). In contrast to the modest elevation of CSF
nitrate and nitrite concentrations previously found in cerebral malaria (Weiss 1998), we found decreased concentrations of nitrate plus nitrite in cerebral malaria, compared to both United Kingdom and local reference populations. Our findings are similar to those found in Tanzanian children with *Plasmodium falciparum* infection (Anstey 1996). In this study, reduced nitrite plus nitrate levels in both plasma and urine were found in children with cerebral malaria, compared with controls and with children with subclinical or uncomplicated malaria. Reduction in concentration of nitrate plus nitrite can only occur as a result of reduced activity of constitutive nitric oxide synthase, or by increased removal of nitric oxide. Within the brain, the activity of both constitutive isoforms of nitric oxide synthase is calcium-dependent and requires tetrahydrobiopterin as a co-factor. (Nathan 1994). We have shown that tetrahydrobiopterin concentrations are increased in cerebral malaria, and co-factor deficiency does not, therefore, seem a likely explanation. It is possible that, in cerebral malaria, the activity of nitric oxide synthase within the brain is limited by reduced concentrations of free intracellular calcium or of the substrate arginine. Both possibilities require further investigation.

The combination of increased quinolinic acid and reduced nitric oxide concentrations within the brain may provide an excitotoxic mechanism that accounts for the symptoms of cerebral malaria. When quinolinic acid is synthesised in excess, it readily enters the extracellular compartment (Speciale 1993). There is neither extracellular metabolism nor active transport of quinolinic acid in cerebral tissue, and toxic quantities of quinolinic acid may therefore accumulate within the synaptic cleft (Foster 1983). Nitric oxide has been shown to protect against NMDA-mediated neurotoxicity by reducing conductance of calcium through the receptor pore (Manzoni 1992; Manzoni 1993). Reduction in nitric oxide production could therefore enhance the neurotoxicity of quinolinic acid (Manzoni 1992; Manzoni 1993).

The mechanisms by which NMDA-toxicity is mediated can be separated into two components, distinguishable on the basis of time course and ionic dependence. An initial influx of sodium causes reversible neuronal swelling, while subsequent influx of calcium leads to delayed neuronal disintegration (Choi 1992). Excitotoxic mechanisms can thus
explain the neurological symptoms of cerebral oedema, namely coma and cerebral oedema that usually resolve and, in a proportion of cases, neurological damage that may be permanent. The graded increment in quinolinic acid concentrations across the three outcome groups lends support to this hypothesis. Although it is possible that the increased concentrations of quinolinic acid found in children who died was a post-mortem artefact, this is unlikely because intracellular accumulation of quinolinic acid does not occur in the central nervous system (Speciale 1993), and the CSF was sampled within 15 minutes of death.

In conclusion, we have found biochemical evidence for an excitotoxic mechanism that may account for the symptoms of cerebral malaria. This is of potential importance because selective NMDA-receptor antagonists are becoming more widely used in medicine, and could provide a novel approach to the treatment of cerebral malaria (Lipton 1993).
6. PHENOBARBITONE PROPHYLAXIS
A randomised, controlled intervention study
Introduction

If anticonvulsant prophylaxis can reduce the incidence of seizures complicating cerebral malaria, this may in turn reduce the risk of death and neurological sequelae (Molyneux 1989; Brewster 1990; Jaffar 1997; van Hensbroek 1997), and have an important impact on the educational potential of children in sub-Saharan Africa (Holding 1999). This chapter describes a randomised, controlled intervention study of anticonvulsant prophylaxis in childhood cerebral malaria.

Phenobarbitone has been used as an anticonvulsant for many years, and is highly effective in the treatment of both partial and generalised seizures (Shorvon 1994b). It is cheap and, unlike the majority of anticonvulsant drugs used for seizure prophylaxis, widely available throughout Africa. It may be given by intramuscular injection, which is a further advantage, since intravenous therapy is not possible in many health facilities throughout the continent. The loading dose recommended for children is 10-20 mg/kg (Rylance 1990; Bone 1993).

The three published studies of phenobarbitone prophylaxis in cerebral malaria have yielded disparate results. In a small, randomised, controlled study on Thai adults, a single intramuscular dose of phenobarbitone 3.5 mg/kg reduced the incidence of seizures by 40% (White 1988a). A single intramuscular dose of phenobarbitone 10 mg/kg given to Indian adults in an open study reduced the incidence of seizures by 20% (Kochar 1997) while, in an open study on Kenyan children, the same dose failed to produce therapeutic blood concentrations, and had no effect on seizure frequency (Winstanley 1992). The randomised, placebo controlled study presented here was designed to assess whether a single intramuscular dose of phenobarbitone 20 mg/kg given on admission to Kenyan children with cerebral malaria could reduce the incidence of seizures complicating the clinical course in hospital. The safety and clinical tolerance of this dose was assessed at the start of the trial.
Methods

Patients
Children aged 9 months to 13 years with cerebral malaria (see Chapter 2) were eligible for the study. Those who had pre-existing afebrile epilepsy or significant neurodevelopmental problems, and those who had received treatment with phenobarbitone or phenytoin during the current illness were excluded.

Sample size
A sample size calculation, assuming 90% power and a significance level of 5%, suggested that a total of 320 children would be required to detect a 50% reduction in seizures occurring prior to recovery of consciousness in the phenobarbitone-treated group. The following seizure categories were considered to be of particular clinical importance:

- Three or more seizures of any duration
- Any seizures lasting for 5 minutes or more
- Any episodes of status epilepticus (defined as seizure activity lasting for 30 minutes or more, or 6 or more seizures within a period of 2 hours).

Randomisation
Using a sequentially numbered register, children were randomised as soon as possible after admission to receive a single intramuscular injection of phenobarbitone 20 mg/kg, or the same volume of identical placebo (90% propylene glycol, the normal vehicle for parenteral preparations of phenobarbitone). Numbered 5ml ampoules of phenobarbitone and placebo were prepared by the pharmacy department of Torbay Hospital, UK. The code identifying drug and placebo was kept at Torbay Hospital, and therefore none of the clinical or scientific staff involved in the study knew which patients had received phenobarbitone. Since previous work (Crawley 1996) had shown an increased frequency
of seizures among younger children with cerebral malaria, randomisation was stratified into two age groups of 24 months or below and above 24 months.

Clinical tolerance
The clinical tolerance of phenobarbitone 20 mg/kg was assessed at the start of the trial. Twenty three children received the study drug (phenobarbitone or placebo) by constant rate intravenous infusion over 4 hours instead of by intramuscular injection. The intravenous route was chosen because the infusion could have been stopped should any adverse events have occurred. As in the main study, the clinical investigators were unaware of which patients had received phenobarbitone. Pulse, respiratory rate, blood pressure, and transcutaneous oxygen saturation were measured at baseline, and at thirty-minute intervals for 5 hours. Blood was taken at the same times for phenobarbitone level, and at hourly intervals for venous gas and lactate. The trial was then unblinded for these 23 patients only, and the phenobarbitone and placebo groups compared with respect to all clinical and biochemical findings.

Investigations
Baseline blood samples were taken for parasite count, full blood count, glucose, electrolytes, blood gas, blood culture, and phenobarbitone level. Further blood samples were taken for phenobarbitone level at 1, 2, 4, 8, 12, 24, 36, and 48 hours. Parasite counts were repeated every 8 hours until discharge, death, or clearance of parasitaemia.

Treatment
Patients received standard therapy for cerebral malaria, as described in Chapter 2. The number and duration of all seizures were recorded using timers pre-set to alarm at 5, 15, and 30 minutes. Seizures lasting for 5 minutes or more were treated in a standardised manner, with intravenous diazepam 0.3 mg/kg, and, as second line therapy, intramuscular paraldehyde 0.2 ml/kg. Anticonvulsants were administered at 5, 15, 30, and 45 minutes, if seizure activity persisted. Intravenous phenytoin 20 mg/kg was given to children who, following randomisation, had received 2 doses of both diazepam and paraldehyde, or had experienced 6 or more seizures within a period of 2 hours.
Follow up

A neurological examination was performed on all patients at the time of discharge from hospital. All patients were asked to return 3 months later for full neurodevelopmental assessment.

Pharmacokinetics

Serial blood samples for phenobarbitone concentration were taken from all patients. Whole blood phenobarbitone concentrations were subsequently assayed by reversed-phase high performance liquid chromatography (Winstanley 1992) in Nairobi. Derived pharmacokinetic parameters, namely maximum concentration (Cmax), time to maximum concentration (Tmax), and area under the concentration / time curve (AUC_{0-12} hours) were obtained after every 10 profiles on patients who had received phenobarbitone by intramuscular injection. Since there was very little variability in the data, with no significant change in the mean derived pharmacokinetic profiles as numbers increased, it was decided that profiles on 50 patients would provide a good representation of the phenobarbitone group as a whole.

Statistical analysis

This is described in Chapter 2. An interim analysis was performed by an independent statistician in January 1997, at which stage 170 patients had been recruited. The results of the analysis were reviewed by the trial monitor, but were not made available to the clinical investigators. The trial was continued until the calculated sample size had been achieved.
Figure 13. Whole-blood phenobarbitone concentrations according to mode of administration

Circles denote intravenous and squares intramuscular administration

Error bars: 1 SD
Results

Recruitment
Between June 1995 and January 1998, 440 children fulfilling the criteria for entry to the study were admitted to the research ward. One hundred were not recruited, for the following reasons. Relatives refused consent (63), death considered imminent (23), clinical pressures made recruitment impossible (14). The remaining 340 children were randomised, and all are included in the main analysis. Eleven children were recruited to the study but were subsequently (before the study code was broken) recognised as fulfilling the criteria for exclusion. Repeat analysis, following exclusion of these cases, did not change the statistical significance of any of the study endpoints.

Clinical tolerance
Twenty three children (10 phenobarbitone, 13 placebo) were assessed for clinical tolerance. Baseline clinical (pulse, systolic blood pressure, respiratory rate, and transcutaneous oxygen saturation) and biochemical (venous blood gas and lactate) characteristics were similar in both groups (p > 0.40 for all comparisons). There were no significant differences between the groups in the mean change between baseline and 5-hour or baseline and maximum / minimum values for any of the parameters measured (p = 0.07 for fall in lactate (0-5 hours) and maximum rise in pH, p > 0.10 for all other comparisons).

Pharmacokinetics
Whole blood concentrations of phenobarbitone obtained by intramuscular injection and intravenous infusion of 20 mg/kg are shown in Figure 13. Intramuscular injection (n = 50) resulted in a median maximum concentration (Cmax) of 25.6 μg/ml (range 10.0 to 53.7 μg/ml, interquartile range 23.6 to 30.2μg/ml). Median time to maximum concentration (Tmax) was 4.0 hours (range 1.0 to 48.0 hours, interquartile range 2.0 to 12.0 hours). Concentrations were maintained above 15 μg/ml, which is within the range (10-30 μg/ml) thought to provide effective seizure prophylaxis (Faero et al. 1979), (Dodson 1984), (Shorvon 1994b), for at least 48 hours. The mean area under the
concentration-time curve for the period 0-12 hours (AUCO-12) following intramuscular injection was 250 µg.hr/ml (95% CI 232.3 to 268.2). There were no significant differences in Cmax, Tmax, and AUC0-12 between children who survived and those who died (p > 0.4 for all comparisons).

Due to technical problems, it was only possible to obtain profiles on 4 of the 10 children who had received phenobarbitone by intravenous infusion. The mean AUCO-12 following intravenous infusion was 196.2 µg.hr/ml (95% CI 154.1 to 238.4). The difference in AUCO-12 between the intravenous and intramuscular routes of administration was of borderline statistical significance (p = 0.05).

**Admission characteristics**

Clinical and laboratory features of all patients in the study are shown in Tables 11 and 12. The placebo and phenobarbitone groups were well matched with regard to the majority of clinical and laboratory parameters. Twenty eight percent of the children (49 placebo, 45 phenobarbitone), had a previous febrile illness that had been complicated by seizures. During the current illness, a significantly higher proportion of children randomized to placebo had a history of seizures prior to admission (148 (87%) versus 133 (78%), p=0.04). The difference in the proportion of children in each treatment group who had been given a dose of diazepam in the 6-hour period prior to admission failed, however, to reach statistical significance (43 (25%) placebo, 32 (19%) phenobarbitone, p = 0.19).
Table 11. Admission clinical features by treatment group

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Placebo (n=170)</th>
<th>Phenobarbitone (n=170)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>35.4 (32.4-38.5)</td>
<td>35.4 (32.7-38.0)</td>
<td>0.97</td>
</tr>
<tr>
<td>Male sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (%)</td>
<td>93 (55)</td>
<td>91 (54)</td>
<td>0.92</td>
</tr>
<tr>
<td>Seizures before admission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any seizures</td>
<td>148 (87)</td>
<td>133 (78)</td>
<td>0.04</td>
</tr>
<tr>
<td>Status epilepticus*</td>
<td>82 (48)</td>
<td>63 (37)</td>
<td>0.05</td>
</tr>
<tr>
<td>Duration of coma prior to Admission (hours)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>5</td>
<td>5</td>
<td>0.95</td>
</tr>
<tr>
<td>Range</td>
<td>1-72</td>
<td>1-72</td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>2-10</td>
<td>2-10</td>
<td></td>
</tr>
<tr>
<td>Blantyre coma score**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 or 1</td>
<td>45 (26)</td>
<td>38 (22)</td>
<td>0.45</td>
</tr>
<tr>
<td>2 or 3</td>
<td>125 (74)</td>
<td>132 (78)</td>
<td></td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>38.9 (38.8-39.1)</td>
<td>38.8 (38.6-38.9)</td>
<td>0.16</td>
</tr>
<tr>
<td>Pulse (per minute)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>149 (146-152)</td>
<td>148 (144-152)</td>
<td>0.60</td>
</tr>
<tr>
<td>Respiratory rate (per minute)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean (95% CI)</td>
<td>41 (39-43)</td>
<td>42 (40-44)</td>
<td>0.69</td>
</tr>
<tr>
<td>Respiratory distress*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (%)</td>
<td>50 (29)</td>
<td>49 (29)</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

* History of multiple seizures ("too many to count"), or of any seizure lasting for 30 minutes or more
** See Table 3
*Increased depth of respiration (Kussmaul's respiration)
Table 12. Admission laboratory investigations by treatment group

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Placebo (n=170)</th>
<th>Phenobarbitone (n=170)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Parasitaemia (per μl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>79620</td>
<td>89600</td>
<td>0.96</td>
</tr>
<tr>
<td>Range</td>
<td>85-1441800</td>
<td>184-1980000</td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>6448-351120</td>
<td>4294-422400</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>7.2 (6.8-7.5)</td>
<td>7.2 (6.9-7.6)</td>
<td>0.78</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>135 (134-136)</td>
<td>134 (133-135)</td>
<td>0.29</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td></td>
<td></td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Median</td>
<td>4.3</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2.8-8.3</td>
<td>2.0-8.0</td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>3.9-4.8</td>
<td>3.8-4.9</td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>Median</td>
<td>4.2</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.2-25.4</td>
<td>1.0-36.0</td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>3.1-6.7</td>
<td>3.2-7.7</td>
<td></td>
</tr>
<tr>
<td>Creatinine* (μmol/l)</td>
<td>63.8 (58.9-69.1)</td>
<td>64.5 (59.6-69.7)</td>
<td>0.85</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.4 (4.9-5.9)</td>
<td>5.3 (4.8-5.8)</td>
<td>0.70</td>
</tr>
<tr>
<td>pH</td>
<td>7.30 (7.28-7.32)</td>
<td>7.31 (7.28-7.33)</td>
<td>0.66</td>
</tr>
<tr>
<td>PCO₂ (kPa)</td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Median</td>
<td>4.1</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.2-11.5</td>
<td>1.1-13.3</td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>3.2-4.8</td>
<td>3.0-4.4</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate (mmol/l)</td>
<td>15.6 (14.8-16.5)</td>
<td>15.1 (14.2-15.9)</td>
<td>0.34</td>
</tr>
<tr>
<td>Base excess</td>
<td>-9.2 (-10.3 to -8.2)</td>
<td>-9.5 (-10.6 to -8.4)</td>
<td>0.73</td>
</tr>
<tr>
<td>Lactate (mmol/l)*</td>
<td>3.7 (3.4 -4.2)</td>
<td>4.0 (3.5- 4.5)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

* Geometric mean
Clinical course
Sixty-three percent (213/340) of the patients achieved parasite clearance before discharge or death. Median (interquartile range) times to parasite clearance were 40.0 (31.5 to 52.8) hours for the phenobarbitone group, and 45.7 (30.6 to 63.0) hours for placebo (p = 0.21, Peto-Peto-Wilcoxon test for equality of survivor function).

There was no difference between the two groups in the time taken to recover consciousness (the ability to localise a painful stimulus (Molyneux 1989)), as shown in Figure 14 (p = 0.39, Log Rank test).

Seizures
Phenobarbitone provided effective seizure prophylaxis, reducing the frequency and duration of seizures by over 50% (Table 13). Multivariate logistic regression analysis, allowing for the slight excess of children in the placebo group who had a history of seizures prior to admission, failed to change the significance of this result (Table 13). Significantly fewer children treated with phenobarbitone subsequently required treatment with phenytoin (14/170 (8.2%), versus placebo 27/170 (15.9%) p = 0.04).

Death
Mortality among children treated with phenobarbitone was more than double that of the placebo-treated group. Of the forty-four deaths that occurred in total (Table 13), 30/170 (18%) were in children given phenobarbitone, compared to 14/170 (8%) children given placebo (odds ratio 2.39, 95% CI 1.28-4.46, p = 0.01). Using multivariate logistic regression analysis, the significance of this result could not be accounted for by minor imbalances in risk factors between the two groups (Table 13). Median time from drug administration to death was 22.5 hours (range 0.42 to 92.7) for children treated with phenobarbitone and 24.2 hours (range 0.4 to 66.7) for those who received placebo (p= 0.72). Kaplan-Meier curves, comparing overall survival in the two groups, are shown in Figure 14.
Table 13. Clinical outcome after treatment with placebo or phenobarbitone

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=170)</th>
<th>Phenobarbitone (n=170)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SEIZURES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three or more seizures</td>
<td>46 (27)</td>
<td>18 (11)</td>
<td>0.32 (0.18-0.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>of any duration</td>
<td></td>
<td></td>
<td>*0.34 (0.19-0.62)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any seizures lasting 5</td>
<td>43 (25)</td>
<td>20 (12)</td>
<td>0.39 (0.22-0.70)</td>
<td>0.002</td>
</tr>
<tr>
<td>minutes or longer</td>
<td></td>
<td></td>
<td>*0.42 (0.24-0.76)</td>
<td>0.004</td>
</tr>
<tr>
<td>Any episodes of status</td>
<td>23 (14)</td>
<td>9 (5)</td>
<td>0.36 (0.16-0.78)</td>
<td>0.01</td>
</tr>
<tr>
<td>epilepticus**</td>
<td></td>
<td></td>
<td>*0.38 (0.17-0.85)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>DEATH / NEUROLOGICAL SEQUELAE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Died</td>
<td>14 (8)</td>
<td>30 (18)</td>
<td>2.39 (1.28-4.64)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>*2.49 (1.19-5.23)</td>
<td>0.02</td>
</tr>
<tr>
<td>Neurological sequelae</td>
<td>33/156 (21)</td>
<td>18/140 (13)</td>
<td>0.55 (0.30-1.02)</td>
<td>0.06</td>
</tr>
<tr>
<td>at discharge</td>
<td></td>
<td></td>
<td>*0.56 (0.30-1.05)</td>
<td>0.07</td>
</tr>
<tr>
<td>Neurological sequelae</td>
<td>15/144 (10)</td>
<td>9/131 (7)</td>
<td>0.63 (0.27-1.47)</td>
<td>0.39</td>
</tr>
<tr>
<td>3 months post discharge</td>
<td></td>
<td></td>
<td>*0.69 (0.29-1.65)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

** Seizure lasting 30 minutes or longer, or a minimum of 6 seizures within a 2-hour period

Figures in italics represent adjusted odds ratios and p values

*Adjusted for seizures prior to admission

*Adjusted for risk factors associated with increased mortality (Blantyre score, respiratory distress, base excess, glucose, urea, creatinine)
Figure 14a. Kaplan-Meier plot of coma resolution
(Circles denote phenobarbitone, and squares placebo)

Figure 14b. Kaplan-Meier plot of overall survival
Children who died were, on admission, more deeply comatose, and significantly more dehydrated, acidotic, and hypoglycaemic compared to those who survived (p < 0.01 for all parameters). Among those who died, mean admission respiratory rate was higher in the placebo group (57 (95%CI 50-64) compared to those given phenobarbitone (47 (95%CI 41-52), p = 0.03). However, respiratory rate at 4 hours (maximum phenobarbitone concentration) was comparable for the two groups (placebo 47 (95%CI 41-43), phenobarbitone 48 (95%CI 43-53) p = 0.76). There were no other significant differences between the groups in admission clinical or laboratory characteristics.

Thirty-three children had a respiratory arrest (cessation of breathing in the presence of normal cardiac function) during their clinical course in hospital, of whom thirty died. Of the 33 who had a respiratory arrest, 22 (66.7%) had received phenobarbitone, and 11 (33.3%) placebo (odds ratio 2.1, 95%CI 1.0-4.5, p = 0.06). When total diazepam dose (which included doses given in the 6 hours prior to admission, plus all doses given in hospital, both before and after randomisation) was divided into four categories, the odds of death for children treated with phenobarbitone rose sharply from less than 0.2 for those given 0, 1, or 2 doses of diazepam to 1.68 (95% CI 0.40 to 6.97, p = 0.004) for those given 3 or more doses. The relationship between death and the interaction between drug group (phenobarbitone or placebo) and diazepam category (less than 3 doses/3 or more doses) was then examined using logistic regression and the Likelihood Ratio test. Diazepam category alone was not significantly associated with an increased risk of death (odds ratio 0.55 95% CI 0.07 to 4.48, p = 0.5). The interaction between diazepam category and drug group (phenobarbitone or placebo) was, however, associated with a greatly increased risk of death (odds ratio 16.47, 95% CI 1.26 to 214.84, p = 0.03). Table 14 compares the difference in mortality between the phenobarbitone and placebo groups following less than 3 and 3 or more doses of diazepam. In this post hoc analysis, a substantial part of the excess mortality in the phenobarbitone group can therefore be accounted for by the interaction between phenobarbitone and multiple (3 or more) doses of diazepam.
Table 14. Mortality in phenobarbitone and placebo groups, according to number of doses of diazepam

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Phenobarbitone</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0, 1, OR 2 DOSES OF DIAZEPAM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>N=150</td>
<td>N=162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 (9%)</td>
<td>13 (9%)</td>
<td>25 (15%)</td>
<td>1.9 (0.9-3.9)</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>3 OR MORE DOSES OF DIAZEPAM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>N=20</td>
<td>N=8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>5 (62%)</td>
<td>31.7 (1.2-814.3)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Neurological sequelae
Fifty-one children (17% of those who survived) had neurological sequelae at the time of discharge from hospital. The commonest problems were hemiplegia, spastic quadriplegia, blindness, speech delay, epilepsy, or a combination of these features. Although the proportion of children with sequelae was reduced in the phenobarbitone group (Table 13), the difference was of borderline statistical significance (placebo 33/156 (21%), phenobarbitone 18/140 (13%), \( p = 0.06 \)). Of 275 children seen 3 months after discharge, 9% had persistent sequelae, and the difference between the two groups was not significant (\( p = 0.39 \), see Table 13).

Discussion
There are many reasons why anticonvulsant prophylaxis is likely to be beneficial in childhood cerebral malaria. Clinical and experimental evidence suggests that prolonged, uncontrolled seizure activity can damage the brain (Aicardi 1983; Meldrum 1973a; Corsellis 1983; Stafstrom 1993). Seizures complicating cerebral malaria are associated with an increased risk of neurological sequelae (Molyneux 1989; Brewster 1990; Crawley 1996) and survivors may, in addition, have significant cognitive problems.

The antiepileptic properties of phenobarbitone were first recognised in 1912, and there is now extensive experience of its use in both adults and children (Treiman, 1998; Shorvon 1994b; Bone 1993; Shaner 1988). Experimental evidence suggests that phenobarbitone can prevent structural damage resulting from uncontrolled seizure activity within the developing brain (Sutula 1992; Mikati 1994). This study shows that a single intramuscular dose of phenobarbitone 20 mg/kg is effective at preventing seizures in children with cerebral malaria. Neurological sequelae were reduced in the phenobarbitone treated group, although the difference failed to reach statistical significance. It is important to remember, however, that the clinical conditions of this study, with rapid treatment and close observation of all children with seizures, were very different from those that prevail in many hospitals throughout Africa. There, inadequate
staffing and paucity of drugs and equipment mean that children may fit for many hours without adequate treatment, so increasing the morbidity and mortality that may be attributed to seizures.

In this study, the unacceptable price of effective seizure prophylaxis was the doubling of mortality in the phenobarbitone treated group. The most plausible explanation is that phenobarbitone-induced respiratory depression has precipitated respiratory arrest in a group of unventilated children who are critically dependent on their respiratory drive. Support for this hypothesis comes from the greater proportion of children treated with phenobarbitone who had a respiratory arrest, and the increased mortality among children treated with both phenobarbitone and multiple (3 or more) doses of diazepam. There was, however, no difference in respiratory rate at 4 hours (the time of peak phenobarbitone concentrations) between the phenobarbitone and placebo groups. Children who continue to have seizures despite phenobarbitone prophylaxis may represent a sub-group with particularly refractory cerebral pathology, who could be unusually susceptible to further sedation with diazepam.

Intravenous diazepam is the drug of first choice for the immediate treatment of status epilepticus, because of its ease of administration and rapid onset of action (Shorvon 1994b). The drug is highly lipid soluble, and is rapidly distributed to cerebral tissue and lipid stores. Following repeated administration, redistribution ceases as tissue stores equilibrate, and further doses result in high, persistent concentrations within the brain, and the consequent risk of sudden respiratory arrest and hypotension. In contrast, phenobarbitone has been shown in two controlled studies (Treiman, 1998; Shaner 1988) to be highly effective in the treatment of status epilepticus, with no associated increase in respiratory depression or hypotension. A retrospective review of children with refractory status epilepticus, who had been treated with phenobarbitone in the dose range 30 to 120 mg/kg (Crawford 1988), demonstrated a striking lack of respiratory depression. Despite case reports in the literature suggesting that the combination of phenobarbitone and diazepam may result in respiratory depression and hypotension (Prensky 1967; Sawyer
1968; Browne, 1973), this is the first study to document an increase in mortality resulting from concomitant use of the two drugs.

Is there a dose of phenobarbitone that is both safe and effective in childhood cerebral malaria? Phenobarbitone is said to provide effective seizure prophylaxis at plasma levels of between 10 and 30 μg/ml. Plasma levels may, however, vary widely between individuals given the same dose (Treiman 1992), a finding confirmed in this study. In addition, phenobarbitone has a pKa of 7.2, and changes in body pH will, therefore, affect the distribution and excretion of the drug. If the pH of the blood is lower than that of the brain, the gradient will favour movement of phenobarbitone into the brain (Simon 1987; Dodson 1984). Status epilepticus disrupts the blood-brain barrier, causing cerebral uptake of phenobarbitone to be enhanced even further (Walton 1989; Ramsay 1979; Brzakovic 1997). The relationship between plasma concentration and effect in cerebral malaria could be different from other conditions without generalised cerebral pathology. It is possible, therefore, that effective anticonvulsant prophylaxis could result from plasma concentrations of phenobarbitone usually considered “sub-therapeutic” (Goldberg 1983). In adults with cerebral malaria, phenobarbitone at doses of both 3.5 mg/kg and 10 mg/kg provided effective seizure prophylaxis (White 1988a; Kochar 1997). Might phenobarbitone 10 mg/kg, in a larger study, prove both safe and effective in childhood cerebral malaria, despite blood concentrations at the lower limit of the “therapeutic range” (Winstanley 1992)?

We have demonstrated that phenobarbitone 20 mg/kg is highly effective at preventing seizures in childhood cerebral malaria, but is associated with an unacceptable increase in mortality. It is not yet clear whether a lower dose may provide effective anticonvulsant prophylaxis without the increased mortality. Other therapeutic options, such as magnesium sulphate (Duley 1995), should be explored. Having found a drug that is both safe and effective, it would then be important to establish, in a sufficiently large study, whether seizure prophylaxis can reduce the incidence of neurological sequelae complicating this important and devastating disease.
7. CONCLUSIONS
Introduction

This chapter summarises the studies presented in this thesis, discusses their limitations, and indicates possible areas for future research.

The role of seizures in the pathogenesis of cerebral malaria

The studies described in this thesis were confined to hospitalised children with cerebral malaria, a group that represents one end of the broad spectrum of clinical disease caused by *Plasmodium falciparum*. In order to gain a better understanding of seizures in cerebral malaria, it is important to consider the role of seizures within the broader context of uncomplicated malaria, in both community and hospital settings.

Community surveys in Kilifi District (where malaria accounts for the majority of fevers in young children) reveal that less than 5% of all febrile episodes in children below the age of 5 years are associated with seizures (V. Marsh, personal communication). Within this rural community, seizures are regarded as a serious condition requiring immediate attention, and social and economic factors determine whether help is sought from the biocultural (local healers) or biomedical (shop-bought drugs, community health workers, clinics, or hospital) sector (Mwenesi 1995a; Molyneux 1999a). Since seizures are a recognised indication for admission to hospital, the proportion of hospitalised children with uncomplicated malaria who have a history of seizures (approximately 25%) is much higher than that observed among febrile children in the community (Waruiru 1996). The proportion of cases of cerebral malaria that are complicated by seizures is, however, even higher. Seizures occur in up to 80% of cases, are often prolonged or multiple, and are associated with an increased risk of neurological sequelae and death (Molyneux 1989; Brewster 1990; Jaffar 1997; van Hensbroek 1997). Although it is possible that all malaria-related seizures have the same pathological basis (sequestration of parasitised erythrocytes within the cerebral vasculature), the work presented here suggests that, in cerebral malaria, seizures themselves play a significant role in the pathogenesis of coma.
Approximately one quarter of the patients recruited to the electroencephalography (EEG) study had recovered consciousness within 6 hours of prolonged or multiple seizures, suggesting that their coma may have been a postictal phenomenon, directly related to seizures. In a similar proportion of patients (and there is overlap between the two groups), coma was associated with continuing subtle seizure activity. In a busy, understaffed hospital in the developing world, subtle seizures could easily be missed, yet their clinical features (tonic eye deviation, nystagmus, hypoventilation, salivation) are sufficiently distinct for them to be recognised by clinical or nursing staff with appropriate training. In most hospital settings in the developed world, the majority of unconscious children are artificially ventilated and paralysed, making it impossible to correlate electroencephalographic findings with clinical signs. The information derived from this observational study may, therefore, provide some valuable insights into a variety of other paediatric encephalopathies.

**Seizures and neurological sequelae**

The studies presented here also confirm the relationship between multiple, prolonged seizures and poor prognosis among patients with cerebral malaria. For the 65 children in the electrophysiological (EEG) study, and the 170 children recruited to the placebo arm of the phenobarbitone study, there was a significant association between the number and duration of seizures and subsequent neurological sequelae at the time of discharge from hospital. A similar trend was observed between seizures and subsequent death, but the smaller number of patients in this category meant that the observed differences failed to reach statistical significance. EEG recordings demonstrated that electrical seizure activity consistently arose from the posterior temporo-parietal region, which is a "watershed" area, lying between territories supplied by the middle cerebral (carotid circulation) and posterior cerebral (vertebro-basilar circulation) arteries. Consequently, it is particularly vulnerable to hypoxia when oxygen delivery to the brain is compromised as a result of sequestration, severe anaemia, or inadequate cerebral perfusion due to hypotension or raised intracranial pressure (MacPherson 1985; English 1997a; Newton 1997b). Because of their anatomical position close to the temporal lobes in the tentorial notch, the posterior cerebral arteries may
be particularly vulnerable to compromise in those patients who have acute intracranial hypertension and incipient transtentorial herniation, as illustrated by Figure 10 in Chapter 3. By initiating the release of excitotoxic mediators such as glutamate or quinolinic acid (Dobbie 2000), local hypoxia may precipitate seizures, which, by raising intracranial pressure and increasing the demand for oxygen and glucose, may exacerbate the situation further. Although there is no evidence for failure of cerebral autoregulation in the majority of these patients, relatively poor collateral circulation in some patients may mean that cerebral blood flow in the border-zone between the carotid and vertebro-basilar territories is insufficient to compensate for increased local demand. In these patients, a vicious cycle might then be generated, leading to intractable partial seizures and eventually to focal infarction. It is of note, therefore, that of the three children in the EEG study who developed a hemiplegia and who subsequently returned for follow-up cerebral computerised tomography (CT), all had evidence of infarction in the contralateral posterior quadrant. Although this hypothesis suggests that seizures may cause infarction, it is equally possible that an area of infarction could act as a focus for subsequent seizure activity. To demonstrate causation, it would be necessary to show, by means of a sufficiently large randomised intervention study, that effective anticonvulsant prophylaxis reduces the incidence of neurological sequelae in children with cerebral malaria.

The aetiology of seizures in cerebral malaria

In addition to hypoxia, there are several other factors that may trigger seizure activity in children with cerebral malaria. Fever is an obvious candidate, yet the majority of seizures in both this and other studies (Waruiru 1996) occurred at temperatures of below 38°C. In addition, many seizures were partial motor, multiple, or prolonged (lasting for more than 30 minutes), unlike the short-lived generalised seizures characteristic of “febrile seizures” commonly observed in young children. Metabolic disturbances (hypoglycaemia, hyponatraemia, hypocalcaemia, hypomagnesaemia) can also precipitate seizures but, with the exception of two children admitted with hypernatraemic dehydration (Na >150mmol/l, urea >20mmol/l) and status epilepticus, no association between metabolic derangement and subsequent seizure activity was found in the studies presented here. At
the time that these studies were carried out, chloroquine was widely used throughout Kenya for the treatment of uncomplicated malaria. A number of case reports (Fish 1988; Jaffe 1988; Luijckx 1992) have suggested an association between seizures and the ingestion of chloroquine yet, in children recruited to the placebo arm of the phenobarbitone prophylaxis study, no association was found between blood chloroquine concentrations on admission and subsequent seizures. Similarly, there was no obvious relationship between the occurrence of seizures and cerebrospinal fluid (CSF) concentrations of quinolinic acid, an endogenous excitotoxin. Compared to a reference UK population, however, children with cerebral malaria had CSF concentrations of quinolinic acid that were significantly raised, and CSF concentrations of nitrate and nitrite, stable breakdown products of nitric oxide, that were significantly reduced. In addition, there was a graded increment in the concentration of quinolinic acid across outcome groups of increasing severity. These studies need to be repeated on a larger number of children, and should include a control group derived from the local population. The mechanism underlying the unexpected reduction in CSF concentrations of nitric oxide metabolites could be explored further by measuring CSF concentrations of arginine (a substrate for nitric oxide synthase) and citrulline (a product of the same enzyme). Quinolinic acid, an N-methyl-D-aspartate (NMDA) receptor agonist, induces the influx of both sodium and calcium into neurones, leading to reversible neuronal swelling and subsequent neuronal disintegration. Nitric oxide is an NMDA receptor blocker, and may provide cerebral protection. The increased CSF concentrations of quinolinic acid and reduced concentrations of nitric oxide metabolites observed in these patients with cerebral malaria therefore lends support to the hypothesis that some of the neurological manifestations of cerebral malaria may be explained by an excitotoxic mechanism. In addition, the advent of NMDA-receptor antagonists, such as magnesium sulphate (Duley 1995), raises the possibility of novel approaches to treatment.
Intervention studies

The best way of proving a causative link between seizures and adverse outcome in children with cerebral malaria is to demonstrate, by means of a randomised, controlled intervention study, that effective anticonvulsant therapy reduces the subsequent incidence of neurological sequelae and death. Phenobarbitone is a highly effective anticonvulsant drug that has been used for years in the treatment of both partial and generalised seizures. It is cheap, widely available throughout Africa, and may be given by intramuscular injection. In children with cerebral malaria, a single intramuscular dose of phenobarbitone 20mg/kg produced blood concentrations above the therapeutic level of 15μg/ml within 2 hours of administration. These levels were subsequently maintained for a minimum of 48 hours. An initial clinical tolerance study showed that, at this dose, phenobarbitone had no adverse effects on pulse, respiratory rate, oxygen saturation, venous blood gas or lactate, when compared to placebo. Phenobarbitone halved the proportion of children with prolonged or multiple seizures, and reduced from 21% to 13% the proportion of children with neurological sequelae at discharge. In many hospitals throughout Africa, prolonged seizures may go untreated, due to shortages of both staff and equipment. The morbidity and mortality of seizures under such “real world” conditions may therefore be higher than that observed in this study. The unexpected and disturbing finding of this study was, however, that the mortality of children treated with phenobarbitone 20mg/kg was double that of the children given placebo. Post hoc analysis demonstrated that mortality was highest among children treated with both phenobarbitone and multiple (3 or more) doses of diazepam, raising the possibility that phenobarbitone had increased mortality by depressing respiratory drive. It is also possible that, if mortality in the phenobarbitone group had been less, the incidence of neurological sequelae among this group might have been higher. These findings pose a dilemma for clinicians treating children with cerebral malaria in Africa. Throughout the continent, phenobarbitone remains the single most widely available prophylactic anticonvulsant drug. In children with cerebral malaria, a dose of 20mg/kg is clearly contraindicated. But is there a lower dose of phenobarbitone that is both effective and safe, or should the assessment of other, more expensive anticonvulsant drugs become a
priority? There is an urgent need for studies that address these questions but, at this stage, it would seem pragmatic to treat children with recurrent, prolonged convulsions with a single dose of phenobarbitone 10-15mg/kg. Under these circumstances, it would also seem advisable to limit the administration of diazepam to a maximum of two doses. Paraldehyde, another cheap and effective drug for the rapid termination of seizures, could be used as an alternative.

In conclusion

Prolonged and multiple seizures, one of the most dramatic characteristics of childhood cerebral malaria, are associated with an increased risk of neurological sequelae and death. Further work needs to be done to clarify the nature of the cognitive problems (Holding 1999) that may also result from uncontrolled seizure activity, and which could have a major impact on the educational potential of children in sub-Saharan Africa. There is an urgent need to find a safe and effective anticonvulsant drug for seizure prophylaxis and to assess, by means of a randomised, controlled intervention study, whether anticonvulsant prophylaxis reduces the incidence of neurological sequelae and death. Fortunately, there is now considerable political and economic interest in attempts to “Roll Back Malaria” (Nabarro 2000), and a renewed urgency to reduce the unacceptable burden of mortality from malaria in Africa. The provision of safe and effective drugs for the prevention and treatment of seizures in childhood cerebral malaria must be a priority for all future case management strategies.


Declaration

I was personally responsible for the design of all the studies presented in this thesis. This included the production of written protocols for subsequent submission to the local and national scientific and ethical co-ordinating committees.

During the clinical and electrophysiological study (chapter 3), I performed all of the electroencephalographic (EEG) recordings. For all ten children who underwent intracranial pressure monitoring, I inserted the subarachnoid catheter and undertook the majority of their clinical care. I filmed all of the video footage in the enclosed CD-ROM.

For the phenobarbitone prophylaxis intervention study, which required the prospective recruitment of a large number of subjects, the majority of patients were recruited and managed by my clinical colleagues, with whom I shared clinical duties.

I was responsible for the compilation and cleaning of all data prior to computer entry, and for subsequent statistical analysis. I wrote all of the papers listed in this Appendix.

Supervisors/Collaborators

Supervisors

Professor Kevin Marsh
Scientific Director, KEMRI Clinical Research Unit, Kilifi, Kenya

Dr Fenella Kirkham
Neurosciences Unit, Institute of Child Health, London, UK

(London Advisor)

Collaborators

Dr Shelagh Smith
Institute of Neurology, London, UK

Mr Peter Muthinji
EEG Department, Kenyatta National Hospital, Nairobi, Kenya

Dr Robert Surtees
Neurosciences Unit, Institute of Child Health, London, UK
Ethical approval and consent

Approval for all studies was obtained from the Kilifi Scientific Coordinating Committee, the KEMRI Scientific Coordinating Committee, and the Kenyan National Ethical Review Committee (see covering letter in this Appendix). Ethical approval for intracranial pressure monitoring was also obtained from the Ethics Committee of the Hospital for Sick Children, Great Ormond Street, London.

Informed, written consent was obtained from the parent or guardian of each child enrolled into a study. An explanation, written in the caretaker’s first language (KiSwahili or KiGiriama), was read aloud by a trained fieldworker who was fluent in the appropriate local language, and caretakers were encouraged to ask questions. It was stressed that failure to grant permission would not in any way compromise the clinical care given to the child, and that consent could be withdrawn at any time without penalty. Prior to the start of all studies, seminars were held for fieldworkers and nurses in order to ensure that they fully understood and approved of the studies, and were competent at answering any questions that might arise.

All children with severe malaria were treated in the same way on the research ward, irrespective of whether they had been recruited into a study, and all medical care was provided free of charge. The presence of the research unit meant that children admitted to Kilifi District Hospital received a higher standard of clinical care than is usually possible in most district hospitals in Africa. The unit had an adequate number of appropriately trained staff, and could provide a regular supply of drugs and equipment. Consequently, many parents would travel a considerable distance from neighbouring districts in order to bring their child to Kilifi District Hospital.
TO WHOM IT MAY CONCERN

Dr Jane Crawley – Research for MD Thesis

I confirm that the research studies described in Dr Crawley’s thesis received full approval from the Kilifi Unit Scientific Coordinating Committee, the KEMRI Scientific Coordinating Committee, and the Kenyan National Ethical Review Committee.

Yours sincerely

[Signature]

Professor Kevin Marsh MBchB FRCP
Scientific Team Leader
KEMRI Centre for Geographic Medicine Research (Coast), Kilifi

20th April 2001
List of publications arising from this research


### SEIZURE SHEET

**Name:**

**Weight:** _____ kg

**Drug doses (Clinician to prescribe):**

- **Diazepam 0.3mg/kg i/v:** _____ mg
- **Paraldehyde 0.2ml/kg i/m:** _____ ml
- **Phenytoin 20mg/kg i/v:** _____ mg

**Clinician’s signature**

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<thead>
<tr>
<th>Date</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Time of onset</td>
<td>PRE</td>
<td>STUDY</td>
<td>DRUG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type¹</th>
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</table>

<table>
<thead>
<tr>
<th>Duration²</th>
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</thead>
</table>

¹**Type:**
- P: Partial seizure
- G: Generalised seizure
- PG: Partial seizure with secondary generalisation

²**Duration:**
- T1: <5 minutes
- T2: 5-14 minutes
- T3: 15-29 minutes
- T4: 30 minutes plus
KILIFI UNIT

FRONT SUMMARY SHEET

To be filled in by clinician

1. KEMRI number .......................................................................................... [___ | ___ | ___ | ___ | ___]
2. First name ...................................................................................................
   Surname ........................................................................................................
3. Date of birth (day/mth/yr) ......................................................................... [___ | ___ | ___ | ___ | ___]
4. Sex (M/F) ..................................................................................................... [___]
5. Date admitted to hospital (day/mth/yr) ....................................................... [___ | ___ | ___ | ___ | ___]
6. Date admitted to KEMRI ward (day/mth/yr) .............................................. [___ | ___ | ___ | ___ | ___]

To be filled in by ward clerk

7. Inpatient surveillance number .................................................................... [___ | ___ | ___ | ___ | ___]
   Hospital number .......................................................................................... [___ | ___ | ___ | ___] / [___]
8. Has the child been admitted to Kilifi District Hospital previously? (Y/N) .... [___]
   When was the last admission? ..................................................................... [___ | ___ | ___ | ___ | ___]
   If records for last admission traced,
   Inpatient surveillance number .................................................................... [___ | ___ | ___ | ___ | ___]
   KEMRI number .......................................................................................... [___ | ___ | ___ | ___ | ___]
9. Date discharged from hospital ................................................................... [___ | ___ | ___ | ___ | ___]
   Alive or dead on discharge? (A/D) ............................................................. [___]
10. Lab sample saved? (Y/N) ............................................................................ [___]
11. **How long has this illness episode lasted?** (in days)
   If more than 30 days, write 99 .......................................................... [___ | ___]

**CURRENT PROBLEMS**

**DRUGS IN LAST 7 DAYS**

**PAST MEDICAL HISTORY**
## HISTORY (continued)

(Please record as complete a history as possible, please write legibly)

### FAMILY HISTORY

### OTHER SIGNIFICANT HISTORY

## CNS BOX

(Fill in for all children with malaria and any other children with disturbed consciousness)

<p>| | | | |</p>
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<tbody>
<tr>
<td>12.</td>
<td>How long has the child been comatose? (in hours)</td>
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<tr>
<td>13.</td>
<td>Has the child ever had seizures prior to (but not including) this illness? (Y/N)</td>
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<tr>
<td></td>
<td>Were these associated with fever? (Y/N)</td>
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<tr>
<td>14.</td>
<td>How many seizures did s/he have this illness?</td>
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<td></td>
<td>How many seizures did s/he have in the past 24 hours?</td>
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<tr>
<td></td>
<td>How long ago was the last seizure (round up to the nearest hour)</td>
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<tr>
<td></td>
<td>If the child has had a fit, ask informant to demonstrate the fit.</td>
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<tr>
<td>15.</td>
<td>What type of seizure:</td>
<td></td>
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<tr>
<td></td>
<td>Generalised P.Partial PG.Partial becoming generalised</td>
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<tr>
<td></td>
<td>If partial, which side was involved? R.Right L.Left</td>
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<tr>
<td>16.</td>
<td>Did the seizure last more than 30 minutes? (Y/N)</td>
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<tr>
<td>17.</td>
<td>Before the child was ill, could s/he:</td>
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<tr>
<td></td>
<td>sit unsupported? (Y/N)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>stand unsupported? (Y/N)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>walk unsupported? (Y/N)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>Is speech normal for age? (Y/N)</td>
<td></td>
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</tbody>
</table>
CLINICAL EXAMINATION FORM

1. Date of examination (day/mth/yr) ................................................ [__ | __ | ___ | ___ | ___ | ___]

2. Time of examination (24 hour clock) ................................................ [__ | ___ | __] [__ | ___]
   (NOTE: midnight is 00:00 of the following day)

3. Temperature: Axillary (centigrade) ................................................ [__ | __ | ___ | ___]
   Rectal (centigrade) ........................................................................ [__ | ___ | ___ | ___]

4. Naked weight (kg) ........................................................................ [__ | ___ | ___ | ___]

5. General condition
   Jaundice? (Y/N) ................................................................................ [__]
   Pallor? (Y/N) .................................................................................... [__]

   Record other general comments below.

CARDIOVASCULAR

6. Pulse: rate / min ................................................................................ [__ | ___ | ___]

7. Blood Pressure
   Systolic ............................................................................................. [__ | ___ | ___]
   Diastolic ............................................................................................. [__ | ___ | ___]
   Cuff size (cm) ....................................................................................

   If ABNORMAL, circle whether
   Gallop Murmur Other (specify) ..........................................................

   Record other cardiovascular observations below.
RESPIRATORY

9. Cough: (Y/N) ................................................................................................................................ [ ]
   Nasal flaring? (Y/N) ................................................................................................................ [ ]
   Respiratory rate: (bpm) ........................................................................................................ [ ]

   Deep breathing? (Y/N) ........................................................................................................ [ ]
   Indrawing (inward movement of bony chest wall)? (Y/N) ....................................................... [ ]
   If indrawing present, is it severe? (Y/N) ........................................................................ [ ]

10. Oxygen saturation % (off oxygen for 5 minutes) .............................................................. [ ]

    If ABNORMAL (including crackles, wheeze, asymmetry), describe in box below.

   

GIT

12. Liver: cm below costal margin in MCL................................................................................ [ ]
    Spleen: cm below costal margin in MCL............................................................................... [ ]

    Record other GIT observations below.

   

NERVOUS SYSTEM EXAMINATION

    Neck stiffness? (Y/N) ........................................................................................................ [ ]

14. Can the child sit unsupported? (Y/N/D)........................................................................... [ ]
    If less than 1 year old, is the child able to breastfeed? (Y/N/D)................................. [ ]
15. Any relevant sedative medication given in last 6 hours? (Y/N).......................... [ ]
   If YES, how many hours ago was it given? ......................................................... [ ] [ ]
   State type, route and dose in box below.

   If ABNORMAL, describe in box below.

17. Position:
   Decerebrate (Y/N) .......................................................................................... [ ]
   Decorticate (Y/N) .......................................................................................... [ ]
   Opisthotonic (Y/N) .......................................................................................... [ ]

COMA SCALES

18. Motor response to painful stimulus (sternal pressure)
   4.Localises pain 3.Flexion 2.Extension 1.None .................................................. [ ]

19. Blantyre scale
   Verbal response: 2.Appropriate cry 1.Moan/inappr. 0.None ................................. [ ]
   Motor response: 2.Localises pain 1.Withdraws 0.Nil/non-spec ............................ [ ]
   Eyes: 1.Directed 0.Not directed ........................................................................ [ ]

20. Is RIGHT fundus adequately seen? (Y/N) ............................................................ [ ]
    If YES, Haemorrhage (Y/N) ................................................................. R: [ ]
    Papilloedema (Y/N) ................................................................. R: [ ]

    Is LEFT fundus adequately seen? (Y/N) .......................................................... [ ]
    If YES, Haemorrhage (Y/N) ................................................................. L: [ ]
    Papilloedema (Y/N) ................................................................. L: [ ]
<table>
<thead>
<tr>
<th>CNS BOX</th>
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<tbody>
<tr>
<td>(Complete for children with suspected CNS disease)</td>
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<tr>
<td>21</td>
<td><strong>Head circumference</strong> (cm) ................................................ [<em><strong>] [</strong></em>] [___]</td>
</tr>
<tr>
<td>22</td>
<td><strong>Pupil reaction</strong> (using strong torch)</td>
</tr>
<tr>
<td>23</td>
<td><strong>Pupil size</strong></td>
</tr>
<tr>
<td>24</td>
<td><strong>Spontaneous eye movements</strong></td>
</tr>
<tr>
<td>25</td>
<td><strong>Corneal reflex</strong></td>
</tr>
<tr>
<td>26</td>
<td><strong>Horizontal oculocephalic</strong> (rotate head side to side)</td>
</tr>
<tr>
<td></td>
<td>1. Normal or full deviation 2. Minimal or none ...................... [___]</td>
</tr>
<tr>
<td>27</td>
<td><strong>Limb tone</strong></td>
</tr>
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<td><strong>Plantars</strong></td>
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Record other CNS observations in box below.
EYES AND ENT

Record observations in box below.

MISCELLANEOUS FINDINGS

30. Other clinical abnormalities? (Y/N) ................................................................. [__]
    If YES, please describe in box below.

[Blank space for description]
WORKING DIAGNOSIS

_____________________________________________________________________

Problems:
_____________________________________________________________________
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MANAGEMENT PLAN
(Please write as clear and complete a plan as possible and include your initials).

Investigations
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Medications:
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Fluids:
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Observations to be made:
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Other comments:
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Chest Xray ordered (Y/N) [__]

Clinician's initials [____|____|____]
DISCHARGE FORM
To be completed on discharge from KEMRI unit

1. KEMRI number.........................................................................................................................
2. Date...........................................................................................................................................
3. Naked weight (kg).......................................................................................................................
4. Height (cm).................................................................................................................................
5. Is the child fully conscious? (Y/N)............................................................................................
6. Is the child's speech back to normal? (Y/N/D)........................................................................
7. Can the child see a small object? (Y/N)....................................................................................
9. Does the child have any abnormal movements? (Y/N).............................................................
   If YES, specify in box

10. Can the child:
    sit unsupported? (Y/N/D)............................................................................................................
    stand unsupported? (Y/N/D)........................................................................................................
    walk unsupported? (Y/N/D)........................................................................................................
11. Any abnormalities of gait? (Y/N).............................................................................................
    If YES, specify in box

12. Does the child have hemiparesis (weakness of one side of the body)? (Y/N).................
13. Further neurological review needed prior to discharge from Ward I? (Y/N)....................
    If YES, arrange for discharge through KEMRI unit. Inform mother and record on transfer notes.
14. On final discharge from hospital, are there any significant neurological sequelae? (Y/N)...
    If YES, specify in box

Appointment date for followup _____/_____/_____.

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Electroencephalographic and clinical features of cerebral malaria

J Crawley, S Smith, P Muthinji, K Marsh, F Kirkham

Abstract
Background—Seizures are a prominent feature of childhood cerebral malaria, and are associated with an increased risk of death and neurological sequelae. We present the electroencephalographic (EEG) findings from a detailed clinical and electrophysiological study.

Methods—Children with cerebral malaria had EEGs recorded within six hours of admission, and at 12 hourly intervals until recovery of consciousness. Ten deeply comatose children underwent intracranial pressure monitoring. Children were not mechanically ventilated, which made it possible to directly correlate the clinical and EEG findings.

Results—Of 65 children aged 9 months and above, 40 had one or more seizures, and 18 had an episode of status epilepticus. Most seizures were partial motor, and spike wave activity consistently arose from the posterior temporo-parietal region, a border zone area lying between territories supplied by the carotid and vertebrobasilar circulations. Fifteen children had seizures that were clinically subtle or electrographic. Clinical seizures were associated with an abrupt rise in intracranial pressure. Fifty children recovered fully, seven died, and eight had persistent neurological sequelae. Initial EEG recordings of very slow frequency, or with background asymmetry, burst suppression, or interictal discharges, were associated with an adverse outcome.

Conclusions—Serial EEG recording has uncovered a range of clinical, subtle, and electrographic seizures complicating childhood cerebral malaria, and has emphasised their importance in the pathogenesis of coma. Further work is required to determine the most appropriate regimen for the prophylaxis and treatment of seizures in cerebral malaria, in order to improve outcome.

Keywords: cerebral malaria; coma; seizures; electroencephalogram

Cerebral malaria, a diffuse encephalopathy caused by Plasmodium falciparum, remains a major cause of death and disability in sub-Saharan Africa. The pathophysiology of the disease is complex and poorly understood. Prolonged, multiple seizures are a prominent clinical feature, and are associated with an increased risk of death and neurological sequelae. Despite their importance, and in common with many other causes of childhood coma, there have been few descriptions of the clinical and electroencephalographic features of seizures in cerebral malaria. Electroencephalography (EEG) can supply the clinician with information on the origin and distribution of seizure activity, detect subtle or subclinical seizures, and may reveal abnormal electrical activity of prognostic significance. It is therefore of potential value in the clinical management of cerebral malaria, and may improve understanding of the underlying pathophysiology. Here we present the EEG findings from a detailed clinical and electrophysiological study of 65 children with cerebral malaria.

Methods

STUDY SITE

The study was conducted at the Kenya Medical Research Institute (KEMRI) unit, located at Kilifi District Hospital, on the coast of Kenya. The hospital serves a predominantly rural population, and approximately 5000 children are admitted to the 35 bed paediatric ward annually. Malaria transmission (of which over 95% is Plasmodium falciparum) occurs throughout the year, with peak transmission following the rainy seasons of April–May and October–November.

PATIENTS

Children aged 9 months and above were eligible for enrolment in the study if they fulfilled the World Health Organisation definition of cerebral malaria, namely unrousable coma not attributable to any other cause in the presence of asexual Plasmodium falciparum parasitaemia. To fulfil the definition of cerebral malaria, coma had to persist for at least one hour after a seizure and/or after the administration of diazepam. Consent was obtained once all procedures had been fully explained to the child’s parent or guardian in their first language.

INVESTIGATIONS

On admission, baseline blood was taken for parasite count, full blood count, electrolytes, glucose, and venous gas. Blood glucose was subsequently monitored every four hours, and parasite count eight hourly. Lumbar puncture was performed once the level of consciousness was starting to improve. In deeply comatose children, intracranial pressure was monitored using a subarachnoid catheter (Camino 110-4B).
CLINICAL MANAGEMENT

Children received standard treatment for cerebral malaria, as detailed previously. Oxygen was available if required, but there were no facilities for mechanical ventilation, a situation common to most hospitals throughout Africa. All clinical seizures were timed and recorded by medical or nursing staff, and classified as generalised tonic-clonic, partial motor, or partial with secondary generalisation. Seizures lasting for more than five minutes were treated with a maximum of three doses of diazepam 0.3 mg/kg, given as a slow intravenous injection over two minutes. Children with recurrent (more than three) seizures or status epilepticus (continuous clinical seizure activity lasting for 30 minutes or more) were treated with a loading dose of intravenous phenytoin 18 mg/kg and, if that failed, with intramuscular phenobarbitone 18 mg/kg. One child with intractable generalised status epilepticus required treatment with intravenous thiopentone 4 mg/kg.

FOLLOW UP

All survivors were seen one month after discharge for neurological examination and repeat EEG. Cerebral computerised tomography (CT) was performed on children with neurological sequelae.

ELECTROPHYSIOLOGY

EEG recordings were made on a 14 channel Medelec 1A94 EEG machine. Silver/silver chloride electrodes were fixed with Elefix and tape to the child's shaved head, and the International 10-20 system used for electrode placement. Recordings were taken within six hours of admission, and at 12 hourly intervals until recovery of consciousness. Continuous recordings using a cerebral function analysing monitor (CFAM, Medaid Ltd, UK) were obtained from selected children who had been unconscious for more than 24 hours or who had prolonged or subtle seizure activity. All EEGs were subsequently analysed by SS, who knew the age of each child and what drugs they had been given, but was blind to any other clinical information.

STATISTICS

Analyses were carried out using stata version 5.0. Student's t test and analysis of variance, with the Scheffe or Bonferroni correction for multiple comparisons, were used for normally distributed quantitative data. Data were log transformed if the distribution was skewed. The Mann-Whitney test was used for data failing to conform to a normal distribution, and Fisher's exact test for categorical data. Multivariate logistic regression analysis was used to examine the relation between specific admission characteristics and subsequent outcome.

Results

ADMISSION CHARACTERISTICS

Sixty five children (age range 9 months to 11 years, median 30 months), were recruited to the study. All children had previous normal development. Prior to hospital admission, 68% (44/65) had seizures which, in 27 cases, were reported to have lasted for at least 30 minutes. In more than half the cases, seizure activity heralded the onset of coma. Children had been comatose for between one and 72 hours (median seven hours) prior to admission and, at the time of admission, 75% were deeply unconscious with a Blantyre coma score of 2 or less. Table 1 details clinical and laboratory features of the children.

CLINICAL COURSE

Fourteen children (22%) recovered consciousness within six hours of multiple or prolonged seizures that had occurred on or immediately prior to hospital admission. Following admission, 62% (40/65) had at least one seizure, while 19 children had more than five seizures. Eighteen children (28%) had an episode of clinical status epilepticus (continuous clinical seizure activity lasting for 30 minutes or more), while three further children had continuous electrical discharges lasting for 30 minutes or more with no apparent clinical correlates (electrographic status epilepticus). Fifty two per cent of the seizures were partial motor, 14% partial with secondary generalisation, and 34% generalised tonic-clonic. Children with generalised seizures were significantly older than those with partial motor seizures (mean age

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ELECTROPHYSIOLOGY

Background activity

Admission EEGs were dominated by high amplitude (>100 µV) slow wave activity with frequencies of 0.5–7 Hz. Admission recordings of very slow frequency (0.5–3 Hz) were obtained from 17/65 (26%) children, and were associated with an increased risk of death (odds ratio 25.6 (95% CI 2.8 to 235); p = 0.004; table 3). This association remained significant despite adjustment for the possible confounding effects of age, glucose, and base excess. Recordings of very slow frequency were not significantly associated with duration of coma, administration of diazepam in the 12 hours prior to admission EEG, age, parasitaemia, glucose, or base excess (p > 0.1 for all comparisons). Ten children had admission recordings that were asymmetric, with increased slow wave activity over one or both hemispheres. Nine of these children had a history of multiple partial motor seizures or status epilepticus in the six hour period prior to admission, and three developed neurological sequelae.

Ictal activity

A striking feature of the EEGs in this series was the consistent localisation of ictal discharges over the posterior temporo-parietal regions. Over 75% of ictal or interictal discharges occurred in this region, sometimes spreading to involve both temporo-parietal regions, or one or both cerebral hemispheres. In eight cases, electrical seizure activity persisted for between two and 140 minutes (median 45 minutes) after successful treatment of a clinical seizure with diazepam. Fifteen children had continuous electrical seizure discharges on EEG, but clinical features that were either extremely subtle or not discernible (electrographic). These children typically presented in coma, with a history of a prolonged partial seizure that had either terminated spontaneously, or as a result of treatment with diazepam. Tonic eye deviation, nystagmus, salivation, and hypoventilation were the most distinctive clinical features. The nystagmus was of large amplitude, with a slow phase of movement that crossed the midline. Respiration was shallow and irregular, and the children were hypoxaemic (arterial oxygen saturation below 80%) and hypercarbic (pCO₂ above 6.5 kPa). EEG showed continuous...
spike wave discharges over the posterior temporo-parietal region contralateral to the direction of eye deviation.

The onset of generalised electrical and clinical seizure activity usually followed an increased frequency of bilateral, predominantly posterior, interictal spike wave discharges. Runs of up to 40 short, generalised seizures, each lasting for 0.5 to 1 minute, were observed in four children, and were followed by notable postictal flattening. Clinical features became increasingly subtle during prolonged generalised seizures. One child was admitted following a generalised seizure at home that had lasted for six hours. Tonic-clonic movements had ceased one hour before admission, and the child was deeply comatose, with irregular respiration, salivation, and priapism. Despite the paucity of clinical features, EEG revealed continuous, generalised seizure activity.

**Interictal epileptic activity**

Interictal spikes and sharp slow waves were observed on admission EEGs from three children, all with a history of prolonged (seizure activity lasting for more than one hour) partial motor status epilepticus. The discharges were bilateral and multifocal, and located predominantly in the temporo-parietal regions (table 2). All of these children developed neurological sequelae.

**Episodic low amplitude events**

Periods of relative attenuation of background activity lasting three to five seconds were seen on admission EEGs from five children. In two cases, these followed administration of intravenous diazepam 0.3 mg/kg for the treatment of seizures. Four of these children recovered normally, while one child died seven hours after admission.

**Burst suppression**

A burst suppression pattern, consisting of bursts of polymorphous complexes occurring synchronously over both hemispheres, and alternating with periods of relative quiescence, was observed on the initial EEGs from two children. One child had been hypotensive and hypoglycaemic on admission, and the other had a history of multiple, prolonged generalised seizures. Both children died.

**Intracranial pressure monitoring and EEG**

Ten children had simultaneous EEG and intracranial pressure (ICP) monitoring. Four children had clinical seizures during ICP monitoring. Intracranial pressure rose by a median of 164% (range 108–285%) during generalised seizures, and by 50% (range 0–186%) during partial seizures. Concomitant rise in mean arterial pressure meant that cerebral perfusion pressure was maintained above 50 mm Hg during clinical seizures. There was no significant rise in ICP during two 90 minute periods of electrographic seizure activity confined to the posterior temporo-parietal regions. At intracranial pressures of below 20 mm Hg, fluctuations in ICP appeared to have no effect on the background frequency
of the EEG. EEG recording from one child with severe intracranial hypertension, however, showed widespread slow wave activity (frequency of 0.5–1 Hz) in association with episodes of opisthotonic posturing, during which ICP rose to peaks of 30 to 40 mm Hg (fig 1A). Within 10 hours, EEG showed attenuation of activity in the right temporo-parietal region (fig 1B), and the right pupil became dilated with sluggish reaction to light. Over the next five hours, despite regular doses of mannitol 0.5 g/kg, the ICP rose to above 80 mm Hg, the cerebral perfusion pressure fell below 30 mm Hg, and the entire EEG became flat and featureless. ICP rose to a peak of 125 mm Hg, and the child died following a respiratory arrest.

Another child aged 3 years was admitted with a 12 hour history of opisthotonic posturing. Initial EEG was reactive, with a background frequency of 2–6 Hz. ICP monitoring was commenced at seven hours, and the child also started continuous CFAM recording. Initial intracranial pressures ranged from 25 to 30 mm Hg, with peaks of 40 mm Hg during episodes of opisthotonus. Over the next four hours, despite inotropic support, mean arterial pressure remained below 75 mm Hg, and rising intracranial pressure consequently caused a progressive decline in cerebral perfusion pressure. Subsequent infusion of mannitol was accompanied by a notable deterioration in clinical condition, and CFAM recording showed a sharp drop in background frequency.

The child died shortly afterwards following a cardiorespiratory arrest.

OUTCOME
Serial EEGs showed a gradual increase in background frequency over the period from admission to recovery of consciousness. The 50 children who made a full recovery had normal EEGs one month after discharge. Children with neurological sequelae were significantly younger (p = 0.02) and had a significantly higher creatinine (p = 0.009) compared to those who recovered fully. Admission base excess was significantly greater (p = 0.04) in children who died compared to those who recovered fully, while four of the seven children who died had sustained opisthotonic posturing during their clinical course in hospital. There were otherwise no significant differences between the three outcome groups in the admission variables, but numbers are small. Follow up EEGs from children with hemiplegia showed decreased activity in the contralateral parieto–temporal region, and cerebral CT scans showed infarction in the same region. Follow up EEGs from two children with spastic quadriplegia and one child with severe cognitive and speech problems were of low amplitude, with a paucity of normal cerebral rhythms, while CT scans from these children showed global cerebral atrophy (table 2).

Discussion
Traditionally, it has been assumed that sequestration of parasitised red blood cells occurs within the cerebral microvasculature of all comatose patients with falciparum malaria. Clinical and experimental work has shown, however, that the pathophysiology of cerebral malaria is highly complex, and that a number of different pathological mechanisms may cause coma. Reduced cerebral perfusion (secondary to raised intracranial pressure and systemic hypotension), severe anaemia, hypoglycaemia, and the local release of cytokines and nitric oxide may all be contributory factors.

Our study suggests that an additional pathophysiological factor, seizures, may play an important role in the pathogenesis of coma. Over one third of the children in this study had more than five seizures or an episode of status epilepticus during their clinical course in hospital. Twenty two per cent recovered consciousness within six hours of prolonged or multiple seizures, suggesting that their coma may have been a postictal phenomenon, directly related to seizures. EEG was essential for identifying the 15 children in whom seizures were either clinically subtle (tonic eye deviation, nystagmus, hypoventilation) or electrographic. Although tonic deviation of the head and eyes commonly occurs during epileptic seizures, nystagmoid eye movements are an unusual ictal manifestation. The clinical characteristics of the nystagmus in these patients were consistent with epileptic nystagmus type two (EN-2), which typically has a seizure focus in the temporo–parietal–occipital cortex, contralateral to the direction of the nystagmus beats. This area overlaps the
putative cortical region of visual motor sensi-
tivity (V5), which is important for the genera-
tion of ipsiversive smooth pursuit. Subtle sei-
zures have not been described in previous
studies of childhood cerebral malaria but are of
great clinical importance, as several of these
children recovered within a few
hours of anticonvulsant treatment. In a busy,
understaffed hospital in the developing world
such seizures are easily missed, yet their clinical
features are sufficiently distinct for them to be
recognised by medical or nursing staff with
appropriate training, without the need for EEG.

The pathogenesis of seizures in childhood
cerebral malaria is likely to be complex and
multifactorial, but our study suggests that cer-
bral hypoxia may be a precipitating factor.
EEG recordings showed that electrical seizure
activity consistently arose from the posterior
temporoparietal region, which is a "water-
shed" area between arteries perfused by the
middle cerebral (carotid circulation) and
posterior cerebral (vertebrobasilar circulation)
arteries. Consequently, it is particularly vulner-
able to hypoxia when oxygen delivery to the
brain is compromised as a result of sequestra-
tion, severe anaemia, or inadequate cerebral
perfusion caused by hypotension or raised
intracranial pressure. Because of their
anatomical position close to the temporal lobes
in the tentorial notch, the posterior cerebral
arteries may be particularly vulnerable to com-
promise in those patients who have acute
intracranial hypertension and incipient tran-
stentorial herniation, as illustrated by the EEGs
in fig 1. Raised intracranial pressure, reduced
cerebral perfusion pressure, and transstentorial
herniation have been documented in cerebral
malaria, and in other paediatric encephalopa-
thies. By initiating the release of excitoto-
toxic mediators such as glutamate or quinolinic
acid, local hypoxia may precipitate seizures,
which, by raising intracranial pressure and
increasing the demand for oxygen and glucose,
may exacerbate the situation further. Although
there is no evidence for failure of cerebral
autoregulation in the majority of these patients,
relatively poor collateral circulation in some
patients may mean that cerebral blood flow in
the border zone between the carotid and
vertebrobasilar territories is insufficient to
compensate for increased local demand. In
these patients, a vicious cycle might then be
generated, leading to intractable partial seizures
and eventually to focal infarction (table 2).

Diffuse background slow wave activity on EEG
occurs in many conditions (metabolic,
toxic, and infectious) that have a generalised
effect on the brain. Background slow wave activity
in cerebral malaria has been described in adults, and in one previous study on chil-
dren. The mechanism for the increased slow
wave activity seen during episodes of intracra-
nial hypertension and opisthotonus is un-
known, but may have been caused by concur-
rent hyperventilation, leading to hypocapnia and
cerebral vasocostriction. Intracranial
pressure rose acutely during seizures, and was
accompanied by a concurrent rise in mean
arterial pressure, suggesting that cerebral
autoregulation was intact. In one case, how-
ever, mean arterial pressure was insufficient to
maintain adequate cerebral perfusion, despite
treatment with pressor agents. Infusion of
mannitol was followed by clinical and electrophysiological deterioration, suggesting that the
integrity of the bloodbrain barrier had been
breached.

EEGs have been used to predict prognosis in
a number of childhood encephalopathies. In
this study, burst suppression or background
activity of very slow frequency was associated
with an increased risk of death. Of five children
with episodic low amplitude events on initial
EEG, four made a full recovery. In two of these
cases, episodic low amplitude events were
associated with the administration of diazepam,
which may partly explain why their
prognosis appears better here than in other
types of encephalopathy. Children with a
history of status epilepticus prior to admission,
and an initial EEG showing asymmetric back-
ground activity or continuous interictal multi-
focal spike waves, had an increased incidence
of neurological sequelae. Overall, almost 70%
of the children with an adverse outcome (death
or neurological sequelae) had one or more poor
prognostic features on initial EEG. Although
this suggests that in some cases significant cer-
bral pathology must have occurred prior to
hospital admission, anticonvulsant prophylaxis
given at the time of admission may prevent fur-
ther cerebral damage. A recent randomised,
controlled study of phenobarbitone prophyl-
axis in childhood cerebral malaria showed
that, at a dose of 20 mg/kg, phenobarbitone
halved seizure frequency but was associated
with an unacceptable doubling of mortality.
Further studies need to be done to find a pro-
phyactic anticonvulsant drug that is both safe
and effective in children with cerebral malaria.

Electrophysiological monitoring using serial
EEG and CFAM has made it possible to iden-
tify a wide variety of seizures (clinical, subtle,
and electrographic) in children with cerebral
malaria, and has emphasised their importance
in the pathogenesis of coma. We have been able
to correlate the electroencephalographic and
clinical findings from this group of uncon-
scious children, something that is not possible
in most parts of the developed world where the
majority of unconscious children are venti-
lated. The information obtained from this
study may, therefore, provide some valuable
insights into a wide variety of other paediatric
encephalopathies.

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Chloroquine is not a risk factor for seizures in childhood cerebral malaria

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Summary

OBJECTIVES There are a number of case reports in the medical literature suggesting an association between the ingestion of chloroquine and subsequent seizure activity. Our study was designed to investigate the relationship between blood levels of chloroquine (CQ), its metabolite desethylchloroquine (DCQ), and seizures in children admitted to hospital with cerebral malaria.

METHODS Serial blood levels of CQ and DCQ were measured over the first 24 h of hospital admission in children with cerebral malaria. The number and duration of all seizures was recorded, and statistical analysis subsequently performed to determine the relationship between seizure activity and blood concentrations of CQ and DCQ.

RESULTS Chloroquine was detected in 92% (100/109) of admission blood samples. 54% (59/109) of the patients had one or more seizures after admission, while 8% (9/109) had an episode of status epilepticus. Median (interquartile range) baseline concentrations of CQ and DCQ were, respectively, 169.4 µg/ml (75.1–374.9) and 352.3 µg/ml (81.9–580.1) for those children who had seizures after admission, compared to CQ 227.5 µg/ml (79.4–430.2) and DCQ 364.0 µg/ml (131.3–709.4) for those who did not have seizures (P > 0.5 for all comparisons). Baseline concentrations of CQ and DCQ were not significantly associated with the occurrence of seizures lasting for 5 min or more. The nine children who had an episode of status epilepticus had significantly lower median admission levels of CQ than those without status epilepticus: 75.1 µg/l (7.4–116.5) vs. 227.5 µg/l (85.6–441.2), P = 0.02. Multivariate logistic regression analysis, taking into account factors likely to affect the risk of seizures in hospital, failed to change the significance of these results.

CONCLUSIONS These findings suggest that chloroquine does not play an important role in the aetiology of seizures in childhood cerebral malaria.

Keywords malaria, chloroquine, seizures

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Introduction

Despite the relentless spread of drug-resistant Plasmodium falciparum, chloroquine continues to be widely used throughout Africa for the treatment and prevention of malaria. The drug is also used in many parts of the world as second-line treatment for hepatic amoebiasis and connective tissue disease. A number of case reports in the medical literature have described an association between seizures and the ingestion of chloroquine, both at therapeutic concentrations (Torrey 1968; Fish & Espir 1988; Luijckx 1992) and following overdose (Cann & Verhulst 1961; Kiel 1964; Jaffe 1988; Riou et al. 1988).

Seizures complicate the clinical course of more than 3% of children admitted to hospital with falciparum malaria (Waruiru et al. 1996). They occur in over 60% of cerebral malaria cases (Crawley et al. 1996) and are associated with an increased risk of death (Jaffar et al. 1997) and neurological sequelae (Molyneux et al. 1989; Brewster et al. 1990). A large proportion of patients admitted to hospital in Kenya with malaria have taken chloroquine, obtained from shops or local dispensaries, prior to attending hospital (Snow et al. 1992; Crawley et al. 1996). The purpose of this study therefore was to investigate the possibility that seizures in childhood cerebral malaria may be caused or exacerbated by chloroquine or its active metabolite desethylchloroquine.
Methods

Study site

The study was conducted at the Kenya Medical Research Institute (KEMRI) unit at Kilifi, on the coast of Kenya.

Consent

The study formed part of a randomised, double-blind, placebo-controlled trial of phenobarbitone prophylaxis in childhood cerebral malaria (Crawley et al. 2000). Approval had been granted by the Kenyan National Ethical Committee. Informed, written consent was obtained from first degree relatives in all cases.

Admission procedure

Children aged 9 months to 13 years were recruited if they had Plasmodium falciparum parasitaemia and were unconscious, as judged by their inability to localize a painful stimulus (Molyneux et al. 1989). Children who had pre-existing epileptic epilepsy or significant neuro-developmental problems, and those who had received treatment with phenobarbitone or phenytoin during the current illness were excluded from the study, as were those found on subsequent lumbar puncture to have meningitis. As soon as possible after admission, children were randomised to receive a single intramuscular dose of phenobarbitone 20 mg/kg or identical placebo. Blood was taken at baseline, 2, 4, 8, 12 and 24 h for measurement of phenobarbitone, chloroquine (CQ), and desethylchloroquine (DCQ). Analysis of the relationship between blood CQ and DCQ concentrations and subsequent seizure activity was confined to the placebo group.

Treatment

Children received standard treatment for cerebral malaria (Crawley 1999). The number and duration of all seizures were recorded using timers pre-set to alarm at 5, 15, and 30 min. Seizures lasting for 5 min or more were treated in a standardized manner, with intravenous diazepam 0.3 mg/kg and, as second-line therapy, intramuscular paraldehyde 62 ml/kg.

Chloroquine assay

Chloroquine (CQ) and desethylchloroquine (DCQ) concentrations were measured in whole blood using a modification of the method of Patchen et al. (1983). Blood samples (100 μl) were extracted with a combination of methyl-tert-butyl-ether and hexane (1:1), centrifuged (1000 × g; 10 min), and the organic layer separated and evaporated in a water bath (37 °C) under a gentle stream of nitrogen. The residue was reconstituted in mobile phase (100 μl) and analysed by high-performance liquid chromatography, using ultraviolet detection at 340 nm. The lower limit of detection for both chloroquine and desethylchloroquine was 5 μg/l.

Statistical analysis

For analysis we used the statistical programme Stata version 5.0 (Stata Corporation, College Station, Texas). Comparisons of normally distributed quantitative data were made by Student's t-test, using logarithmic transformation for data that had a skewed distribution. Data failing to conform to a normal distribution were compared with the non-parametric Kruskal–Wallis test and Spearman's rank correlation coefficient. Categorical data was compared with Fisher's exact test. Multivariate logistic regression analysis was used to examine the effect of potential confounding variables measured at admission on the association between chloroquine levels and the occurrence of seizures.

Results

Samples

Of 340 patients recruited to the main phenobarbitone prophylaxis study, 170 received placebo. Sufficient blood was available for measurement of CQ and DCQ on 109 (64%) of these patients. There was no significant difference in either the frequency or duration of seizures between patients who had levels measured and those who did not (P > 0.1 for all comparisons; Fisher's exact test). Chloroquine was detected in 100/109 (92%) of the baseline blood samples. Median (interquartile range) baseline concentrations were 214.9 μg/l (79.4-577.3) for CQ and 349.3 μg/l (90.3-594.7) for DCQ. Of 80 children who had three or more CQ levels taken during their hospital course, 29 (36%) were thought to have received CQ in the period immediately (< 24 h) before hospital admission, since the concentration of CQ in their blood fell by more than 50% during the first 24 h of admission (White et al. 1988). These children had median (IQR) baseline concentrations of CQ and DCQ that were significantly higher than those given chloroquine more than 24 h prior to admission (CQ 374.9 μg/l (194.9-888.3) vs. 114.8 μg/l (41.0-185.8), P = 0.0001; DCQ 580.1 μg/l (312.3-1156.2) vs. 284.1 μg/l (52.6-518.9), P = 0.001; Kruskal–Wallis).

Seizures

54% (59/109) of the children had one or more witnessed seizures after admission. Table 1 compares the baseline characteristics of those who had seizures after admission with...
Proportions were compared by Fisher's exact test, means by Student's t-test, and medians by the Kruskal–Wallis test.

those who did not. Children who had seizures after admission had lower median baseline levels of CQ and DCQ than those who did not have seizures, although the difference failed to reach statistical significance (Table 1). Children who had received chloroquine less than 24 h prior to admission had a similar frequency and duration of seizures as those who had received it earlier (P > 0.4; Fisher's exact). Of the 109 children in the study, 32 (29%) had three or more seizures after admission. There was no significant correlation between the total number of seizures after admission and median baseline concentrations of CQ (Spearman's rho = 0.089, P = 0.36) or DCQ (Spearman's rho = 0.039, P = 0.69). Of children with seizures, 76% (45/59) had a seizure lasting for 5 min or more, of whom nine had an episode of status epilepticus (clinical seizure activity lasting for 30 min or more). Median (IQR) baseline levels of CQ and DCQ and the corresponding area under the concentration curves (AUC0–12) were not significantly associated with the occurrence of seizures lasting for 5 min or more (P > 0.5 for CQ, DCQ, and AUC0–12; Kruskal-Wallis). In the nine children who had an episode of status epilepticus, however, median baseline CQ concentrations and AUC0–12 CQ were significantly reduced (Table 2). Multivariate logistic regression analysis, taking into account the factors likely to affect the risk of seizures in hospital (Table 1), failed to change the significance of any of the above results.

### Table 1 Comparison of baseline admission characteristics in children with cerebral malaria (CQ: chloroquine; DCQ: desethylchloroquine)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (interquartile range) unless stated</th>
<th>Seizures after admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n = 50)</td>
<td>Yes (n = 59)</td>
</tr>
<tr>
<td>Age (months)</td>
<td>32.7 (28.0–38.1)</td>
<td>28.7 (25.1–32.7)</td>
</tr>
<tr>
<td>Seizures before admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%)</td>
<td>39/50 (78%)</td>
<td>56/59 (95%)</td>
</tr>
<tr>
<td>Anticonvulsants before admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%)</td>
<td>7/50 (14%)</td>
<td>17/59 (29%)</td>
</tr>
<tr>
<td>Seizures before admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%)</td>
<td>16/49 (33%)</td>
<td>32/59 (54%)</td>
</tr>
<tr>
<td>Anticonvulsants before admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%)</td>
<td>22/50 (44%)</td>
<td>33/59 (56%)</td>
</tr>
<tr>
<td>Baseline CQ (µg/l)</td>
<td>227.5 (79.4–430.2)</td>
<td>169.4 (75.1–374.9)</td>
</tr>
<tr>
<td>Baseline DCQ (µg/l)</td>
<td>364.0 (131.3–709.4)</td>
<td>352.3 (81.9–580.1)</td>
</tr>
<tr>
<td>AUC0–12 CQ (µg.hr/l)</td>
<td>2058.9 (1014.1–5469.4)</td>
<td>2057.6 (802.8–2536.8)</td>
</tr>
<tr>
<td>AUC0–12 DCQ (µg.hr/l)</td>
<td>4534.8 (2420.3–6416.6)</td>
<td>4285.2 (1985.0–5740.6)</td>
</tr>
<tr>
<td>Parasitaemia (µl)</td>
<td>64805 (29987–140049)</td>
<td>42293 (22473–79598)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.1 (3.0–5.3)</td>
<td>4.1 (3.3–5.3)</td>
</tr>
<tr>
<td>Base excess</td>
<td>- 7 (-12 to -4)</td>
<td>- 8 (-13 to -3)</td>
</tr>
</tbody>
</table>

Proportions were compared by Fisher's exact test, means by Student's t-test, and medians by the Kruskal–Wallis test.

### Table 2 Baseline levels of chloroquine (CQ), desethylchloroquine (DCQ), and area under the concentration curve for 0–12 h (AUC0–12) for children with and without status epilepticus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Status epilepticus after admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n = 100)</td>
</tr>
<tr>
<td>Baseline CQ (µg/l)</td>
<td>227.5 (85.6–441.2)</td>
</tr>
<tr>
<td>Baseline DCQ (µg/l)</td>
<td>375.9 (134.8–701.2)</td>
</tr>
<tr>
<td>AUC0–12 CQ (µg.hr/l)</td>
<td>2170.1 (1085.9–4053.6)</td>
</tr>
<tr>
<td>AUC0–12 DCQ (µg.hr/l)</td>
<td>4769.4 (2420.3–6416.6)</td>
</tr>
</tbody>
</table>

Proportions were compared by Fisher's exact test, means by Student's t-test, and medians by the Kruskal–Wallis test.
Discussion

There are many possible explanations for the high incidence of seizures in childhood cerebral malaria. Fever, known to precipitate seizures in young children (Wallace 1988), is an almost universal feature of cerebral malaria, although many of the seizures in cerebral malaria occur at temperatures of at least 38 °C (Crawley et al. 1996). Hypoglycaemia (White et al. 1987), hypotension (English et al. 1996), and cerebral hypoxia, secondary to severe anaemia and inadequate cerebral perfusion (Newton et al. 1997), may also precipitate seizures.

Throughout Africa, chloroquine continues to be widely used in the treatment of febrile illnesses at the community level, illustrated by this and by previous studies (Snow et al. 1996; Crawley et al. 1996). Baseline chloroquine concentrations in this study were generally at the low end of the therapeutic range, and were well below concentrations (2000 μg/l and above) considered toxic (Riou et al. 1988). The association between chloroquine and seizures has been described in both adults and children, at therapeutic concentrations (Torrey 1968; Fish & Espir 1988; Luijckx et al. 1992) and after overdose (Cann & Verhulst 1961; Kiel 1964; Jaffe 1988; Riou et al. 1988). Seizures may occur within a few hours of overdose (Cann & Verhulst 1961; Kiel 1964; Jaffe 1988), or between one day and several weeks after the start of treatment with therapeutic doses of chloroquine (Luijckx et al. 1992).

The concentration of chloroquine within the brain is approximately four times that of plasma (Titus 1989), and experimental evidence suggests that chloroquine may precipitate seizures by attenuation of gamma-aminobutyric acid pathways (Amabeoku 1992), and by the enhancement of dopaminergic neurotransmission (Amabeoku & Chikuni 1993).

In this study, however, there was no association between baseline levels or area under the concentration curve (which reflects both absorption and subsequent elimination) of chloroquine or its active metabolite desethylchloroquine, and number or duration of seizures in children with cerebral malaria. However, baseline levels of chloroquine and desethylchloroquine were reduced in the small subgroup of children who had an episode of status epilepticus. There are a number of limitations to this study. The dose and route of chloroquine administration were not known, and it was only possible to obtain a rough estimate of the time of ingestion in 73% of the patients. Information on seizures that occurred prior to admission was based on clinical history, which can be unreliable, and analysis was therefore confined to seizures that were witnessed by clinical staff at hospital. In addition, it is not known whether the reported association between chloroquine ingestion and seizures is a dose-related or idiosyncratic phenomenon. Although this study suggests that chloroquine is not an important determinant of seizures of childhood cerebral malaria, more definitive evidence would come from prospective longitudinal studies. Such studies could assess the prevalence of seizures complicating cerebral malaria in relation to changing patterns of chloroquine usage. The rapid spread of chloroquine resistance, and the urgent need to change to alternative antimalarial drugs (White 1998), is likely to provide such an opportunity in the near future.

Acknowledgements

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References


J. Crawley et al.  Chloroquine and seizures in cerebral malaria


Act of phenobarbital on seizure frequency and mortality in childhood cerebral malaria: a randomised, controlled prevention study

Crawley, Catherine Waruiru, Sadik Mithwani, Isaiah Mwangi, William Watkins, David Ouma, Peter Winstanley, J Peto, Kevin Marsh

Introduction

Every year, more than one million children in sub-Saharan Africa die or are disabled as a result of cerebral malaria. Seizures complicate a high proportion of cases and are associated with an increased risk of death and neurological sequelae. The most common sequelae are hemiplegia, spastic quadriplegia, visual impairment, and epilepsy, but a much wider range of motor and cognitive impairments occurs. If anticonvulsant prophylaxis can lower the frequency of seizures complicating cerebral malaria, this benefit might in turn reduce the risk of death and neurological sequelae, and have an important impact on the educational potential of children in sub-Saharan Africa.

Phenobarbital has been used as an anticonvulsant for many years, and is highly effective in the treatment of both partial and generalised seizures. It is cheap and, unlike most anticonvulsant drugs used for seizure prophylaxis, widely available throughout Africa. It can be given by intramuscular injection, which is a further advantage, since intravenous therapy is not possible in many health facilities throughout the continent. The loading dose recommended for children is 10–20 mg/kg.

There have been two previous studies of phenobarbital prophylaxis in cerebral malaria. In a small, randomised study on adults in Thailand, three of 24 patients treated with a single dose of phenobarbital (3.5 mg/kg) had seizures after admission to hospital, compared with 13 of 24 given placebo. However, in a pharmacokinetic study on Kenyan children with cerebral malaria, phenobarbital 10 mg/kg did not produce blood concentrations (15 mg/L and above) known to provide effective prophylaxis against febrile seizures.

We undertook a randomised, placebo-controlled study to assess whether a single intramuscular dose of phenobarbital (20 mg/kg) given on admission to Kenyan children with cerebral malaria could lower the frequency of seizures complicating the clinical course in hospital. The safety and clinical tolerance of this dose were assessed at the start of the trial.

Methods

Study participants

The study took place at the Kenya Medical Research Institute (KEMRI) unit, at Kilifi District Hospital, on the coast of Kenya. The hospital serves a predominantly rural population, and about 3000 children are admitted to the 35-bed paediatric ward each year. Malaria transmission (of which over 95% is Plasmodium falciparum) occurs throughout the year, with peak transmission after the rainy seasons of April to May and October to November.

The study was approved by the Kenyan National Ethical Committee. Informed, written consent was obtained from first-degree relatives in all cases.
phenytoin during the current illness, and those found on phenobarbital-treated group. The seizure categories judged to localise a painful stimulus: Blantyre score 3 or less') in the cerebral malaria defined as unrousable coma (inability to light microscopy. Children who had had seizures before single intramuscular injection of phénobarbital 20 mg/kg. We calculated, assuming 90% power and a significance level of lumbar puncture to have meningitis.

Those who had received treatment with phénobarbital or admission were assessed a minimum of 30 min after the end of seizure activity. We excluded children who had pre-existing afebrile epilepsy or significant neurodevelopmental problems, those who had received treatment with phenobarbital or phenytoin during the current illness, and those found on phenobarbital-treated group. The seizure categories judged to localise a painful stimulus: Blantyre score 3 or less') in the cerebral malaria defined as unrousable coma (inability to light microscopy. Children who had had seizures before single intramuscular injection of phénobarbital 20 mg/kg. We calculated, assuming 90% power and a significance level of lumbar puncture to have meningitis.

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Figure 1: Trial profile

Eligible children were aged 9 months to 13 years and had cerebral malaria defined as unrousable coma (inability to localise a painful stimulus: Blantyre score 3 or less') in the presence of Plasmodium falciparum parasitaemia, detected by light microscopy. Children who had had seizures before admission were assessed a minimum of 30 min after the end of seizure activity. We excluded children who had pre-existing afebrile epilepsy or significant neurodevelopmental problems, those who had received treatment with phenobarbital or phenytoin during the current illness, and those found on lumbar puncture to have meningitis.

Design and procedures

We calculated, assuming 90% power and a significance level of 5%, that a sample size of 320 children would be required to detect a 50% reduction (from 30% to 15%) in seizures occurring before recovery of consciousness in the phenobarbital-treated group. The seizure categories judged to be of particular clinical importance were: three or more seizures of any duration; any episode of status epilepticus (defined as seizure activity lasting for 5 min or longer; or six or more seizures within a period of 2 h).

By means of a sequentially numbered register and as soon as possible after admission, children were randomly assigned a single intramuscular injection of phenobarbital 20 mg/kg (200 g/L), or the same volume (0.1 mL/kg) placebo. The placebo, 90% propylene glycol (the vehicle for parenteral preparations of phenobarbital), had the same viscosity and colour as the phenobarbital preparation. Numbered 5 mL ampoules of phenobarbital and placebo were prepared by the pharmacy department of Torbay Hospital, Torbay, UK. The code identifying drug and placebo was kept at Torbay Hospital, and therefore none of the clinical or scientific staff involved in the study knew which patients had received phenobarbital. Since previous work had shown an increased frequency of seizures among younger children with cerebral malaria, randomisation was stratified into two age-groups (24 months or younger and above 24 months).

The clinical tolerability of phenobarbital 20 mg/kg was assessed at the start of the trial. 23 children were given the study drug (phenobarbital or placebo) by constant-rate intravenous infusion over 4 h instead of by intramuscular injection. The intravenous route was chosen because an infusion could have been stopped if any adverse events occurred. As in the main study, the clinical investigators were unaware of which patients had received phenobarbital. Paediatric respiratory rate, blood pressure, and transcutaneous oxygen saturation were measured at baseline and every 30 min for 1 h. Blood was taken at the same times for measurement of phenobarbital concentration, and every 1 h for venous and capillary measurements. The trial was then unmasked for 23 patients only, and the phenobarbital and placebo groups compared in terms of all clinical and biochemical findings.

Baseline blood samples were taken for quantitative plasma count, full blood count, measurement of glucose, electrolytes, and phenobarbital concentrations, blood gases, and blood culture. Further blood samples for phenobarbital measurement were taken from all patients at 1 h, 2 h, 4 h, 8 h, 12 h, 36 h, and 48 h. Parasite counts were repeated every 8 h until discharge, death, or clearance of parasitaemia. Severe intracranial hypertension is a feature of cerebral malaria; lumbar puncture was delayed until the neurological status of the child had improved, or was done post mortem for those who died. Cerebrospinal fluid was assessed by microscopy and culture, and all samples with a white-cell count of above 10 per μL were screened for antigens of Haemophilus influenzae, Streptococcus pneumoniae.

Children received antimalarial chemotherapy with a single dose of intravenous quinine dihydrochloride (15 mg/kg) and subsequent doses of 10 mg/kg every 12 h. All children treated with intravenous benzylpenicillin (60 mg/kg every 4 h) and chloramphenicol (25 mg/kg every 6 h) until the results of lumbar puncture were available. Intravenous fluids and drugs were given as clinically indicated. Children with temperatures above 38.5°C were treated with paracetamol (15 mg/kg per rectum every 6 h). Hypoglycaemia (blood glucose below 2.2 mmol/L) was corrected with i.v. bolus of 0.5 g/kg dextrose. The number of duration of all seizures were recorded by means of timer pads to alarm at 5 min, 15 min, and 30 min. Seizures lasting 5 min or longer were treated with diazepam 0.1 mg/kg per i.v. injection over 2 min. After two doses of diazepam had been given without improvement, intravenous paraldehyde 0.2 mL/kg was used as second-line treatment. Anticonvulsants were administered at 5 min, 15 min, 30 min, and 45 min if seizure activity persisted. Phenobarbital 20 mg/kg infused intravenously over 20 min if a child had received doses of both diazepam and paraldehyde without resolution. It had experienced six or more seizures within 2 h.

Vital signs (temperature, pulse, respiratory rate, and transcutaneous oxygen saturation), Blantyre coma score, blood glucose (BM Stix, Roche Diagnostics, Lewes, Sussex, UK) were monitored every 4 h. Oxygen was usually required, but there were no facilities for artificial ventilation.

All patients were neurologically examined at the time of discharge from hospital. All patients were asked to return 3 months later for full neurodevelopmental assessment.

Serial blood samples were taken from all patients at phenobarbital concentration. Whole-blood phenobarbital concentrations were measured in Nairobi, Kenya, by reverse-phase high-performance liquid chromatography. Patients in whom phenobarbital was detected in the blood samples taken 1 h, 2 h, and 4 h were assumed to have received placebo. Categorisation was verified at the end of the study when the code was broken. We derived pharmacokinetic one maximum concentration, time to maximum concentration, and area under the concentration/time curve [AUC] for every ten profiles on patients who had received phenobarbital. Since there was very little variability in the data, a significant change in the mean derived pharmacokinetic parameters numbers increased, we decided that profiles would provide a good representation of the phenobarbital profile as a whole. In this way, we avoided the cost and time of analysing samples from all 170 children who received phenobarbital.
I have characteristics that met criteria for exclusion.

Clinical tolerance
Among the 23 children (ten phenobarbital, 13 placebo) assessed for clinical tolerance, baseline clinical and biochemical characteristics were similar in both groups (p>0.40 for all comparisons). There were no significant differences between the groups in the mean change between baseline and 5 h or baseline and maximum or minimum values for any of the variables measured (p=0.07 for fall in lactate 0–5 h and maximum rise in pH, p>0.10 for all other comparisons).

Pharmacokinetics
Whole-blood concentrations of phenobarbital obtained by intramuscular injection and intravenous infusion of 20 mg/kg are shown in figure 2. Intramuscular injection (n=50) resulted in a median maximum concentration of 25.6 mg/L (IQR 23.6–30.2). Maximum whole-blood concentration was 4.0 h (2.0–12.0). Concentrations were maintained above 15 mg/L, which is within the range (10–30 mg/L) thought to provide effective seizure prophylaxis for at least 48 h. The mean AUC_{[0,4]} after intramuscular injection was 250 mg h L^{-1} (95% CI 232.3–268.2). Maximum phenobarbital concentrations were slightly lower in eight children who died than in 42 survivors (median 25.1 [range 15.3–33.1] vs 26.6 [10.0–44.9] mg/L). There were no significant differences in pharmacokinetic variables between survivors and those who died (p>0.4 for all comparisons), but numbers are small.

For technical reasons, we could obtain pharmacokinetic profiles on only four of the ten children.
who had received phenobarbital by intravenous infusion. The mean AUC_\text{g,2} after intravenous infusion was 196.2 mg h L^{-1} (95% CI 154.1–238.4). The difference in AUC_\text{g,1,2} between the intravenous and intramuscular routes of administration was of borderline statistical significance (p=0.05).

Outcomes

213 (63%) of the 340 patients achieved parasite clearance before discharge or death. The placebo and phenobarbital groups did not differ in the median time to parasite clearance (40.0 [IQR 30.6–52.8] vs 45.7 [30.6–63.0] h; p=0.21). There was no difference between the groups in the time taken to recover consciousness (the ability to localise a painful stimulus; figure 3, p=0.39, log-rank test).

Phenobarbital provided effective seizure prophylaxis, decreasing the frequency and duration of seizures by over 50% (table 2). Multivariate logistic regression analysis, with allowance for the slight excess of children in the placebo group who had a history of seizures before admission, did not affect this finding. Significantly fewer children assigned phenobarbital than those assigned placebo subsequently required treatment with phenytoin (14/170 [8.2%] vs 27/170 [15.9%], p=0.04).

Mortality among children assigned phenobarbital was more than double that of the placebo group (table 2). Of the 44 children who died, 30 were in the phenobarbital group, compared with 14 in the placebo group (odds ratio 2.39 [95% CI 1.28–4.46], p=0.01). Use of multivariate logistic regression analysis showed that this difference could not be accounted for by minor imbalances in risk factors between the groups. Median time from drug administration to death was 22.5 h (range 0–4–92.7) for children in the phenobarbital group and 24.2 h (0–4–66.7) for those in the placebo group (p=0.72). Kaplan-Meier curves, comparing overall survival in the two groups, are shown in figure 3.

Children who died were, on admission, more deeply comatose and significantly more dehydrated, acidotic, and hypoglycaemic than those who survived (p<0.01 for all variables). Among children who died, mean admission respiratory rate was higher in the placebo group than in the phenobarbital group (57 [95% CI 50–64] vs 47 [41–52], p=0.03). However, respiratory rate at 4 h (median time to maximum phenobarbital concentration) was similar for the two groups (47 [41–43] vs 48 [43–53], p=0.76). There were no other significant differences between the groups in admission clinical or laboratory characteristics.

33 children had a respiratory arrest (cessation of breathing in the presence of normal cardiac function) during their clinical course in hospital; of these 30 died. Of the 33 who had a respiratory arrest, 22 had received phenobarbital and 11 placebo (odds ratio 2.1 [95% CI 1.0–4.3], p=0.05). In analyses according to total diazepam dose (in four categories; this total includes doses given in the 6 h before admission, plus all doses given in hospital), the odds of death for children treated with phenobarbital rose from 0.2 for those given fewer than three doses of diazepam to 1.7 for those given three or more doses of diazepam.

Table 2: Clinical outcome

<table>
<thead>
<tr>
<th>Seizures</th>
<th>Placebo (n=170)</th>
<th>Phenytoin (n=170)</th>
<th>Unadjusted analyses</th>
<th>Adjusted analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seizures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three or more of any duration</td>
<td>46 (27%)</td>
<td>18 (11%)</td>
<td>0.32 (0.18–0.58)</td>
<td>0.34 (0.19–0.62)*</td>
</tr>
<tr>
<td>Any lasting 5 min or longer</td>
<td>43 (25%)</td>
<td>20 (12%)</td>
<td>0.39 (0.22–0.70)</td>
<td>0.42 (0.24–0.76)*</td>
</tr>
<tr>
<td>Any episode of status eplilepticus†</td>
<td>23 (14%)</td>
<td>9 (5%)</td>
<td>0.36 (0.16–0.78)</td>
<td>0.38 (0.17–0.86)*</td>
</tr>
<tr>
<td>Death</td>
<td>14 (8%)</td>
<td>30 (18%)</td>
<td>2.39 (1.29–4.46)</td>
<td>2.49 (1.29–4.55)</td>
</tr>
<tr>
<td><strong>Neurological sequelae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At discharge</td>
<td>33/156 (21%)</td>
<td>18/140 (13%)</td>
<td>0.55 (0.30–1.02)</td>
<td>0.56 (0.30–1.05)*</td>
</tr>
<tr>
<td>3 months after discharge</td>
<td>15/144 (10%)</td>
<td>9/131 (7%)</td>
<td>0.63 (0.27–1.47)</td>
<td>0.69 (0.29–1.65)*</td>
</tr>
</tbody>
</table>

*Adjusted for seizures before admission.
†Lasting >30 min or more than six within 2 h.
‡Adjusted for factors associated with increased mortality (Blantyre score, respiratory distress, base excess, glucose, urea, creatinine).

Figure 3: Kaplan-Meier plots of coma resolution and overall survival

Note that the scales on both axes differ between the two parts of the figure.

Table 2: Clinical outcome
The interaction between drug group and diazepam category, a measure of the difference in mortality between the phénobarbital and placebo groups after fewer than three doses (p=0-004 for unequal odds). The relation between treatment and death and the interaction between drug group (phénobarbital or placebo) and diazepam category (one or more doses) was then assessed by logistic regression and the likelihood ratio test. The interaction between treatment category and drug group (phénobarbital or placebo) was, however, associated with a greatly reduced risk of death (16-5 [1-3-21-5], p=0-03). The likelihood ratio test, comparing the logistic regression model with and without the interaction term, was significant (p=0-015) and, after adjustment for the interaction between drug group and diazepam category, mortality in the phénobarbital group was lower (odds ratio 1-92 [0-94-3-91], p=0-07). Table 2 compares the difference in mortality between the phénobarbital and placebo groups after fewer than three doses (p=0-06) and after more than three doses (p=0-004 for unequal odds). The relation between the number of doses of diazepam and mortality from hospital. Although the proportion of children with sequelae was lower in the phénobarbital than in the placebo group (table 2), the difference was of borderline significance (p=0-06). Of 275 children treated with quinine, benzylpenicillin, and, in most cases, paracetamol. Winstanley and colleagues found that, in children with refractory status epilepticus, who had been treated with phénobarbital (30–120 mg/kg), showed a striking lack of respiratory depression. Published case reports suggest that the combination of phenobarbital and diazepam may result in respiratory depression and hypotension. In contrast, two controlled studies have shown that phenobarbital is highly effective in the treatment of status epilepticus, with no associated increase in respiratory depression or hypotension. A retrospective review of children with refractory status epilepticus, who had been treated with phénobarbital (30–120 mg/kg), showed a striking lack of respiratory depression. Published case reports suggest that the combination of phenobarbital and diazepam may result in respiratory depression and hypotension, and this study documents an increase in mortality resulting from concomitant use of the two drugs. Other drug interactions should be considered, since all children were treated with quinine, benzylpenicillin, chloramphenicol, and, in most cases, paracetamol. Winstanley and colleagues found that, in children with severe malaria, intravenous quinine did not affect the disposition of intramuscular phenobarbital. We know of no evidence that benzylpenicillin, chloramphenicol, or paracetamol may interfere with the action of phenobarbital. Seizures complicating cerebral malaria are said to provide effective seizure prophylaxis at plasma concentrations of between 10 mg/L and 30 mg/L. Plasma concentrations may, however, vary widely between individuals given the same dose, a finding confirmed in this study. In addition, phenobarbital has a pKa of 7-2, and changes in body pH therefore affect the distribution and excretion of the drug. If the pH of the blood is lower than that of the brain, the gradient will
favour movement of phenobarbital into the brain.\textsuperscript{10,12} Status epilepticus disrupts the blood-brain barrier, increasing cerebral uptake of phenobarbital even further.\textsuperscript{12,13} The relation between plasma concentration and effect in cerebral malaria could be different from that in other disorders without generalised cerebral pathology. Effective anticonvulsant prophylaxis might result from plasma concentrations of phenobarbital generally judged subtherapeutic.\textsuperscript{12} Phenobarbital 3-5 mg/kg provided effective seizure prophylaxis in adults with cerebral malaria.\textsuperscript{12} Phenobarbital 10 mg/kg, in a larger study, might prove effective in childhood cerebral malaria, despite blood concentrations below the "therapeutic" cut-off of 15 mg/L.\textsuperscript{10}

Other therapeutic options, such as magnesium sulphate, have also been explored in children with cerebral malaria. Once a drug that is both safe and effective has been found, the next step would be to establish, in a sufficiently large study, whether seizure prophylaxis can lower the frequency of neurological sequelae complicating this devastating disease.

Contributors
Jane Crawley was responsible for trial design and coordination, pharmacokinetic data. David Ouma was responsible for phenobarbital assays and calculation of derived pharmacokinetic data. David Ouma was responsible for phenobarbital assays. Peter Wynstanley was involved in trial design, pharmacokinetics, and clinical tolerance. Timothy Peto was trial monitor and gave statistical advice. Kevin Marsh was involved in trial design, clinical care of patients, and data collection. Jane Crawley prepared the paper, which was then critically appraised by all the other investigators.

Acknowledgments
We thank the Director of KEMRI for permission to publish this paper; the nurses on the paediatric research ward at Kilifi Hospital; Boneface Muam bi, Steven W ale, Gladys Binns, Anderson Kahindi, Naomi Lewa, Elizabeth Mwangi, Mercy Mwalongo, Susan Mwangi, Simon Kenga, Kevin Marsh in involved in trial design, clinical care of patients, and data collection. Jane Crawley prepared the paper, which was then critically appraised by all the other investigators.

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Abnormal respiratory patterns in childhood cerebral malaria

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Abstract

Of 295 children with cerebral malaria, 117 (40%) had an abnormal respiratory pattern; 15 children exhibited more than one pattern during their clinical course. Four distinct patterns were seen. (i) Deep breathing (80 children); this was associated with severe metabolic acidosis, and resolved following treatment with intravenous fluids and/or blood. (ii) Hypoventilation with nystagmus and salivation (18 children); simultaneous transcutaneous and electroencephalographic recording revealed continuous electrical seizure activity, demonstrating that these children were in subtle status epilepticus; anticonvulsant treatment resulted in return to normal of blood gases and recovery of consciousness. (iii) Hyperventilation with extensor posturing (20 children), which was associated with varying degrees of intracranial hypertension. (iv) Periodic respiration (14 children); all had clinical features suggestive of transtentorial herniation, and died following a respiratory arrest. Abnormal respiratory patterns can alert the clinician to complications of cerebral malaria that require treatment. Recognition of these patterns and rapid initiation of appropriate supportive therapy may help to reduce the high mortality rate of this disease.

Keywords: malaria, cerebral malaria, Plasmodium falciparum, respiratory patterns, children, Kenya

Introduction

Cerebral malaria, a febrile encephalopathy caused by Plasmodium falciparum, has a mortality rate of between 10 and 40%, with 10% of survivors being left with permanent neurological sequelae. The pathophysiology is complex and, in addition to coma, children may present with seizures, which are often prolonged and multiple (MOLYNEUX et al., 1989; BREWSTER et al., 1990; CRAWLEY et al., 1996; WARUIRU et al., 1990), anaemia, metabolic acidosis (TAYLOR et al., 1993; ENGLISH et al., 1997), and hypoglycaemia (WHITE et al., 1983; MARSH et al., 1995). Most deaths occur within the first 24 h after admission, highlighting the need for rapid assessment and treatment. In many African hospitals, however, diagnostic facilities are extremely limited, and clinical signs may provide the only indication of underlying pathophysiology requiring treatment. As a result of a series of research studies, we have come to recognize 4 distinct respiratory patterns and their associated pathophysiology which have proved helpful in the clinical management of children with cerebral malaria.

Methods

The studies were conducted at the Kenya Medical Research Institute (KEMRI) Research Unit at Kilifi District Hospital on the Kenyan coast. Three thousand to 4000 children are admitted each year to the 36 beds general paediatric ward, while children with severe malaria (WHO, 1990) are transferred to the 6 beds paediatric high dependency unit. Cerebral malaria was defined as an inability to localize pain (MOLYNEUX et al., 1989) in the presence of P. falciparum parasitaemia. Clinical assessment included close observation of respiratory pattern and rate, depth of coma, posture, and focal neurological signs. Blood was taken for parasite count, full blood count, determination of glucose, electrolytes, lactate and venous blood gas levels, and blood culture. Children received standard antimarial treatment with intravenous quinine or intramuscular artesunate. Investigations that have been carried out during the course of research studies include the measurement of transcutaneous oxygen saturation, central venous pressure (ENGLISH et al., 1996b), electroencephalography (EEG; 14-channel Medelec 1A94 machine) (CRAWLEY et al., 1996), cerebral function monitoring (CIFAM, channel EEG, Meded Ltd.) and, with selected children, intracranial pressure monitoring using a subarachnoid catheter (model 110–4B, Camino Laboratories) (NEWTON et al., 1997). All studies were carried out with the approval of the KEMRI Scientific Co-ordinating Committee, and with written informed consent from parents.

Results

Between January 1993 and September 1995, 295 children were admitted with cerebral malaria. One hundred and seventeen (40%) had an abnormal respiratory pattern, of whom 15 had more than one pattern during their clinical course. The 4 patterns were (i) acidotic or deep breathing (Kussmaul's respiration), (ii) hypoventilation with associated nystagmus, (iii) hyperventilation with extensor posturing, and (iv) periodic respiration.

Deep breathing

Deep breathing, defined as a persistent increase in the depth and rate of respiration, was observed in 80 children. It is dealt with only briefly here, since details have already been published (ENGLISH et al., 1996a, 1996b). Deep breathing was associated with severe metabolic acidosis (base excess -12 or more), with a sensitivity of 91% and a specificity of 83% (ENGLISH et al., 1996a). These children had higher mean blood lactate, urea and creatinine levels and osmolality, and lower mean pH and haemoglobin level, than those without deep breathing (ENGLISH et al., 1997), suggesting that hypovolaemia, anaemia, and renal impairment were important contributing factors. Central venous pressure was normal (<6 cm of water) in 24 of these children (ENGLISH et al., 1996b), and rapid transfusion (over 1-4 h) of 20 mL/kg of normal saline or blood (for those with a haemoglobin level <5 g/dL) was followed by a dramatic fall in lactate level and clinical improvement in the majority of patients. Of the 80 children in this group, 80% went on to make a full recovery, and 20% died.

Hyperventilation with associated nystagmus

Eighteen children presented in deep coma with shallow, irregular respiration. Transcutaneous oxygen saturation was below 90% in all children, while the median pCO2 of 15 children who had concurrent blood gas analysis done was 7.0 kPa (range 5.5-11.1 kPa). Other important clinical features were bilateral consensual eye
deviation with nystagmus, and salivation. Eight children had simultaneous electrophysiological recordings made by EEG or CFAM, and all showed electrical evidence of continuous seizure activity (Crawley et al., 1996). Although electroencephalographs from 5 children revealed continuous unilateral spike-wave discharges in the parieto-temporal region, sometimes spreading throughout the hemisphere, there was either no manifestation on the contralateral limbs, or minimal, intermittent clonic movements of a digit, eyebrow, or angle of the mouth. CFAM confirmed continuous seizure activity in these patients, and demonstrated seizure activity in 1 who did not have EEG. These children were therefore in subtle status epilepticus. Seizure activity resolved spontaneously in one patient and, with the others, anticonvulsant treatment was followed by an improvement in respiratory effort and blood gases. CFAM recordings, as judged by their ability to localize a painful stimulus, a median of 6 h (range 2-70) after anticonvulsant treatment. Two children, both of whom had prolonged partial seizures, had mild hemiplegia on discharge. One child, who was profoundly amnesic and anoxic, subsequently died (see below). Fifteen children made a full recovery.

Two cases were of particular interest. One child of 5 months was hypoventilating on admission despite profound metabolic acidosis (base excess -30). Following treatment with diazepam, the child developed deep, rapid respiration, and the PCO₂ fell from 7-6 to 2-6 kPa, providing appropriate partial compensation for the metabolic acidosis. Another child, of 51 months, had been unconscious for 4 h following a prolonged generalized seizure at home. There was no clonic activity, and the child was deeply unconscious with shallow irregular respiration, eye deviation, nystagmus, salivation, and pria-pism. EEG revealed generalized electrical status, which was unresponsive to 4 doses of diazepam and loading doses of phenytoin and phenobarbitone. Seizure activity was finally abolished after 2 intravenous doses of thiopentone 3 mg/kg, and the child recovered consciousness 6 h later.

Full clinical details of these children can be obtained from the first author.

Hyperventilation with extensor posturing

Twenty children had episodes of hyperventilation (increases in rate and/or depth of respiration), associated with extensor posturing. The posturing varied in severity from intermittent increases in extensor tone (decerebrate rigidity) through to full-blown opisthotonic posturing of patients, who did not have EEG. These children had concomitant arterial blood gas levels which were suggestive of primary hyperventilation in the absence of metabolic acidosis, pH being 7-48 or above, median PCO₂ 2-6 kPa (range 1-3-5 kPa), median pO₂ 16-5 kPa (range 13-6-19-5), and median base excess -2 (range -7 to zero). In 13 of these children, hyperventilation and posturing were intermittent. Four children had concurrent EEG, evidence of seizure activity, while monitoring of intracranial pressure (ICP) in 2 children (Newton et al., 1997) demonstrated mild intracranial hypertension, the ICP being between 10 and 20 mmHg. Intrathoracic perfusion pressures (CVP) above 50 mmHg. No child received treatment with mannitol. One child, with a history of status epilepticus, was left with mild hemiplegia, one went on to develop sustained opisthotonic posturing (see below), and 11 made a full recovery.

Seven children in this group had severe, sustained opisthotonic posturing and hyperventilation on admission, while one child developed it while in hospital. There was no electrical evidence of seizure activity on EEG in 7 of these children, but 2 children who underwent ICP monitoring had severe intracranial hypertension (ICP> 40 mmHg for at least 15 min, in association with CPP <40 mmHg). Although intravenous mannitol (0-5-0-75 g/kg over 20 min) was given to 7 children, only 2 received more than 4 doses, the other 5 receiving one or 2 doses just before death. All children died (see below) following respiratory arrest in the presence of a normal pulse and absent pupillary responses, features suggestive of transtentorial herniation.

Periodic respiration

Fourteen children (including 8, described above, who had sustained opisthotonic posturing) had a respiratory pattern that changed abruptly a median of 12 h after admission. Breathing became shallow and irregular, with intermittent gasping. Pupillary responses were sluggish and the oculocephalic response absent. Nine received one or more doses of mannitol, but all went on to become apnoeic without initial cardiac arrest. Despite attempted resuscitation, all these children died.

Discussion

This series of children with cerebral malaria illustrated how anomalies in respiratory pattern can provide the clinician with valuable information on pathophysiology that may result in a beneficial change of treatment. The findings are of particular relevance to hospitals in the rural tropics, where diagnostic facilities are limited.

In humans, respiration is under dual control from metabolic and neural influences. Metabolic respiratory control is directed principally at maintaining arterial oxygenation and acid–base balance, and is regulated by respiratory 'centres' in the lower brain stem. Neural control originates from preponine structures lying mainly at the forebrain level. The 2 controlling systems are integrated largely in the lower brain stem, and each system projects by distinct descending pathways to the spinal respiratory motor neurones. The presence of this dual control mechanism means that diseases causing coma commonly induce respiratory abnormalities that can reflect either the pathological anatomy or chemistry of the illness (Plum & Posner, 1982). The pathological hallmark of cerebral malaria is sequestration of parasitized red blood cells in the cerebral microvasculature (MacPherson et al., 1985). Neuronal damage may result from interference with microcirculatory flow and consequent hypoxia, intracranial hypertension secondary to increased cerebral blood volume (Newton et al., 1997), or the local release of toxic mediators such as cytokines (Grau et al., 1989; Kwiatkowski et al., 1990; White & Ho, 1992), nitric oxide (Clark & Rockett, 1996), and excitotoxic neurotransmitters (R. Surtees, personal communication). Prolonged, multiple seizures are a common complicating factor (Crawley et al., 1996). Abnormalities in respiratory pattern may therefore arise either as a direct result of the diffuse encephalopathy, or secondary to metabolic disturbances (anaemia, acidosis, hypoglycaemia) that complicate the disease process.

Deep breathing is the commonest respiratory abnormality observed in children with severe malaria in coastal Kenya. It is a clinical sign with good inter-observer agreement amongst clinicians who regularly care for children with severe malaria (English et al., 1995) and is reliably associated with metabolic acidosis (Lunt et al., 1996a) which may be caused by anaemia, dehydration, or a combination of both. Detection may alert the clinician to the need for urgent treatment with blood transfusion (English et al., 1996b) and/or intravenous fluids.

Hyperventilation leading to hypoxia and hypercapnia is a potentially life-threatening situation which is easily overlooked in many clinical settings. When diazepam has been given before admission it is tempting to attribute the ensuing hyperventilation to drug-related res-
ABNORMAL RESPIRATORY PATTERNS IN MALARIA

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Announcements

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Seizures and status epilepticus in childhood cerebral malaria

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Summary
Prolonged, multiple seizures complicate a high proportion of cases of childhood cerebral malaria, and several studies have shown an association between these and neurological sequelae. We prospectively studied 65 patients (38 female) admitted to Kilifi Hospital in 1994. Electroencephalographic recordings (EEGs) were made at 12-hourly intervals, with continuous recordings made on a cerebral function analysing monitor (CFAM). Survivors were seen one month after discharge. Cerebral computerized tomography was performed on children with neurological sequelae. Sixty-two percent of patients had seizures following admission, of whom half had an episode of status epilepticus. Fifty-two percent of seizures were partial motor, 34% generalized tonic-clonic, and 14% partial with secondary generalization. In 22%, coma appeared to be due to a prolonged postictal state. Ten children had subtle motor seizures. Posterior parieto-temporal discharges were the most common EEG finding. Seven children died, eight developed neurological sequelae, and 50 (77%) recovered fully. Status epilepticus was associated with the development of neurological sequelae. Prolonged, multiple seizures may play an important part in the pathogenesis of coma in childhood cerebral malaria, and are likely to contribute to both the morbidity and mortality of this disease.

Introduction
Malaria is a major cause of morbidity and mortality in sub-Saharan Africa, causing the death of over one million children each year.¹ Approximately 1% of clinical infections with Plasmodium falciparum result in severe disease, of which one of the most serious forms is cerebral malaria,²,³ with a mortality of 10–40%.⁴ Although most survivors make a full recovery, neurological sequelae (hemiplegia, speech problems, cortical blindness, epilepsy) occur in 5–15%.⁵,⁶

Although the cause of coma in cerebral malaria is not known, the essential pathological feature is sequestration of parasitized red blood cells in the cerebral microvasculature.⁷ Neuronal damage may result from interference with microcirculatory flow and consequent hypoxia, intracranial hypertension secondary to increased cerebral blood volume,⁸ or the local release of toxic mediators such as cytokines, nitric oxide and excitotoxic neurotransmitters.⁹,¹⁰

Patients present with a diffuse encephalopathy, and seizures, which are often prolonged and multiple, occur in up to 80% of cases.¹¹,¹² Uncontrolled seizure activity can damage the brain by aggravating hypoxia, hypoglycaemia and intracranial hypertension,¹³ and several studies have suggested an association between status epilepticus and neurological sequelae in childhood cerebral malaria.⁵,⁶

Despite their high prevalence and potential pathogenic importance, there have been no detailed studies of seizures in cerebral malaria. Here we describe the clinical and electrophysiological spectrum of seizures in childhood cerebral malaria in an area of Kenya where malaria is endemic.
Methods

Study site
The study was conducted on the 5-bed KEMRI paediatric research ward at Kilifi District Hospital, Kilifi, Kenya, between January and September 1994. The characteristics of malaria transmission and the population from which patients were drawn have been described previously.14

Patients
Children aged 9 months and above were eligible for enrolment in the study if they fulfilled the World Health Organisation (WHO) definition of cerebral malaria, namely unrousable coma not attributable to any other cause in the presence of asexual Plasmodium falciparum parasitaemia.15 To fulfil the definition of cerebral malaria, coma had to persist for at least 1 h after a seizure and/or after the administration of diazepam. Depth of coma was quantified using the Blantyre coma score,3 in which a coma score of 5 denotes full consciousness, and 0 complete absence of any response to painful stimulus. One hundred and ten children who fulfilled this definition of cerebral malaria were admitted during the study period. Sixty-five (60%) were recruited, since for technical reasons it was only possible to study two children at a time.

Clinical investigations and management
A clinical history and complete physical examination was performed on all children. Blood was taken for baseline assessment of parasite count, full blood count, urea and electrolytes, glucose, lactate, blood gas, and plasma chloroquine level. Electroencephalographic (EEG) recordings were made on a 14-channel Medelec 1A94 EEG machine. Silver/silver chloride electrodes were fixed with Elefix and tape to the child’s shaved head, and the International 10–20 system was used for electrode placement. Recordings were taken within 6 h of admission, and at 12-h intervals until recovery of consciousness (Blantyre coma score 5). Continuous recordings using a CFAM (cerebral function analysing monitor, Medaid Ltd) were obtained from children unconscious for more than 24 h. Any unusual neurological signs or witnessed seizure activity were recorded on video. In deeply comatose children, intracranial pressure was monitored using a subarachnoid catheter (Camino 110-4B). Intracranial hypertension is a feature of childhood cerebral malaria8 and, to reduce the risk of transtentorial herniation, lumbar puncture was performed once the clinical condition of the child had improved, or was done post-mortem in those who died. Children received standard antimalarial treatment with intravenous quinine 20 mg/kg loading dose and 10 mg/kg 8-hourly, or intramuscular artemether 3.2 mg/kg loading dose and 1.6 mg/kg every 24 h, as part of a multicentre study comparing the efficacy of quinine and artemether in the treatment of childhood cerebral malaria. Intravenous benzyl penicillin and chloramphenicol were administered until the results of lumbar puncture were known. Intravenous fluids and blood were given as clinically indicated. Seizures lasting more than 5 min were treated with intravenous diazepam 0.3 mg/kg. Recurrent (>3) seizures were treated with a loading dose of intravenous phenytoin 18 mg/kg and, if that failed, with intramuscular phenobarbitone 18 mg/kg. Children with continuous seizure activity lasting for 30 min or more were considered to have status epilepticus.16

Follow-up
All survivors were seen one month after discharge for neurological examination and repeat EEG. Cerebral computerized tomography (CT) was performed on all children with neurological sequelae.

Analysis
EEGs were analysed by SS who knew the age of each child, but was blind to any other clinical information. Statistical analysis was carried out using SPSS (version 5.0; 1992). Analysis of variance and Student’s t test were used to compare means of normally distributed data. Data not conforming to a normal distribution were compared by analysis of variance after logarithmic transformation, or by the Mann-Whitney U test. Proportions were compared by the χ² test.

Results
Sixty-five children were studied, of whom 38 were female. Ages ranged from 9 months to 11 years (median 30 months). All had previously normal development. Children were unconscious for between 1 and 72 h (median 7 h) prior to admission. In over half the cases, onset of coma coincided with the onset of epileptic seizures.

On admission
Presenting clinical features and investigations are shown in Tables 1 and 2. Seventy-five percent of the children were deeply unconscious on admission, with a Blantyre coma score of 2 or less. Thirteen children (20%) had received diazepam on or up to 6 h prior to admission, in doses of between 0.2–1 mg/kg. In five cases, diazepam had been given
Seizures and cerebral malaria

Table 1  Clinical comparison of children with and without seizures after admission (n = 65)

<table>
<thead>
<tr>
<th></th>
<th>No seizures after admission (n = 25)</th>
<th>Partial seizures (n = 28)</th>
<th>Generalized seizures (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)*</td>
<td>38.5 (29.9-47.1)</td>
<td>26.7 (20.6-32.9)</td>
<td>64.0 (44.7-83.5)</td>
</tr>
<tr>
<td>Past history of febrile seizures</td>
<td>2 (8%)</td>
<td>5 (18%)</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>Pre-admission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any seizures</td>
<td>15 (60%)</td>
<td>22 (78%)</td>
<td>7 (58%)</td>
</tr>
<tr>
<td>Status epilepticus</td>
<td>11 (44%)</td>
<td>13 (46%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Admission coma score**</td>
<td>2 (2-3)</td>
<td>2 (1-4)</td>
<td>2 (0-4)</td>
</tr>
<tr>
<td>Admission temperature (°C)</td>
<td>38.8 (38.3-39.3)</td>
<td>38.4 (38.0-38.8)</td>
<td>39.4 (38.4-40.5)</td>
</tr>
<tr>
<td>Status epilepticus after admission</td>
<td>--</td>
<td>14 (50%)</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>Neurological sequelae</td>
<td>3 (12%)</td>
<td>4 (14%)</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Death</td>
<td>4 (16%)</td>
<td>2 (7%)</td>
<td>1 (8%)</td>
</tr>
</tbody>
</table>

Data expressed as frequency (proportion) or mean (95% CI).
* p<0.001 for comparison between the three groups. For all other variables there was no statistically significant difference at the 5% level.
** Admission coma score expressed as median (range).

Table 2  Laboratory parameters on admission: comparison of children with and without seizures after admission (n = 65)

<table>
<thead>
<tr>
<th></th>
<th>No seizures post-admission (n = 25)</th>
<th>Seizures post-admission (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitaemia (per µl)*</td>
<td>41523 (15306-112645)*</td>
<td>44846 (22315-90129)*</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>7.0 (6.1-7.9)</td>
<td>6.8 (5.8-7.7)</td>
</tr>
<tr>
<td>Na (mmol/l)</td>
<td>137 (133-140)</td>
<td>136 (134-137)</td>
</tr>
<tr>
<td>K (mmol/l)</td>
<td>4.4 (4.0-4.8)</td>
<td>4.4 (4.1-4.7)</td>
</tr>
<tr>
<td>Urea (mmol/l)*</td>
<td>4.8 (3.2-7.2)*</td>
<td>4.7 (3.7-6.0)*</td>
</tr>
<tr>
<td>Creatinine (µmol/l)*</td>
<td>68 (54-86)*</td>
<td>54 (45-66)*</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.4 (3.5-5.3)</td>
<td>5.9 (4.6-7.2)</td>
</tr>
<tr>
<td>Corrected calcium (mmol/l)</td>
<td>2.05 (1.95-2.15)</td>
<td>2.10 (2.02-2.18)</td>
</tr>
<tr>
<td>pH</td>
<td>7.36 (7.32-7.40)</td>
<td>7.32 (7.28-7.36)</td>
</tr>
<tr>
<td>Base excess</td>
<td>-7.3 (--10.7 to -0.9)</td>
<td>-7.2 (--9.7 to -4.7)</td>
</tr>
<tr>
<td>Lactate (mmol/l)*</td>
<td>3.3 (2.4-4.7)*</td>
<td>4.0 (3.1-5.0)*</td>
</tr>
<tr>
<td>Osmolality (mOsmol/kg)</td>
<td>289 (279-299)</td>
<td>289 (284-294)</td>
</tr>
<tr>
<td>Chloroquine (ng/ml of free base)**</td>
<td>80 (0-1570)**</td>
<td>43 (0-902)**</td>
</tr>
</tbody>
</table>

Data expressed as means (95% CI).
* Geometric mean (95% CI).
** Median (range); n = 54, of whom 38 (70%) had detectable levels of plasma chloroquine. There were no statistically significant differences at the 5% level between the two groups for any of the above variables.

in intramuscularly at a health centre. Fourteen children (22%) had multiple seizures or status epilepticus on or immediately prior to recruitment, but had regained consciousness within 6 h of cessation of seizure activity.

Clinical course

Forty children (62%) had between one and more than 20 clinical seizures (median five), of duration 0.5–600 min (median 3 min). Eighteen children (28%) had one or more episodes of status epilepticus. Seventy-six percent of all seizures occurred within 24 h of admission. Fifty-two percent of the seizures were partial motor, 14% partial with secondary generalization, and 34% generalized tonic-clonic. Three children had both partial and generalized seizures during their clinical course. Partial seizures were slightly (64%), but not significantly, more common on the right side of the body. Only 42% of the 310 seizures documented were associated with a rectal temperature of 38 °C or above. Two were associated with hypoglycaemia. Ten children (25% of those with seizures after admission), with a median age of 26 months (range 9–60), had seizures that were clinically very subtle. In nine children these consisted of nystagmoid eye movements, salivation, and shallow, irregular respiration with concurrent hypoxaemia (arterial oxygen saturation below 80%) and hypercarbia (pCO₂ > 6.5 kPa). Although EEGs revealed continuous unilateral spike-wave discharges in the parieto-temporal region, sometimes
spreading to involve the whole of that hemisphere, there were either no manifestations in the contralateral limbs, or intermittent minimal clonic movements of a digit, eyebrow, or mouth. One child was admitted in deep coma, with irregular respiration, priapism, and salivation, following 6 h of generalized status epilepticus at home. EEG revealed generalized seizure activity, yet there were no other clinical accompaniments.

Twenty-six children received between one and five doses of intravenous diazepam, 0.3 mg/kg. In the majority of cases, cessation of clinical seizures occurred within 5 min of drug administration. In eight children, electrographic seizure activity persisted for between 2 and 140 min despite cessation of the clinical seizure with treatment. Intravenous phenytoin 18 mg/kg was given to 24 children, of whom 63% had no further seizures. Two out of four children who received intramuscular phenobarbital also had no further seizure activity. In one child, intravenous thiopentone 4 mg/kg was effective at stopping generalized status epilepticus that had been unresponsive to three doses of diazepam and 18 mg/kg of both phenytoin and phenobarbital.

Intracranial pressure (ICP) was monitored in 10 children, of whom four had a total of 31 (range 1-16) seizures during the course of monitoring. Both generalized and partial seizures caused a rise in intracranial pressure, the median rise in ICP (+164%, range 108-285) being greater during 18 generalized seizures than during 13 partial seizures (+50%, range 0-186). The magnitude of the rise was not affected by seizure duration. In all cases, a concurrent rise in mean arterial pressure meant that cerebral perfusion pressure was adequately maintained above 50 mmHg. Generally, intracranial pressure fell at the end of each seizure, but remained high (above 30 mmHg) for 2 min following the cessation of one generalized seizure. One period of electrographic seizure activity with no associated clinical manifestations was not associated with a significant rise in intracranial pressure.

Electrophysiology

Two hundred and seventy EEG and 30 CFAM recordings were made. During coma, the EEG was dominated by high-amplitude slow waves (4 Hz and below). Fifteen of 28 children with partial seizures had an EEG recorded during a clinical seizure. In all cases, ictal spike-wave discharges arose from the posterior parieto-temporal region (Figure 1). In eight of these cases there was considerable disparity between clinical and electrical seizure activity, with electrical activity spreading to involve all of one or both hemispheres despite the clinical seizure remaining partial. A further eight recordings from seven children with generalized seizures showed generalized ictal discharges. All children who made a full recovery had normal EEGs at one month follow-up.

Outcome

Fifty children (77%) made a full recovery, seven (11%) children died, and eight (12%) had persistent neurological sequelae one month after discharge. Among survivors, median time to localize a painful stimulus was 21.5 h (range 4-111 h). Of the eight children with sequelae, four had a hemiplegia, two had spastic quadriplegia, one had severe cognitive and speech problems, and one had epilepsy. The children with sequelae were significantly (p=0.02) younger (mean age 20.4 months, 95% CI 6.2-34.5 months) than those who recovered fully (39.8 months, 95% CI 33.2-46.3 months). Six (75%) had status epilepticus before or after admission, as did 27 (54%) normal survivors. Two children who developed a spastic quadriplegia had severe hypernatraemic dehydration on admission. There were otherwise no significant differences in clinical or laboratory parameters between those with sequelae and normal survivors, but numbers are small. Seven of the eight children with sequelae had CT scans at one month follow-up and, with the exception of the scan from the child with epilepsy, all showed abnormalities. Scans from 3/4 children with hemiplegia revealed an area of infarction in the contralateral posterior parieto-temporal region (Figure 2). During their clinical course in hospital, two of these children had developed partial status epilepticus with electrical discharges arising from the same area. One child with partial motor status epilepticus and subsequent hemiplegia failed to attend for CT scan. CT scans on the two children with spastic quadriplegia and one with profound cognitive and speech problems showed generalized cerebral atrophy. Follow-up EEGs from children with residual hemiplegia showed low-amplitude slow wave activity in the contralateral parieto-temporal region, while EEGs from children with spastic quadriplegia were featureless and of low amplitude.

Two (29%) of the seven children who died had status epilepticus during their clinical course. Those who died were significantly more acidotic on admission (p values 0.04 and 0.01 for pH and base excess, respectively) than those who survived. One had Haemophilus influenzae septicaemia (but normal cerebrospinal fluid) and three had one or more episodes of hypoglycaemia. Laboratory parameters were otherwise unremarkable.

Discussion

The WHO definition of cerebral malaria attempts to delineate a homogenous encephalopathic syndrome
associated with high levels of morbidity and mortality. Our data suggest that, in a proportion of cases, recurrent seizures play an important role in the pathogenesis of coma. Eighty-five percent of children in this series had at least one seizure during their clinical course, with the onset of coma associated with seizures in over half the cases. For the 22% who regained consciousness within 6 h of admission, coma appeared to result from prolonged or repeated seizures, and these children are likely to represent a different pathophysiological entity from those with prolonged coma. Following a prolonged seizure, the brain enters a phase of 'cortical exhaustion', with depletion of adenosine triphosphate, glucose, oxygen and the accumulation of lactic acid. In the postictal phase the EEG may be flattened, or show diffuse slow wave activity, the duration of the postictal phase being increased after prolonged or multiple seizures.

This study has also shown that in a proportion of children with cerebral malaria, coma is due to continuing subtle seizure activity which is likely to go undetected, but which is responsive to anticonvulsant drugs. Ten children (25% of those with seizures on or after admission) had seizures with minimal clinical manifestations, a recognized feature of prolonged generalized status epilepticus. The important point here is that the ictal features would be easily missed unless specifically looked for, yet uncontrolled seizure activity can damage the brain. Increased cerebral metabolism and hypercapnia both cause an increase in cerebral blood flow and therefore cerebral blood volume, so potentially exacerbating the intracranial hypertension that can complicate cerebral malaria. Subtle seizures are an easily treated cause of coma, and five of these children regained consciousness within 6 h of treatment with intravenous diazepam. Most children with cerebral malaria are admitted to busy, understaffed hospitals in the developing world, where subtle seizures are likely to be missed unless health personnel are trained in their detection.

What are the possible causes of seizures in cerebral malaria? Fever is known to precipitate seizures in young children. However, more than half the seizures documented after admission occurred when the rectal temperature was below 38 °C. Hypoglycaemia and electrolyte imbalance can cause seizures, yet only two seizures were associated with a blood glucose <2.2 mmol/l. It is likely that hypernatraemic dehydration was partly responsible for the status epilepticus and subsequent spastic quadriplegia that developed in two children. Although mild hyponatraemia and hypocalcaemia occurred in a
Cerebral CT scan obtained one month after discharge, showing extensive areas of infarction (numbered 1 and 2) in the left posterior temporal/occipital region, with associated cerebral atrophy. This 9-month-old child had multiple right partial motor seizures (including several episodes of status epilepticus) during her clinical course, and subsequently developed a dense right hemiplegia.

Figure 2. Cerebral CT scan obtained one month after discharge, showing extensive areas of infarction (numbered 1 and 2) in the left posterior temporal/occipital region, with associated cerebral atrophy. This 9-month-old child had multiple right partial motor seizures (including several episodes of status epilepticus) during her clinical course, and subsequently developed a dense right hemiplegia.

of hypoxia, or by initiating the release of excitotoxic mediators such as glutamate or quinolinic acid. However, whilst sequestration appears to be a global phenomenon, the majority of patients in this study had partial seizures. Electrographically, these seizures were associated with ictal spike-wave activity in the posterior parieto-temporal region. This is a 'watershed' area, lying between territories supplied by the posterior and middle cerebral arteries. It is therefore particularly vulnerable to ischaemia when oxygen delivery to the brain is compromised as a possible result of severe anaemia, or inadequate cerebral blood flow due to hypotension, raised intracranial pressure or impaired autoregulation. CT scans on two children with partial seizures and subsequent hemiplegia showed infarction in this area. Although it is difficult to know whether the seizures caused infarction or vice versa, it is clear that uncontrolled seizure activity, with the concomitant increased demand for oxygen and glucose, is likely to exacerbate the situation.

Several studies of cerebral malaria have shown an association between status epilepticus and neurological sequelae, which occur in 5-15% of survivors. There is a large clinical and experimental literature to support the hypothesis that prolonged seizure activity can damage the brain, causing deficits in both motor and cognitive function. The hippocampus, which plays an important role in short- and long-term memory, is particularly prone to seizure-induced damage. The gross neurological sequelae described in this and other studies of cerebral malaria are likely to represent one end of a spectrum of a much wider range of handicaps. Many 'normal' survivors of cerebral malaria may have significant cognitive problems, with serious implications for their subsequent educational potential.

Can anticonvulsant prophylaxis reduce the incidence of status epilepticus complicating cerebral malaria? An ideal candidate drug would need to be cheap, safe, effective, and preferably administered by the intramuscular route. Phenobarbitone fulfills these criteria, yet its role in seizure prophylaxis in cerebral malaria is unclear, since two small published studies have produced somewhat conflicting results. If prophylactic phenobarbitone can reduce the incidence of status epilepticus complicating cerebral malaria, it is possible that this will also have an impact on the incidence of subsequent neurological sequelae. A definitive study of intramuscular phenobarbitone would therefore seem to be an important next step.

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SEIZURES IN CHILDHOOD
CEREBRAL MALARIA

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