Reduced bactericidal capacity of polymorphs in iron deficiency*

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Chandra, R. K. (1973). *Archives of Disease in Childhood*, **48**, 864. **Reduced bactericidal capacity of polymorphs in iron deficiency.** Intracellular bacterial killing by polymorphonuclear leucocytes and reduction of nitro-blue tetrazolium were defective in 12 children with iron deficiency anaemia. Opsonic activity of plasma and phagocytosis were normal. The abnormalities were corrected quickly by parenteral administration of iron.

The relation between infection and malnutrition is well established on the basis of clinical and epidemiological observations. Recent studies (Smythe *et al.*, 1971; Chandra, 1972) indicate that in protein-calorie malnutrition several immune functions are adversely affected, including intracellular bacterial killing by polymorphonuclear leucocytes (PMNs) (Seth and Chandra, 1972; Selvaraj and Bhat, 1972). Interpretation of studies on malnourished human subjects is difficult because there are usually multiple nutritional deficiencies, some clinically manifest, others covert but nevertheless significant. Occasionally isolated deficiencies occur which permit the study of the effect of the deficiency of a single nutrient on each immune function. We have studied bacterial phagocytosis and metabolic activity of PMNs and the opsonic function of plasma of children with iron deficiency anaemia.

Subjects and methods

The diagnosis of iron deficiency anaemia was established in 12 children, 1 to 8 years old, on the basis of a haemoglobin level less than 10 g/100 ml, microcytosis, and hypochromia of peripheral red blood cells, serum iron <50 µg/100 ml, and reduced bone marrow iron. On the serum iron levels, the patients were classified arbitrarily as moderately (21–49 µg/100 ml) and severely (≤20 µg/100 ml) iron deficient. Serum albumin was >3.2 g/100 ml in each case, and anthropometric measurements were above 80% of the 50th centile for age on Harvard growth charts. In the 7 cases studied serum vitamin B₁₂ and folate levels were within the normal range.

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The presenting symptoms included progressive pallor in 12 patients, breathlessness in 5, and abdominal pain in 3. The duration of symptoms varied from 1 to 18 months. Dietary intake of iron was low in all. 5 children showed hookworm ova in the stool. At the time of testing none had any significant infection, though 4 had a history of frequent bacterial infections.

Age-sex matched healthy children attending the Well Child Clinic who were bled routinely for periodic haemogram and serum protein estimations were used as controls.

The bactericidal test used was essentially that described by Quie *et al.* (1967). We used surface counting of *Staphylococcus aureus* colonies on nutrient agar plates. The opsonic activity of plasma was estimated by the number of bacteria phagocytosed by PMNs of healthy controls at 20 minutes of culture in the presence of 10% plasma from the patient or the control. The bactericidal capacity of PMNs was calculated by the ratio, number of viable intracellular bacteria at 140 minutes/number at 20 minutes of culture. The quantitative nitro-blue tetrazolium test was performed by the method of Baehner and Nathan (1968). Samples from patients and controls were run in parallel.

All patients were admitted to hospital and received the usual hospital diet. No antibiotic was administered to any child. The tests were repeated 4 to 7 days after total-dose parenteral administration of iron.

Statistical analysis was done by the Student's 't' test.

Results

Phagocytosis by PMNs and opsonic activity of plasma were comparable ($P > 0.1$) in patients with iron deficiency anaemia and healthy controls (Table I). However, intracellular bacterial killing by PMNs and oxidative reduction of nitro-blue tetrazolium were both significantly ($P < 0.001$) less in the iron-deficient children than in the healthy

TABLE I
Opsonic activity of plasma and phagocytosis by polymorphonuclear leucocytes (PMNs)

Group	No. tested	Plasma opsonic activity (bacteria/10 ⁶ normal PMNs)		PMN phagocytosis (bacteria/10 ⁶ PMNs in normal plasma)	
		Range	Geometric mean	Range	Geometric mean
Healthy controls	10	7.3 × 10 ⁴ to 1.4 × 10 ⁶	9.2 × 10 ⁴	7.3 × 10 ⁴ to 1.4 × 10 ⁵	9.2 × 10 ⁴
Iron-deficient cases	12	4.2 × 10 ⁴ to 1.1 × 10 ⁵	7.8 × 10 ⁴	5.6 × 10 ⁴ to 9.8 × 10 ⁴	6.9 × 10 ⁴

children (Table II). The abnormality in the nitro-blue tetrazolium test was more pronounced in the group with severe iron deficiency and there was a significant positive correlation with serum iron concentration. The bactericidal defect was slightly, but not significantly, greater ($0.05 < P < 0.1$) in the

group of children with moderate iron deficiency, and there was no obvious correlation with serum iron level.

Therapy with iron corrected the abnormalities in both the bactericidal and the nitro-blue tetrazolium tests in each patient (Table II).

TABLE II
Bactericidal capacity of polymorphonuclear leucocytes and quantitative nitro-blue tetrazolium test in children with iron deficiency anaemia and in healthy controls

Age (yr) and sex	Serum iron ($\mu\text{g}/100\text{ ml}$)	Bactericidal capacity (no. of viable intracellular bacteria at 140 min/no. at 20 min of culture)		Quantitative nitro-blue tetrazolium test (Δ optical density)	
		At admission	After therapy	At admission	After therapy
<i>Healthy control</i>					
7 M	110	0.09	—	0.32	—
6½ F	95	0.05	—	0.23	—
8 M	90	0.11	—	0.20	—
1 M	80	0.03	—	0.14	—
5 F	80	0.11	—	0.19	—
4½ M	65	0.09	—	0.32	—
6 M	—	0.05	—	0.31	—
7½ F	—	0.01	—	0.34	—
4 M	—	0.17	—	0.37	—
3 M	—	0.21	—	0.39	—
Mean		0.097		0.292	
<i>Moderate iron deficiency</i>					
5 F	40	0.49	0.23	0.06	0.23
8 M	35	0.66	0.21	0.06	0.31
4 M	35	0.39	0.09	0.19	0.26
6½ F	30	0.21	0.04	0.13	0.32
7 M	24	0.73	0.11	0.11	0.29
1 M*	24	0.81	0.33	0.03	0.18
Mean		0.550	0.168	0.097	0.265
<i>Severe iron deficiency</i>					
7½ F*	20	0.37	0.13	0.03	0.14
4½ M*	15	0.62	0.17	0.02	0.30
8 M	12	0.47	0.19	0.06	0.21
6 M	12	0.31	0.07	0.01	0.23
5 F*	4	0.39	0.11	0.02	0.21
3 M	4	0.43	0.20	0.01	0.19
Mean		0.432	0.145	0.025	0.213

*Hookworm ova present in stools.

Discussion

Iron and iron-binding proteins have a complex role in host immunity. Iron deficiency may have a direct effect on the development of lymphoid tissue (Schmidt, 1928). Patients with kwashiorkor are prone to serious infections, and there is a correlation between low serum siderophilin and death (Antia, McFarlane, and Soothill, 1968). Human milk exerts a powerful bacteriostatic effect against *Esch. coli*; this is mediated in part through iron binding protein lactoferrin and possibly transferrin as well, acting in concert with specific *Esch. coli* antibody (Bullen, Rogers, and Leigh, 1972). Wells *et al.* (1972) and Higgs and Wells (1972) reported iron deficiency in patients with chronic mucocutaneous candidiasis, most of whom improved when treated with iron. They suggested that chronic lack of tissue iron may result in defective epithelial formation, with secondary defects in the immune response.

We are aware of no previous study of polymorph function in iron deficiency. Our study has shown that bacterial killing by PMNs of subjects with iron deficiency was significantly impaired. The oxidative reduction of nitro-blue tetrazolium was also defective, and was lower in the severely iron-deficient children than in the moderately deficient ones. In contrast, there was no obvious relation between the level of serum iron and the bacterial killing in the iron-deficient children, and the mean value for the severely deficient children was higher than that for the moderately deficient group, though not significantly so. There are at least two possible explanations for this surprising finding. Perhaps the defect of the critical iron-dependent function involved in bacterial killing is already maximal in the moderately iron-deficient group, or perhaps iron concentration within the polymorph is insufficient for optimal bacterial growth.

The mechanism through which iron deficiency causes dysfunction of PMNs is not certain. Lack of iron diminishes the activity of several enzymes that either contain iron or are dependent on it. These include myeloperoxidase (Higashi *et al.*, 1967), which contributes significantly to the metabolic processes activated inside PMNs for bacterial destruction (Klebanoff, 1970).

The patients were given parenteral iron in a dose adequate to correct the deficiency and to build up body stores. The abnormalities in bacterial killing and the nitro-blue tetrazolium test were corrected within 4 to 7 days, when the tests were repeated, before any appreciable change in haemoglobin concentration would have occurred; this points to a

causal role for iron deficiency itself rather than anaemia. It is likely that these children had multiple dietary deficiencies, but no supplements other than iron were given, and it is unlikely that hospital diet for a few days only would have produced any other significant alteration in the environment to have contributed to the reversal of PMN dysfunction. Normal levels of serum albumin and anthropometric measurements exclude protein-calorie malnutrition as a cause of leucocyte abnormalities.

It is possible that dysfunction of PMNs due to iron deficiency may be one of the pathogenetic mechanisms of susceptibility of malnourished children to infections.

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