NON-HAEMATOLOGICAL ASPECTS OF IRON NUTRITION

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INTRODUCTION

The Ebers Papyrus, dating from about 1500 BC, describes remedies containing iron. Fe was used by Hippocrates and the ancient Roman physicians. Following the detailed description of chlorosis by Lange in 1554 and Lazarus Riverins in 1640, Fe was popularized by Sydenham, in 1681, as the specific remedy (Fairbanks *et al.* 1971). Fe has been easily measured for many years, and is perhaps the most studied of the nutrients. Indeed, it could be argued that the investigation of clinical Fe deficiency is the historical basis for the whole discipline of haematology. This has led to the impression that the only condition of importance, in which Fe is involved, is Fe-deficiency anaemia. The clinician often goes further and equates anaemia itself with Fe deficiency. If asked what was the most common deficiency in the world today many would answer 'Fe'.

Despite the years of study, it is only recently that we have begun to appreciate that the effects of Fe deficiency are much more widespread than simply to cause anaemia. Further, we are finding that the iron sword is indeed double-edged.

The problems of excess Fe are not confined to relatively rare occurrences of haemochromatosis, multiple blood transfusions and the particularly high Fe intakes from Bantu beer or poisoning with Fe tablets. Evidence is accumulating that a damaging excess body burden of Fe is not uncommon, and that this increases the individual's susceptibility

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to invasion by infecting organisms. More surprisingly it seems that Fe, by virtue of its reactive nature, is intimately involved in the relatively common clinical syndrome of kwashiorkor. Excess local deposits of Fe are involved in certain chronic inflammatory diseases such as rheumatoid arthritis.

The other edge of the sword, Fe deficiency, is also associated with increased infection, in this case due to impaired ability to kill those invaders that have developed pathogenic mechanisms to obtain the Fe they, themselves, need from a depleted host. Fe deficiency of a degree common in humans may also affect both physical work capacity and mental ability.

It is the purpose of the present review to examine some of the evidence for these non-haematological effects of Fe nutrition. Reviews on various aspects of Fe metabolism have recently been published (Bothwell *et al.* 1979; Jacobs & Worwood, 1980; Finch & Huebers, 1982; Jacobs, 1982; Oppenheimer & Hendrickse, 1983; Halliwell & Gutteridge, 1984, 1985*a*; Dallman, 1986; Jacobs, 1987).

CHEMISTRY AND METABOLISM OF IRON

The importance of Fe derives from the fact that it exists in two stable, interchangeable forms, ferrous (Fe[II]) and ferric (Fe[III]). This basic chemical characteristic explains both the essentiality and the toxicity of Fe. Because there is a single electron change between the two stable states of Fe, it is used extensively for single electron transfer reactions in the body (copper is also used to a lesser extent). The transfer of electrons down the mitochondrial respiratory chain, by single electron steps, is achieved by oxidation and reduction of the Fe contained in the cytochromes. This explains the extreme toxicity of some Fe-binding chemicals such as cyanide and azide. Many other oxidases, peroxidases and dehydrogenases in the body rely on Fe at the active site.

The chemistry of Fe is intimately related to the chemistry of free radicals because Fe reactions involve the transfer of a single electron. A free radical is simply an atom or molecule which has an unpaired electron (Halliwell & Gutteridge, 1985*a*; Slater *et al.* 1987): they are amongst the most reactive species of chemical known. Each electron avidly seeks to combine with a second electron which has the opposite spin. They can add on to other molecules in order to share their electrons to form an 'adduct'. Alternatively, they can abstract an atom, usually hydrogen, from an adjacent molecule. This in turn leaves an unpaired electron on the molecule which has been attacked; this chain reaction goes on until the latest radical meets a second radical with which it can 'share' its electron, or until a radical is produced which is relatively stable, a so-called scavenger or antioxidant.

Several free radical species are produced in vivo during various metabolic events. For example, the inflammatory response by polymorphonuclear neutrophils (Babior, 1978*a*, *b*, 1984) and the synthesis of prostaglandins and leukotrienes involve the production of a multitude of free radical species (see Halliwell & Gutteridge, 1985*a*). If free radicals are not effectively neutralized they could react with and severely damage cellular components, as described previously. In particular, the reaction between free radicals and unsaturated fatty acids leads to the generation of lipid hydroperoxides and subsequent membrane damage (Halliwell & Gutteridge, 1985*a*; Slater *et al.* 1987).

As Fe is involved in single electron transfer reactions, each of these reactions can lead to the production of a free radical; these include harmful oxygen derivatives such as peroxide, superoxide and hydroxyl radicals (Aisen & Listowsky, 1980; Hershko & Peto, 1987). Clearly, each of these reactions is potentially dangerous: they must be, and are, rigorously

controlled to contain the radicals. There must also be mechanisms to deal effectively with radicals which leak from the enzymic sites, where they are harnessed, and to repair any damage which occurs. Moreover, Fe itself must be stored and transported in a form which prevents it from undergoing single electron changes (from the ferric to ferrous state). Our love–hate relationship with Fe is the cost we incur to have an aerobic metabolism. If there is insufficient Fe we suffer loss of function: if there is too much, or the control and repair mechanisms are defective, we suffer damage.

Fe is attached to its transport and storage proteins in the ferric state. When it is in the ferrous state it is available to be oxidized during a radical producing reaction. When reducing agents such as superoxide, ascorbic acid or NADPH are present, Fe can undergo repeated cycles of oxidation and then reduction. The oxidation step will produce a free radical and then the ferrous-Fe is regenerated by the reducing agent: so-called redox cycling. At each turn of the catalytic cycle potentially damaging free radicals are produced (Halliwell & Gutteridge, 1984, 1985*a*, *b*, 1986; Slater, 1984). This catalytic property, which is the basis of the toxicity of Fe, is deliberately used by the body to kill invading organisms.

Clearly, with too much Fe we will damage ourselves and with too little we may have difficulty with energy production and with killing pathogens. Equally clearly, the biological effects of Fe will be determined by both the metabolism of the proteins which bind and control Fe and the integrity of the mechanisms which prevent and repair free radical damage.

The main reaction by which Fe mediates its toxicity in vivo is thought to involve the Haber–Weiss reaction, the sequence of which is shown in the following scheme (see Halliwell & Gutteridge, 1984, 1985*a*, *b*; Slater, 1984):

$$O_2^{\cdot-} + Fe^{3+} \text{-----} \rightarrow O_2 + Fe^{2+} \quad (1)$$

$$2O_2^{\cdot-} + 2H^+ \text{-----} \rightarrow O_2 + H_2O_2 \quad (2)$$

$$Fe^{2+} + H_2O_2 \text{-----} \rightarrow Fe^{3+} + OH^- + {}^{\cdot}OH. \quad (3)$$

In this scheme the Fe(III) is reduced to Fe(II) by the superoxide anion, $O_2^{\cdot-}$, although other reducing agents, such as ascorbate, are effective, reaction 1. Superoxide is produced during the respiratory burst of phagocytic cells, by leakage from the cytochrome chain and from some enzymic reactions (Babior, 1978*a*, *b*, 1984). Although hydrogen peroxide is produced by several reactions in the body (Chance *et al.* 1979), reaction 2 catalysed by superoxide dismutase (*EC* 1.15.1.1) (McCord & Fridovich, 1969) is probably the most important quantitatively. In reaction 3, known as the Fenton reaction, ferrous-Fe reacts with H_2O_2 to generate the extraordinarily reactive hydroxyl radical ($^{\cdot}OH$). The hydroxyl radical reacts with the first molecule it encounters to abstract a hydrogen; this forms water and a new radical from the attacked molecule. The hydroxyl radical cannot, itself, diffuse far from its point of production precisely because it is so reactive, therefore it may not directly damage any vital macromolecule, depending on where it is produced. However, its reaction initiates a series of secondary radicals which can diffuse throughout the cell and reach important structures.

Recently, Braughler *et al.* (1986) and Minotti & Aust (1987) have demonstrated that the extent to which lipid peroxidation occurs in vitro is particularly dependent on the ratio Fe^{2+}:Fe^{3+}, as well as on the concentration of H_2O_2. Almost certainly, under oxidizing conditions in vivo hydroxyl radicals are produced.

Under normal conditions the availability of Fe for participation in redox cycling, in vivo, is limited by an elaborate system of Fe-binding proteins (Aisen & Listowsky, 1980; Weinberg, 1984; Crichton & Charloteaux-Wauters, 1987). Non-protein-bound Fe only

exists in vivo in conditions of Fe overload, when Fe-binding systems are overwhelmed (Jacobs, 1977; Hershko & Peto, 1987) or when the Fe-binding proteins themselves are not synthesized in adequate amounts. Under these conditions Fe causes a greatly increased rate of production of free radicals (Gutteridge *et al.* 1983; Halliwell & Gutteridge 1985*a*, *b*). The biochemistry of the Fe storage proteins, which bind and control Fe, has been comprehensively reviewed (Jacobs & Worwood, 1975; Aisen & Listowsky, 1980; Crichton & Charloteaux-Wauters, 1987; Theil, 1987).

IRON AND INFECTION

Both an excess and a deficiency of Fe may lead to an increased susceptibility to infection, each by different mechanisms and with different organisms.

Micro-organisms themselves have stringent Fe requirements for their own growth and metabolism (Weinberg, 1984; Barclay, 1985; Bullen & Griffiths, 1987). Indeed, the particular organisms which infect normal man are pathogenic by virtue of possessing proteins which bind Fe sufficiently strongly to obtain Fe from the host's own binding proteins. Part of the host's response to infection is to desaturate the circulating binding proteins and to sequester the Fe in the liver, in an attempt to deprive the invader. When organisms are encountered which can strip Fe from the host's proteins, host Fe deficiency or sequestration does not provide an adequate defence.

In vitro, the bactericidal capacity of neutrophils from Fe-deficient animals and humans is usually found to be impaired (Braggs & Miller, 1973; Chandra & Saraya, 1975; Moore & Humbert, 1984), although this is not always so (Kulapongs *et al.* 1974). Walter *et al.* (1986) examined the neutrophils from Fe-deficient infants. They found that the bactericidal capacity of the cells was impaired, although opsonization was normal. The defect was corrected when the infants were given Fe. It appears that Fe deficiency may affect the respiratory burst and, therefore, the bactericidal capacity of neutrophils (Murakawa *et al.* 1987). In the neutrophil, killing of ingested organisms depends on the generation of superoxide, H_2O_2 and hypochlorous radical, all of which are Fe dependent. It is to be expected that a chronic deficiency of Fe would lead to a persistence of infection (Bullen, 1987).

Although Fe deficiency does not seem to affect antibody production (Brock & Mainou-Fowler, 1986), it almost certainly affects lymphocyte function. Reports of impaired cell-mediated immunity in Fe deficiency have been frequently made (see Strauss, 1978; Swarup-Mitra & Sinha, 1984; Brock & Mainou-Fowler, 1986; Bullen & Griffiths, 1987). In human studies there is circumstantial evidence to suggest that Fe deficiency may predispose to particular infections; however, problems with study designs make it difficult to draw definite conclusions (Keusch & Farthing, 1986). It is likely that there is an increased susceptibility to certain specific infections: those in which the organism is able to acquire adequate Fe for its own purposes, and against which the cell-mediated immune system and phagocytic killing are the important modes of defence. Unfortunately, the studies that have been done have not taken these basic biological variations into consideration in their design.

The other side of the coin is excess Fe. Those organisms which do not possess the ability to remove Fe from transferrin and will, therefore, not infect an Fe-deficient, or even an Fe-adequate host, will clearly become a potential threat to the host as his transferrin becomes more saturated with Fe.

Barry & Reeve (1977) found an increased incidence of Gram-negative (*Escherichia coli*) sepsis in Polynesian neonates who were treated with intramuscular Fe dextran. More

recently, Oppenheimer *et al.* (1986*a*, *b*) have reported a carefully controlled clinical trial of the effects on morbidity of Fe supplementation in a chronically Fe-deficient population. Because of the degree of Fe deficiency they hypothesized that Fe would have a beneficial effect on infection morbidity; on the contrary they found that the children who were given Fe had an increased morbidity; in particular, they had a higher prevalence of malaria and respiratory infection with undefined organisms. Murray *et al.* (1975, 1978, 1980) have provided evidence from studies in East Africa that there is an increased risk of malaria and tuberculosis when Fe supplements are given to humans. These reports have been supported by animal studies (Keusch & Farthing, 1986; Bullen & Griffiths, 1987). Thus, several studies provide strong evidence that Fe deficiency can protect against particular infections and Fe supplementation may increase the risk of these infections.

Much of the confusion and argument that surrounds the relationship between Fe status and infection stems from a failure to appreciate the heterogeneity of infections, the different strategies adopted by organisms, the relative importance of the various host defences, the different ways in which the host is compromised, and the consequences of changes in Fe metabolism on both host and parasite. It is obviously not good to be Fe deficient, no more than it is good to have glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49) deficiency. However, depending on the prevalent infecting organisms in a community, it may indeed be advantageous to be deficient in Fe, just as it confers an advantage to have glucose-6-phosphate dehydrogenase deficiency in a malarious district. Studies done in the relatively aseptic industrialized world may reach opposite results from those done in disadvantaged societies. Indeed, the proper and expedient advice, in terms of whether to treat Fe deficiency, may be (correctly) opposite depending on the community under consideration.

The corollary of this is that the Fe status of the community may be a determinant of the types of infection that are most prevalent. This sort of mechanism may underlie the observation that malnourished children, although almost invariably infected, have a different spectrum of infecting organisms from well nourished children. For example, malnourished children rarely seem to develop meningitis (Christie *et al.* 1985). Various points of view have been argued by different authors (Stockman, 1981; Weinberg, 1984; Barclay, 1985; Brock & Mainou-Fowler, 1986; Dallman, 1986; Keusch & Farthing, 1986; Griffiths & Bullen, 1987). Clearly, whether or not to advocate Fe supplementation in a community must depend on accurate appraisal of the local ecology and the weighing of many factors apart from the average Fe status of the population.

In malnourished populations, particularly where kwashiorkor is the predominant form of malnutrition, it may be disadvantageous to give Fe prophylaxis (McFarlane *et al.* 1970; Golden & Ramdath, 1987). Malnourished children have reduced levels of transferrin, and an increased transferrin saturation (see below). Fe supplements may overwhelm the compromised Fe-binding proteins and provide Fe for free radical reactions as well as for bacteria (Weinberg, 1984; Bullen & Griffiths, 1987). Severely malnourished children often have septicaemia and infection of the hollow viscera with multiple organisms; it is in these same children that transferrin is saturated. We have, for example, observed group-D streptococcus septicaemia (ten cases) only in children with fully saturated transferrin (Golden & Heikens, unpublished results).

IRON AND KWASHIORKOR

Kwashiorkor is a form of severe malnutrition characterized by generalized oedema and fatty infiltration of the liver; the skin is often hypopigmented and may desquamate; hair discoloration and mental changes are also characteristic of kwashiorkor (Williams, 1935).

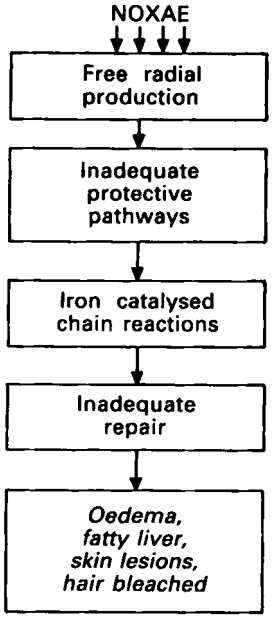

Fig. 1. Proposed mechanism leading to the clinical signs of kwashiorkor (from Golden, 1985).

A detailed description of this syndrome, along with a summary of the observations that are not explained by the classical aetiological hypotheses, has been presented by Golden (1985) and Golden & Ramdath (1987).

Golden (1985) proposed that the clinical symptoms of kwashiorkor resulted from an imbalance between the generation of free radicals in vivo and their subsequent safe disposal. The various steps in this hypothesis are outlined in Fig. 1. Central to this hypothesis is the availability of Fe in vivo for promoting free radical production. This is not because Fe excess is an essential part of the hypothesis, for it is not; however, the degree of stress and the profundity of the deficiencies can be very much less, to produce the same damage, in the presence of Fe. This means that any child in the community with excess Fe will be particularly vulnerable. We have found that the defences against free radicals are indeed compromised in children with kwashiorkor (Golden & Ramdath, 1987). These findings are summarized in Fig. 2. The question that now arises is whether Fe is usually available in vivo to catalyse the excess formation of free radicals in these children.

PLASMA IRON

Table 1 shows the reported Fe levels in serum or plasma from children with oedematous malnutrition. The values from Jamaica are somewhat higher than those reported by other workers studying malnourished children (Kondi *et al.* 1963; Adams *et al.* 1967; Lynch *et al.* 1970; Caballero *et al.* 1985). It is clear, however, that plasma Fe concentration, *per se*, does not differ much from normal anywhere in the world that it has been studied.

TRANSFERRIN

Transferrin, the major Fe-binding protein in plasma, has a very high affinity for Fe. It is normally present in excess so that about 70% of the transferrin is *not* associated with Fe (Aisen & Listowsky, 1980; Crichton & Charloteaux-Wauters, 1987; Griffiths & Bullen,

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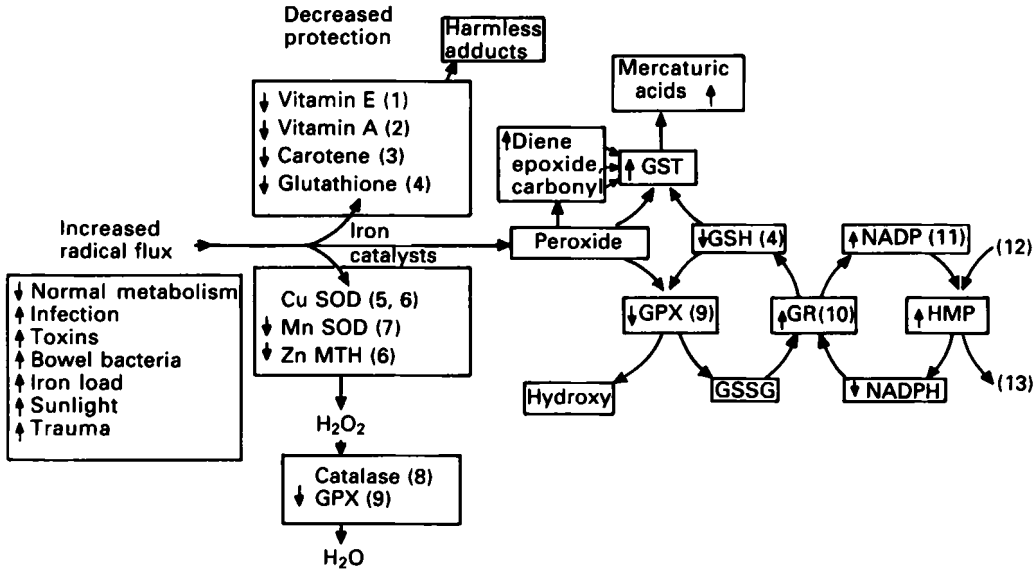

Fig. 2. diagram showing the mechanisms of radical production and subsequent metabolism. ↑ or ↓ beside the substrates, products and enzymes show whether they have been demonstrated to be increased or decreased respectively in children with kwashiorkor. The essential nutrients involved are: (1) vitamin E, (2) vitamin A, (3) carotene, (4) sulphur amino acids, (5) copper, (6) zinc, (7) manganese, (8) iron, (9) selenium, (10) riboflavin, (11) nicotinic acid, (12) magnesium and phosphorus, (13) thiamin. Cu SOD, Cu–Zn superoxide dismutase (*EC* 1.15.1.1); Mn SOD, manganese superoxide dismutase; Zn MTH, Zn metallothionein; GST, glutathione S-transferase (*EC* 2.5.1.18); GPX, glutathione peroxidase (*EC* 1.11.1.9); GSH, glutathione (reduced); GSSG, glutathione (oxidised); GR, glutathione reductase (NAD(P)H) (*EC* 1.6.4.2); HMP, hexose-monophosphate shunt (glucose-6-phosphate dehydrogenase) (*EC* 1.1.1.49), 6-phosphogluconic acid dehydrogenase (*EC* 1.1.1.43, 1.1.1.44); CAT, catalase (*EC* 1.11.1.6).

Table 1. *Serum and plasma iron levels reported for malnourished children*

Reference	Fe (μg/l) Mean	Range	Diagnosis	Country
Kondi *et al.* (1963)	470	130–1120	Oedematous	Kenya
Adams & Scragg (1965)	540	70–1020	Oedematous	S. Africa
Sandstead *et al.* (1965)	490	120–910†	Oedematous	Egypt
Adams *et al.* (1967)*	510	120–1520	Oedematous	S. Africa
Lynch *et al.* (1970)	550	170–930	Oedematous	S. Africa
Caballero *et al.* (1985)	630	260–990†	Oedematous	Guatemala
Ramdath & Golden (unpublished results)*	760	150–1160	Kwashiorkor	Jamaica
	700	180–1530	Marasmic kwashiorkor	
	650	140–1580	Marasmic	
Paediatric reference values from *Geigy Scientific Tables* (Lentner, 1984)	590	350–830	7–12 months	
	750	310–1190	1st year	

* Plasma values.
† Range derived from mean and 2 SD.

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Table 2. *Iron-binding capacity of malnourished children*

(Means and standard deviations with study groups shown in parentheses)

	Fe-binding capacity (μg/l)				
	Malnourished		Comparison		
Reference	Mean	SD	Mean	SD	Country
Edozien & Udeozo (1960)	119 (Oedematous)	23	180 (Controls)	47	Nigeria
Mönckeberg *et al.* (1962)	148 (Grade 3 malnutrition)	35	260 (Controls)	37	Chile
Kondi *et al.* (1963)	120 (M, MK, K)		288 (Recovered)		Kenya
Adams & Scragg (1965)	95 (K)	43	348 (Recovered)	79	S. Africa
Sandstead *et al.* (1965)	91 (K)	52	407 (Recovered)	74	Egypt
Lynch *et al.* (1970)	154 (K)	97	358 (Recovered)	144	S. Africa
Ramdath & Golden (unpublished results)	124 (K)	123	355 (Controls)	58	Jamaica
	161 (MK)	114			
	250 (M)	175			

M, marasmus; MK, marasmic kwashiorkor; K, kwashiorkor.

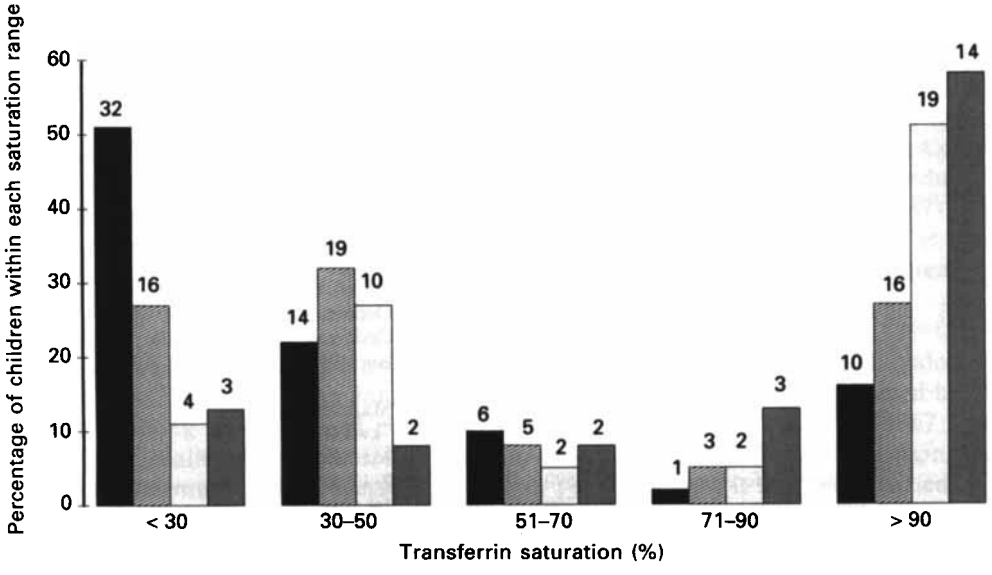

Fig. 3. Percentage of malnourished Jamaican children falling within various ranges of percentage transferrin saturation. Values shown indicate number of children in each group. Normal saturation of transferrin is approximately 30%. (■), Marasmus; ▨, marasmic-kwashiorkor; (▩), kwashiorkor; (▧), dead.

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1987). The reserve Fe-binding capacity of transferrin is, therefore, substantial. It serves two purposes: first, it binds any Fe which enters the plasma and prevents it from participating in redox cycling reactions; second, it deprives bacteria of Fe and is, therefore, bacteriostatic. Transferrin levels may be assessed either directly by immunological methods or, more commonly, indirectly by measuring the Fe-binding capacity of plasma. This is done by mixing an excess of inorganic Fe with a known volume of plasma, followed by removal of the unbound Fe. The Fe bound to plasma is then quantified and expressed as the total Fe-binding capacity.

Antia *et al.* (1968) and McFarlane *et al.* (1970) found low levels of transferrin in Nigerian children with kwashiorkor. Those who died had a much lower concentration (330 mg/l) than those who survived (1300 mg/l). Similarly low levels of transferrin were reported from Uganda by Masawe & Rwabwogo-Atenyi (1973). Mean plasma transferrin levels for Jamaican children with malnutrition (marasmus 1700 mg/l, marasmic kwashiorkor 1100 mg/l, kwashiorkor 840 mg/l, children who died 760 mg/l) are similar to those reported from elsewhere in the developing world. It is clear that transferrin levels are abnormally decreased in malnourished children, especially in those with oedematous malnutrition; furthermore the transferrin level seems to indicate the prognosis of the individual child.

Clearly, if the plasma Fe levels are normal and the transferrin is low then the transferrin saturation will be high and the capacity to bind more Fe will be reduced: this is what is found. Table 2 shows a summary of the reported Fe-binding capacity of malnourished children. In African children with kwashiorkor, the transferrin saturation ranged from 8% to 100% (Kondi *et al.* 1963; Adams *et al.* 1967). In the study by Adams *et al.* (1967) one-third of the children had a transferrin saturation of greater than 70%. Lynch *et al.* (1970) reported transferrin saturation ranging from 5% to 72% and Caballero *et al.* (1985), in Guatemala, reported a mean transferrin saturation of 57%. The proportion of Jamaican children with kwashiorkor, and other forms of malnutrition, that fall into different ranges of transferrin saturation is shown in Fig. 3. It is apparent that transferrin saturation is higher in oedematous malnutrition and is related to mortality.

McFarlane *et al.* (1970) suggested that septicaemia due to Fe becoming available for bacteria was the cause of death in these children. To this possibility must be added the toxic, peroxidative effects of the Fe itself, the pro-oxidant stresses of bacterial toxins and the radical species elaborated by leucocytes in an attempt to fight the infection. We have recently examined the plasma of malnourished children directly for Fe which is available for redox cycling using the bleomycin assay of Halliwell & Gutteridge (1985*a*, *b*). All but one of the children that died had free Fe present in their plasma at the time of admission (Ramdath & Golden, unpublished results).

FERRITIN

Although the measurement of plasma ferritin is said to reflect Fe stores accurately (Lipschitz *et al.* 1974; Jacobs & Worwood, 1975; Milman *et al.* 1983), this is only true for otherwise healthy individuals. Plasma ferritin values are known to be increased in infection, malignancy and other forms of inflammation. Indeed, ferritin values seem to be particularly high in patients with malaria! Obviously ferritin values in kwashiorkor, with the ubiquitous infection (Rowland *et al.* 1977; Christie *et al.* 1985), and frequent liver dysfunction (Alleyne *et al.* 1977), must be cautiously interpreted.

Nevertheless, ferritin values do seem to be increased in kwashiorkor. Fig. 4 shows admission ferritin levels in children consecutively admitted for severe malnutrition and in healthy children. Compared to the normal paediatric range (Cook *et al.* 1976) the majority of healthy Jamaican children appear to be Fe deficient. In contrast, in the malnourished

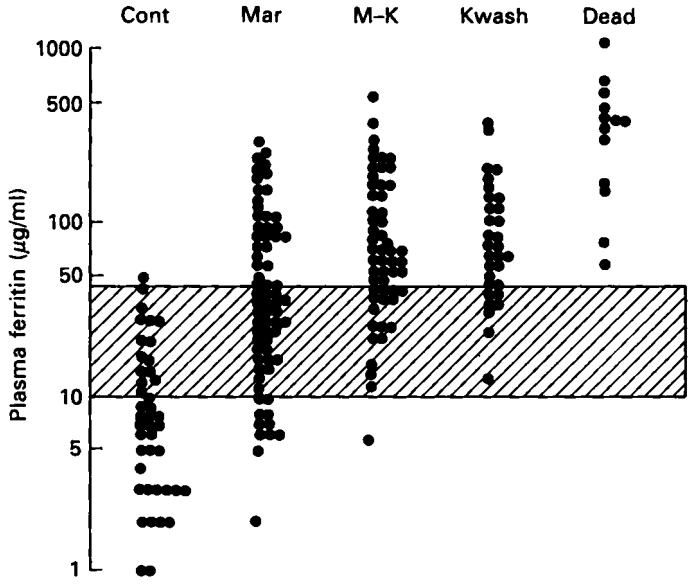

Fig. 4. Plasma ferritin values in control children (Cont), malnourished children with marasmus (Mar), marasmic kwashiorkor (M-K), kwashiorkor (Kwash) and those who died. Each point represents a separate child. Of the children who died four were Kwash, and nine M-K. No child with Marasmus died. (▩), Paediatric control range of Cook *et al.* (1976). (From Golden *et al.* 1985*a*.)

groups 70% had plasma ferritin values above the normal paediatric range. Even the children with marasmus rarely had low ferritin values: 43% had values higher than normal. In the oedematous groups all but one patient had either normal or elevated plasma ferritin. More than 70% of oedematous children had values above the normal range. The relationship between mortality and plasma ferritin values is shown in Fig. 5. Of children with plasma ferritin values greater than 250 μg/l 64% died, as opposed to a mortality of 3% in those who had lower ferritin values. Plasma ferritin levels have also been found to be increased in India (Srikantia, 1958), Guatemala (Caballero *et al.* 1983, 1985) and Senegal (Guiro *et al.* 1987), indicating that this is a general and not a local phenomenon.

The relationships between high ferritin levels, infections and abnormal liver functions have been examined in malnourished children by Golden *et al.* (1985*a*). Plasma ferritin levels were measured on admission and again after the resolution of infections and hepatic abnormalities: the ferritin remained high. Even at discharge about half the children had values above the normal range. This supports the view that the high ferritin values, in general, reflect high body Fe stores and are not simply a response to infection.

Worwood *et al.* (1979) demonstrated that the majority of ferritin in normal serum is glycosylated. This probably represents secreted ferritin. Ferritin that has leaked from damaged cells is not glycosylated. We have found that on admission the percentage of ferritin that is glycosylated is between 60 and 80 (Hudson-Thomas *et al.* 1988); this is a normal value. It is, thus, unlikely that the high ferritin is due to tissue necrosis.

IRON STORES OF MALNOURISHED CHILDREN

Direct measurements of trace elements in tissues of children who have died from kwashiorkor show that of all the minerals only Fe and sodium are present at higher than normal concentrations (Waterlow, 1948; Miles *et al.* 1988). The mean level of hepatic Fe

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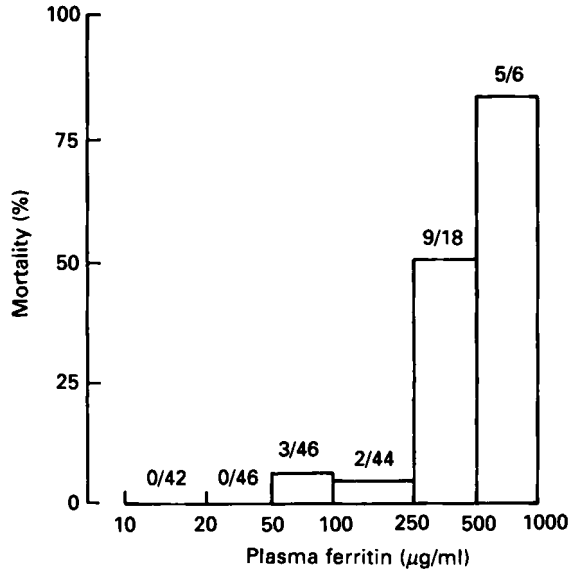

Fig. 5. Relationship between plasma ferritin levels (μg/l) and mortality in Jamaican malnourished children. Values about the columns indicate the no. of children who died relative to the total no. of children within each plasma ferritin range.

was approximately three times higher than in Jamaican controls (Fig. 6). Liver Fe has been found to be increased in India, Africa, Lebanon, as well as Jamaica (Table 3). In contrast, malnourished children have decreased levels of liver zinc, Cu and manganese (Warren *et al.* 1969), and muscle Zn, magnesium (Cheek *et al.* 1970) and potassium levels (Nichols *et al.* 1969).

Because children with kwashiorkor are anaemic several workers have examined bone marrow for the presence of stainable Fe. The marrow Fe of these children was almost always either normal or abundant. Harju *et al.* (1984) have estimated that the minimum serum ferritin level required before stainable Fe is found in bone marrow is between 20 and 25 μg/l. These findings, therefore, support the proposition that there is a high storage Fe in kwashiorkor (also see Table 3).

STUDIES WITH DESFERRIOXAMINE (DFO)

Although the high transferrin saturation, high levels of ferritin, stainable Fe in the marrow and excess liver Fe in the dead children are indicative of Fe overload in kwashiorkor, we need independent confirmation by a quantitative method which is not subject to the errors of ferritin or the bias of post-mortem material. What was the Fe status of the children that did not die?

The Fe-chelating drug DFO is a siderophore produced by *Streptomyces pilosus* and is at present the drug of choice for treating Fe overload (Cohen, 1987). Administration of the drug to patients with various forms of Fe overload results in the urinary excretion of Fe. Under normal circumstances the urinary Fe excretion following DFO administration is negligible (Dagg *et al.* 1966); it can, therefore, be regarded as an independent measure of the amount of Fe in the body that can be easily mobilized. In other words it is a measure of both the Fe burden and of the amount of Fe which is easily mobilized and, hence, potentially available for redox cycling.

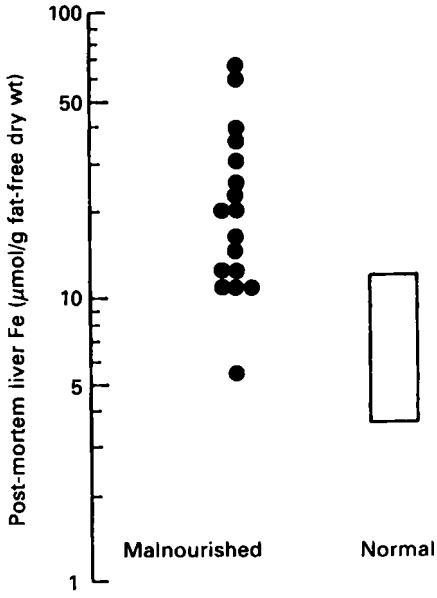

Fig. 6. Iron content of post-mortem livers (μmol/g fat-free dry liver weight) from Jamaican malnourished and normal children (Waterlow, 1948).

Table 3. *Studies of iron stores in kwashiorkor*

Reference	Country	Sample	Finding
Waterlow (1948)	Jamaica	Liver	Either increased or normal levels of Fe
Gillman & Gillman (1951)	W. Africa	Liver	
Mukherjee & Sarkar (1958)	India	Liver	
McLaren *et al.* (1968)	Lebanon	Liver	
Miles *et al.* (1988)	Jamaica	Liver	
Waterlow (1948)	Jamaica	Marrow	Stainable Fe found in variable proportions of the subjects studied
Kondi *et al.* (1963)	Kenya	Marrow	
Adams & Scragg (1965)	S. Africa	Marrow	
Sandstead *et al.* (1965)	Egypt	Marrow	
Adams *et al.* (1967)	S. Africa	Marrow	
Lynch *et al.* (1970)	S. Africa	Marrow	

Table 4 shows the 24 h urinary Fe following administration of 500 mg DFO intramuscularly and the corresponding plasma ferritin value. In the groups diagnosed as having kwashiorkor or marasmic kwashiorkor the mean urinary Fe outputs were 987 and 708 μg Fe/24 h respectively. These values were much higher than the corresponding mean for children with marasmus: 367 μg Fe/24 h. The question of normal values arises as these have not been determined for the paediatric age range. There is a close relationship between plasma ferritin and urinary Fe (Hudson-Thomas *et al.* 1988). The urinary Fe excretion which corresponds to the normal paediatric range of ferritin is 30–400 μg/24 h. In adults, with their much larger Fe pool, the excretion of 1000 μg after DFO is regarded as diagnostic of Fe overload. These results confirm that children with oedematous malnutrition are, as a group, suffering from Fe overload. Obviously, they should not be

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Table 4. *Urinary iron excretion following desferrioxamine**
(Mean values with their standard errors, ranges in parenthesis)

Type of Malnutrition	Urinary Fe (μg/24 h)			Plasma ferritin (μg/l)		
	mean	SE	Range	Mean	SE	Range
Marasmus ($n = 8$)	367	90	(103–836)	82	40	(12–355)
Marasmic kwashiorkor ($n = 11$)	708	224	(70–2465)	300	128	(13–1250)
Kwashiorkor ($n = 10$)	987	219	(582–2862)	329	222	(12–2320)

* Desferrioxamine (500 mg) given intramuscularly.
† Plasma ferritin assayed using an enzyme immunoassay (Spectro Ferritin method; Ramco Laboratories, Texas, USA).

given therapeutic doses of Fe; indeed, one may argue that these children should be treated initially with DFO until infections are treated and the Fe-binding proteins are resynthesized.

We have had the opportunity to examine one child's liver with the electron microscope. Ferritin molecules were abundant in the cytoplasm of the hepatocytes and not in the Kuppfer cells (M. H. N. Golden & S. Brooks, unpublished results). This is in distinction to thalassaemia and other forms of overload where the Fe is in the Kuppfer cells.

EFFECTS OF IRON OVERLOAD

Elevated Fe stores increase the possible occurrence of free Fe in vivo (Jacobs 1977). This is associated with lipid peroxidation, the production of cytotoxic compounds and subsequent membrane damage (see Esterbauer, 1982, 1985; Halliwell & Gutteridge, 1985*a*, *b*; Slater *et al.* 1987). It is precisely with this sort of damage, where the function of the protein synthetic machinery, the packaging and export of lipoproteins, and the transfer of acyl groups into the mitochondrion are globally impaired, that one would expect to find grossly diminished hepatic export protein levels and fatty accumulation in the liver: these are the hallmarks of kwashiorkor.

In animals the peroxidation of hepatic mitochondria has been extensively studied in Fe overload (Vladimirov *et al.* 1980). It is now well established that peroxidation of the rat mitochondrial membrane is enhanced as hepatic Fe increases (Robotham & Lietman, 1980; Bacon *et al.* 1985; Cheeseman *et al.* 1985; Masini *et al.*, 1985; Trenti *et al.* 1986). Robotham *et al.* (1974) have suggested that mitochondrial accumulation of Fe results in uncoupling of oxidative phosphorylation or inhibition of electron transport. This has also been reported in liver biopsies from malnourished children (Waterlow, 1961). In support, Bacon *et al.* (1985) found that oxygen consumption, state 3 respiration and overall respiratory control ratio were all decreased in the mitochondria of rats fed on high levels of Fe. Massive Fe overload in humans is associated with overwhelming liver damage, cardiac disease, diabetes mellitus, hormonal changes, skin changes and increased risk of infection (Halliwell & Gutteridge 1985*b*; Gordeuk *et al.* 1987; Cohen, 1987).

THE ENIGMA OF IRON OVERLOAD IN KWASHIORKOR

The problem that we are now left with is: why do some children in many impoverished Third World countries get Fe overload, when much of the developed world and most

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children in the developing world have a problem of Fe deficiency? Where does the Fe come from? How do these children acquire sufficient Fe to cause Fe overload? We do not know the answers to these questions. Clearly there are intrinsic differences between individuals, and in any community it is rarely more than a few percent of the children that succumb to kwashiorkor.

The work of Lynch *et al.* (1970) has shown that Fe absorption is not abnormal in children with kwashiorkor. A similar conclusion was drawn by Caballero *et al.* (1985). We could speculate that it is precisely the degree of bacterial contamination of the immediate environment and food preparation practices that lead to the difference. Malnutrition is closely associated with poverty. The diets are very heavily contaminated with faecal bacteria: the poorest families prepare feeds infrequently and retain unconsumed food for later use. The absorption of Fe from these real feeds with their heavy bacterial contamination has not been measured. The bacteria growing in the paps and gruels secrete siderophores in order to obtain Fe for themselves. The effect of these siderophores on Fe homeostasis in man is unexplored. DFO clearly alters Fe metabolism in a profound way; however, if this particular siderophore is given orally it simply leads to increased absorption and then urinary excretion of Fe without any effect on Fe burden. Members of the other four classes of siderophore may behave quite differently. We have preliminary findings, from an indirect assay, showing increased siderophore levels in feeds given to malnourished children. Whether feed siderophore content is adequate to explain the Fe overload of malnourished children, or to explain why these particular children are different from the majority who have Fe deficiency, remains to be investigated.

EFFECTS OF IRON DEFICIENCY

It has been assumed that with Fe deficiency haemoglobin is the only vulnerable protein, and that all the other quantitatively minor Fe proteins are conserved at the expense of haemoglobin. This has lead to the widely held view that anaemia is the only clinical effect that needs to be taken into account. Carefully conducted animal studies suggest that this contention is not true.

Finch *et al.* (1976) assessed work performance of Fe-deficient and control rats using the time taken to reach exhaustion on a treadmill. As expected rats fed on an Fe-deficient diet for 4 weeks showed a moderate fall in haemoglobin levels (from 76 to 55 g/l). Dramatically, there was a decrease in running time from 19 min to 3 min. When the rats were transfused with blood to raise the haemoglobin values to normal it made no difference, the running time remained at about 2 min. However, after the rats were given one injection of Fe dextran their running times returned to more than 20 min. Clearly, the effect of Fe deficiency on the performance of these rats was not due to changes in the blood. In the muscle, there were decreased concentrations of myoglobin and of the cytochromes, with functional impairment of the mitochondria (Finch *et al.* 1976).

This work has been extended and repeatedly confirmed. Davies *et al.* (1984) showed that severe Fe deficiency brought about an impairment of mitochondrial function in muscle, which closely correlated with the impaired capacity for extended periods of exercise. Reduction of oxidative phosphorylation in muscle mitochondria from Fe-deficient rats has also been reported by Mackler *et al.* (1984) and Hagler *et al.* (1981). It appears that changes in the capacity for oxidative phosphorylation, in the Fe-deficient rat, occur mainly in the skeletal muscle (Salmon, 1962; Dallman & Schwartz, 1965; McKay *et al.* 1983), and relate directly to the severity of the Fe deficiency (Koziol *et al.* 1978). Dallman (1986) examined the oxidative enzymes from different tissues of Fe-deficient rats; the effects of Fe deficiency were most severe in the mitochondria of skeletal muscle and intestinal mucosa; effects in

the liver were moderate, whereas the heart was only mildly affected. Mackler *et al.* (1978) detected no alteration in mitochondrial function, monoamine oxidase or catalase activity in the brains of Fe-deficient rats.

The defects in Fe deficiency include an impaired ability of animals to maintain body temperature when challenged with a cold stress (Beard *et al.* 1984; Mayfield *et al.* 1987), an effect which may be mediated through a diminished production of thyroid-stimulating hormone (Beard *et al.* 1984).

Animal studies have separated the effects of anaemia *per se* from low tissue levels of Fe. The relevant question is how do these changes apply to Fe deficiency in human populations? Detailed reviews have been prepared by Viteri & Torun (1974), Scrimshaw (1984), and Cook & Lynch (1986). Studies in humans as well as in rats demonstrate that Fe deficiency is accompanied by an increased cardiac output, a higher extraction rate of O_2 from arterial blood and an impairment in aerobic and physical work capacity (Davies *et al.* 1973; Viteri & Torun, 1974; Gardner *et al.* 1975; Edgerton *et al.* 1977; Gardner *et al.* 1977). Gardner *et al.* (1977) carefully related the effect of variable haemoglobin levels to work capacity and heart rate. Subjects were grouped according to haemoglobin levels from 60 to 130 g/l, in increments of 10 g/l. Each 10 g/l haemoglobin decrement was associated with an increase in heart rate of 4·7%; from 60 to 90 g/l each 10 g led to an increase in work capacity of about 1·8 min. Above 90 g/l there was no increment in work capacity.

Patients with severe anaemia undoubtedly have an impairment of physical capacity. Many intervention programs (see: Basta *et al.* 1979; Edgerton *et al.* 1979; Scrimshaw, 1984) have demonstrated that functional capacity and productivity are increased with Fe supplementation. These studies show the practical utility of treating an Fe-deficient population: they do not tell us about the nature of the defects. Often the subjects have no dietary differences in Fe, but may have different intakes of, for example, ascorbate (Basta *et al.* 1979) and different burdens of parasites. These are not unimportant points: it does not seem appropriate to treat intestinal parasites or low ascorbate intakes with Fe supplements.

The effects of Fe deficiency in humans on thermoregulation, energy expenditure and intestinal function, independently of anaemia, have not been adequately addressed. The patient who tells the doctor that he is tired and demands an Fe tonic may be absolutely right, and the doctor who measures that patient's haemoglobin and 'reassures' his patient that everything is all right and no treatment is required is totally wrong: the patient is more likely to be right that something is wrong; whether or not it is commonly Fe deficiency only careful investigation can tell us.

IRON AND MENTAL FUNCTION

Fe undoubtedly has an important role in the normal development and function of the brain (Mackler & Finch, 1982; Sourkes, 1982; Evans, 1985; Sandstead, 1986). Studies in animals (Mackler *et al.* 1978; Weinberg, 1982) have suggested that Fe deficiency may impair normal mental development. This relationship has been explored quite extensively in human studies from Chile (Walter *et al.* 1983), Costa Rica (Lozoff *et al.* 1987), Guatemala (Lozoff *et al.* 1982*a*, *b*; Pollitt *et al.* 1986), Indonesia (Soemantri *et al.* 1985), India (Agarwal *et al.* 1987), Israel (Palti *et al.* 1985) and in the USA (Webb & Oski, 1973; Oski & Honig, 1978; Deinard *et al.* 1981, 1986; Oski *et al.* 1983; Pollitt *et al.* 1983). The results of these studies have been reviewed by Pollitt & Leibel (1982), Evans (1985), Pollitt *et al.* (1986) and Cook & Lynch (1986).

Despite the numerous studies, the relationship between mental performance and Fe status is still not clear. This may be attributed, partly, to variations in study designs.

Unfortunately, studies which were thought to have had an adequate design have also produced equivocal results. A universal finding, however, is that the ability of anaemic children to concentrate (attention span) is diminished.

The relationship between anaemia and mental development is confounded by the time frame required to obtain a measurable response, as well as the criteria for defining anaemia. For example Deinard *et al.* (1981) found no difference in overall mental performance of 11–13-month-old children who had varying levels of stored Fe. This group used ferritin and packed cell volume to define their groups. Studies by Oski & Honig (1978) and Oski *et al.* (1983) have shown that a single intramuscular dose of Fe resulted in a rapid (7 d) improvement of mental performance. On the other hand Agarwal *et al.* (1987) showed that whereas nutritional status affected IQ scores, haemoglobin levels had no effect. However, performance scores for digit span, coding and mazes of children with haemoglobin levels of greater than 120 g/l were significantly higher than those of anaemic children (haemoglobin < 100 g/l).

In order to be of functional relevance, the relationship between Fe status and mental development must be clearly defined, using appropriate measures of Fe status. Lozoff *et al.* (1987) showed that children with haemoglobin levels of less than 100 g/l had mental development index (MDI) scores that were significantly lower than those of children with haemoglobin levels between 101 and 105 g/l. Psychomotor development index (PDI) scores, however, were only slightly lower in the former group. In this study MDI and PDI were repeated after 1 week and 3 months of Fe supplementation. At 1 week there was mean increase in haemoglobin of 10 g/l; however, neither MDI nor PDI changed significantly with Fe supplementation (Lozoff *et al.* 1987). This is in contrast to the results of Oski *et al.* (1983). The question as to whether changes in MDI and PDI are related to haemoglobin or Fe stores remains unanswered.

Lozoff *et al.* (1987) found that 3 months of Fe supplementation increased haemoglobin levels by 37 g/l; this is good evidence to indicate Fe deficiency. However, the response to Fe supplements was not universal and it may be that other nutrients were limiting. MDI and PDI scores remained low in children who failed to respond to Fe supplementation. This suggests that other factors, such as those involved in Fe uptake and utilization, may also influence these performance tasks.

Studies of Fe deficiency and mental impairment are often done on impoverished populations, where malnutrition and psychosocial deprivation frequently occur (Birch & Richardson, 1972). Pollitt & Leibel (1976) showed that children from lower socio-economic strata were more likely to have haemoglobin levels of less than 100 g/l. In poor communities intakes of all nutrients may be suboptimal; however, we are able to identify deficiencies of Fe because of fairly well established clinical and biochemical indices. Agarwal *et al.* (1987) found that, of all variables examined, nutritional status *per se* had the greatest influence on the outcome of mental performance.

Quite clearly the possibility of trace element deficiencies occurring in a malnourished population is high (Golden *et al.* 1985*b*); however, there is limited knowledge of deficiencies of trace elements (Hambidge, 1985).

Fe may, therefore, be only one of the several nutrients limiting performance. Experimental Zn deficiency in animals, for example, is associated with gross abnormalities in brain structure and alteration in neurotransmitter levels (Sandstead, 1986). In particular, levels of noradrenaline are increased (Fe-deficient children have increased urinary excretion of noradrenaline; Voorhess *et al.* 1975). In humans Zn deficiency has been implicated in neurophysiological impairment (for a review, see Sandstead 1986). Zn is only one example, however.

It is tempting to speculate that changes in mental performance could occur when a

malnourished child is supplemented with Zn, or indeed any other essential nutrient. It appears that Fe may play a role in certain mental tasks; however, it is absolutely essential that this relationship be elucidated by carefully conducted, well designed studies that address the mechanisms involved and assess the effects of all the confounding variables.

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