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Studies in iron supplementation of preschool children

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1. The effect of daily supplements of 20–30 mg inorganic iron as ferrous sulphate on the growth, activity and haematological status of preschool children was studied for 3·5, 7 and 12 months and compared to that of children who served as controls. All children were given their daily requirements of energy and protein. In addition, they received 5 μg cyanocobalamin and 200 μg folic acid.

2. Fe supplementation increased the haemoglobin, serum Fe and percentage saturation of transferrin and reduced the unsaturated Fe-binding capacity significantly compared to corresponding values for the controls.

3. Height and activity were unaffected by Fe supplements.

4. Of the children 45% had haemoglobin values below 110 g/l at the end of 7–12 months of Fe supplementation.

Iron-deficiency anaemia is the commonest nutritional deficiency disease and is widely prevalent among south Indian children (Rao, Tasker & Ramanathan, 1954; Rao, Rao & Baker, 1959; Rao, Swaminathan, Swarup & Patwardhan, 1959; Swaminathan, Apte & Rao, 1960; Nutritional Research Laboratories, 1967; Ratnaswamy, Webb & Pereira, 1967). Therapeutic supplementation with Fe would presumably increase the haemoglobin levels in these anaemic subjects. There are reports from other countries that even small doses of Fe are of benefit to Fe-deficient children (Bradfield, Jensen, Gonsales & Garrayar, 1968; Bradfield, Jensen, Quiroz, Gonsales, Garrayar & Hernandez, 1968). The present studies were undertaken as a preliminary trial to assess the effects of fairly small Fe supplements on the growth, activity and haematological status of preschool children.

MATERIALS AND METHODS

The subjects were apparently-normal children, 2–5 years of age, resident in an orphanage. Informed consent was obtained from the children's parents or legal guardians for their participation in the trials. On admission to the orphanage, each child was medically examined and a skiagram of the chest was obtained. Children with tuberculosis and other chronic diseases were not admitted. None of the children had hookworm infestation.

The diet in the orphanage was based on cereals (rice and wheat). Vegetables, pulses and condiments were also eaten and crude cane sugar (jaggery) was used for sweetening. Foods of animal origin were excluded. Four meals were served through the day. The diet provided 2 g vegetable protein and 334–376 J energy/kg body-weight, thus satisfying the energy and protein requirements of this age-group (WHO, 1973). The diet was found by analysis to provide 12·4–16 mg Fe/child per d.

First study

Blood samples were taken without venous occlusion at the start of the study for estimation of haemoglobin, packed cell volume, serum Fe, unsaturated Fe-binding capacity, serum vitamin B_{12} and serum folic acid. Haemoglobin was estimated by the cyanmethaemoglobin method, using a photoelectric colorimeter checked periodically against an international reference standard (International Committee for Standardisation in Haematology). Values

for packed cell volume were determined using a standardized microhaematocrit centrifuge. Serum Fe was estimated by the method recommended by the International Committee for Standardisation in Haematology (1971). The unsaturated Fe-binding capacity was determined by the method of Herbert, Gottlieb, Lau, Fisher, Gevirtz & Wasserman (1966) and the serum vitamin B_{12} assayed using *Euglena gracilis* Z strain (Hutner, Bach & Ross, 1956). Serum folic acid was determined by the technique of Waters & Mollin (1961) using *Lactobacillus casei* as the test organism.

The estimations were repeated 3·5 and 7 months later.

The children were paired on the basis of their heights and serum Fe concentrations. One child of each pair was assigned to the experimental group and the other child to the control group.

Supplements were given daily. All children received 5 μg cyanocobalamin and 200 μg folic acid. In addition the children in the experimental group were given 20 mg inorganic Fe as ferrous sulphate between meals.

The heights of the children were measured at monthly intervals and were the average of three readings on each occasion. Weights were taken every 2 weeks on three consecutive days and the mean value recorded. The illnesses that occurred among the children were recorded every day by trained resident staff.

The activity of each pair of children was assessed at the beginning, at 3·5 months and at 7 months, during the morning and afternoon free-play sessions by the same two observers recording their activity (lying, sitting, standing, walking and running) at 5 min intervals throughout the total play period of 7 h.

Second study

Children were assigned, on the same basis as the first study, to the experimental and control groups. The children in the experimental group were given daily supplements of 30 mg inorganic Fe as ferrous sulphate with 5 μg cyanocobalamin and 200 μg folic acid, while the children in the control group were given only the cyanocobalamin and folic acid supplements. Heights, weights and illnesses were recorded in the same way as in the first study. Blood samples were taken for haematological estimations at the beginning and end of the study which lasted for 12 months. Ten pairs of children completed 12 months in the trial.

RESULTS

First study

Twenty-seven children were supplemented with 20 mg Fe for 3·5 months. The values for heights, weights and haematological values at the beginning and end of the study together with those for the twenty-seven children who served as controls are shown in Table 1.

For statistical analysis, each child served as his own control. The difference between the initial and final measurements for each individual was determined, the mean difference and standard deviation of the mean for each group was determined and compared by Student's *t* test. The mean difference between the initial and final values for haemoglobin and packed cell volume was significantly greater in the Fe-supplemented than in the control group ($P < 0{\cdot}005$ and $P < 0{\cdot}025$ respectively). Both groups of children showed an increase in values for serum folic acid and vitamin B_{12} after supplementation with these vitamins.

The activity of the children in both the groups was assessed at the beginning and after 3·5 months of supplementation. The difference between the two groups of children was not significant.

At 3·5 months after the start of the study, some children left the orphanage to enter schools elsewhere. Twelve pairs of children continued in the study for a total period of 7 months.

Table 1. *The heights, weights and haematological values of apparently-normal preschool south Indian children given iron supplements (20 mg inorganic Fe as ferrous sulphate/d) and of the control group at the beginning and end of the first study**

(Mean values and one standard deviation for twenty-seven children/group)

	Height (m)		Wt (kg)		Haemo-globin (g/l)		Packed cell volume		Serum Fe (μg/l)		Unsaturated Fe-binding capacity (μg/l)		Percentage saturation of transferrin		Serum folic acid (ng/ml)		Serum vitamin B_{12} (pg/ml)	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fe-supplemented:																		
Initial	0·922	0·066	12·65	2·58	99	17	0·351	0·038	565	230	2899	779	18·2	9·0	13·7	3·8	170·5	78·7
Final	0·937	0·068	13·05	1·67	116	7	0·374	0·018	833	330	2458	548	26·0	11·1	29·2	7·9	559·4	100·1
Control:																		
Initial	0·928	0·067	12·72	1·98	103	11	0·358	0·028	592	248	2744	701	20·4	15·1	16·7	5·8	210·9	102·6
Final	0·942	0·069	12·86	2·05	109	13	0·361	0·028	755	305	2645	609	22·6	10·0	29·9	11·1	494·1	109·1

* For details, see p. 494.

Table 2. *The haematological values and increases in height and weight of apparently-normal preschool south Indian children given 20 or 30 mg Fe as ferrous sulphate/d at the beginning and the end of the two studies**

(Mean values and one standard deviation for twenty-two children/group)

	Haemoglobin (g/l)		Packed cell volume		Serum Fe (μg/l)		Unsaturated Fe-binding capacity (μg/l)		Percentage saturation of transferrin		Increase in: Height (m/month)		Increase in: Wt (kg/month)	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fe-supplemented:														
Initial	98·0	13·0	0·352	0·033	471	181	3122	611	13·8	7·0	5·2	1·6	0·090	0·078
Final	109·0	6·0	0·366	0·022	880	290	2326	638	28·3	11·3				
Control:														
Initial	101·0	10·0	0·359	0·028	493	184	3093	626	14·3	6·2	4·6	1·8	0·077	0·074
Final	103·0	13·0	0·354	0·036	596	281	3247	903	17·0	13·4				

* For details, see p. 494.

Table 3. *Episodes of illness among the apparently-normal preschool south Indian children in the iron-supplemented and control groups**

Group	Respiratory infections	Fever	Diarrhoea, dysentery	Skin infections	Scabies	Otitis	Gingivitis	Con-junctivitis	Measles	Chicken-pox	Hepatitis	Nephritis
Fe-supplemented	73	24	34	25	11	13	14	48	6	6	—	—
Control	82	22	24	21	3	16	14	27	3	7	1	2

* For details, see p. 494.

The activity of the twelve pairs of children was reassessed at the end of the trial period. The difference in activity of the children in the two groups was not significant.

Combined evaluation

Since the numbers of children who took part in the first and second trials for 7 and 12 months respectively were small, the results for the two groups of Fe-supplemented children were combined and compared with those of the two groups not supplemented with Fe (Table 2). The values were analysed as for the first study. The differences between the means of the individual differences between the initial and final values for haemoglobin, serum Fe, percentage saturation of transferrin and unsaturated Fe-binding capacity were significant ($P < 0.025$, $P < 0.001$, $P < 0.001$ and $P < 0.01$ respectively). The differences in values for height and packed cell volume were not significant. The initial (± 1 SD) serum folic acid was 15.1 ± 4.1 ng/ml for the Fe-supplemented group and 17.5 ± 5.7 ng/ml for the control group. The final values for the children in both groups who were supplemented with Fe for 12 months were > 50 ng/ml and the range of values after 7 months of Fe supplementation was 11·5–> 50 ng/ml for the Fe-supplemented group and 16·0–> 50 ng/ml for the control group. The initial serum vitamin B_{12} was 190.8 ± 96.9 pg/ml for the Fe-supplemented group and increased to 433.6 ± 144.4 pg/ml. The corresponding initial and final values for the control children were 223.5 ± 11.7 pg/ml and 415.2 ± 366.6 pg/ml respectively.

The record of illness among the Fe-supplemented and control children is shown in Table 3. The pattern of illness was similar in both groups. The greater number of respiratory episodes than expected in both the Fe-supplemented and control children was due to the inclusion of two children with 'wheezy' bronchitis in the Fe-supplemented group who accounted for twenty-three episodes between them and three children similarly affected in the control group (twenty-nine episodes). Three children, two in the Fe-supplemented and one in the control group, with chronic suppurative otitis media also increased the numbers of this disease. The child in the control group had six episodes and the two children in the Fe-supplemented group had eight episodes between them. Twenty-four children in the Fe-supplemented group had conjunctivitis and four of them had recurrent attacks, bringing the number of episodes to forty-eight. In the control group, twenty-three children were affected, but only one of them was subjected to recurrent disease.

DISCUSSION

The Fe status of children who received supplements of 20–30 mg Fe was improved, compared with that of the children who served as controls. The Fe-supplemented children showed a significant increase in haemoglobin, serum Fe and percentage saturation of transferrin and a decrease in their unsaturated Fe-binding capacity.

It was surprising that, despite supplementation for 7–12 months with Fe, folic acid and cyanocobalamin, which brought these nutrients in the blood to adequate levels, the mean final haemoglobin concentration was 109 g/l and 45 % of the children had a haemoglobin concentration of < 110 g/l and were therefore still anaemic by the criteria of the WHO (1972). Since the haemoglobin measurements were periodically checked against an international haemoglobin standard the results cannot be attributed to laboratory error. It should be emphasized that none of the children had intestinal parasites. There are several possible explanations for the failure of the haemoglobin to increase further. Many of the children had several illnesses, albeit minor ones, during the months of supplementation. It is possible that these illnesses affected haemopoiesis. However, the number of illnesses experienced by each child did not show any correlation with the final haemoglobin concentration or with final serum Fe. T. J. John (personal communication) has found that in

apparently-healthy preschool children in this area, viral isolates can be obtained from 58 % of randomly-collected stool specimens. Such inapparent infections may also depress haemopoiesis.

The children were all receiving a diet which excluded animal proteins. Although their growth was satisfactory, it is theoretically possible that the absence of animal protein adversely affected haemopoiesis. The necessity for animal as distinct from vegetable protein for haemopoiesis has not however been demonstrated.

If these explanations are not applicable, the results of this study must call into question the validity of the criteria of WHO (1972) for the haemoglobin concentration below which anaemia is likely to be present in this age-group. It is not stated on what values the WHO (1972) recommendations are based. It has been suggested that there may be racial differences in normal haemoglobin concentrations (Owen, Lubin & Garry, 1973; Garu, Smith & Clark, 1974, 1975) which account for the findings in this study. It should be emphasized that the final test for the presence of nutritional anaemia is the demonstration in otherwise healthy individuals of an increase in haemoglobin concentration after the administration of haemopoietic nutrients (Garby, Irnell & Werner, 1969). By this criterion at the end of the trial the children could not be considered to be anaemic.

Mackay (1931) found more episodes of respiratory infection in children with Fe deficiency than in Fe-supplemented children. In the present study, the number of episodes of respiratory illness in the Fe-supplemented and control groups of children was similar. There were more episodes of conjunctivitis in the Fe-supplemented group in the second study. There was an epidemic of conjunctivitis during the period of the study, and although the number of affected children was similar in both groups, more of the affected Fe-supplemented children had recurrent attacks. It is not known whether this was related to the Fe administration but Masawe, Muindi & Swai (1974) had suggested that Fe supplementation might increase susceptibility to some infections.

The daily supplement of 20–30 mg Fe given in this study probably represented a greater Fe intake than could readily be achieved by food fortification. Supplemental inorganic Fe (15 mg as ferrous sulphate) given to parasitized schoolchildren in the Amazon for 10 weeks was effective in increasing values for haemoglobin and packed cell volume in anaemic children whose initial haemoglobin concentration was < 100 g/l (Bradfield, Jensen, Quiroz *et al.* 1968). In another study, even supplements of 5 mg inorganic Fe resulted in an improvement in values for haemoglobin and for packed cell volume and serum Fe, when given to schoolchildren for 10 weeks (Bradfield, Jensen, Gonsales *et al.* 1968). It is important to determine whether the smaller amounts of Fe such as used by Bradfield and his colleagues (Bradfield, Jensen, Gonsales *et al.* 1968) and which could be achieved by food fortification, would produce adequate results in Indian children. Because of the high content of phytate and other inhibitors of Fe absorption in south Indian diets, smaller doses than those used in this study might be ineffective.

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