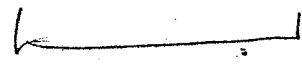


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Iron Deficiency Anemia: Adverse Effects on Infant Psychomotor Development

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ABSTRACT. In a double-blind, placebo-control prospective cohort study of 196 infants from birth to 15 months of age, assessment was made at 12 months of age of the relationship between iron status and psychomotor development, the effect of a short-term (10-day) trial of oral iron vs placebo, and the effect of long-term (3 months) oral iron therapy. Development was assessed with the mental and psychomotor indices and the infant behavior record of the Bayley Scales of Infant Development in 39 anemic, 30 control, and 127 nonanemic iron-deficient children. Anemic infants had significantly lower Mental and Psychomotor Developmental Index scores than control infants or nonanemic iron-deficient infants (one-way analysis of variance, $P < .0001$). Control infants and nonanemic iron-deficient infants performed comparably. No difference was noted between the effect of oral administration of iron or placebo after 10 days or after 3 months of iron therapy. Among anemic infants a hemoglobin concentration < 10.5 g/dL and duration of anemia of > 3 months were correlated with significantly lower motor and mental scores ($P < .05$). Anemic infants failed specifically in language capabilities and body balance-coordination skills when compared with controls. These results, in a design in which intervening variables were closely controlled, suggest that when iron deficiency progresses to anemia, but not before, adverse influences in the performance of developmental tests appear and persist for at least 3 months despite correction of anemia with iron therapy. If these impairments prove to be long standing, prevention of iron deficiency anemia in early infancy becomes the only way to avoid them. *Pediatrics* 1989;84:7-17; iron deficiency, development, infant, anemia, nutrition.

Iron deficiency, the world's most prevalent nutritional disorder, can no longer be considered a simple anemia readily reversed by iron therapy.¹ Numerous systemic effects have been described, among which behavioral derangements may be the most worrisome.² Their occurrence during early infancy is a cause for further concern because of the coincident latter part of the growth spurt of the brain during this period as well as numerous biologic and behavioral changes. Moreover, it is unclear whether deficits arising during this stage can be completely reversed after nutritional rehabilitation.³

It has become evident in recent years that iron deficiency causes impairment in the way infants perform the Bayley Scales of Infant Development, the test used in most previous studies.⁴⁻¹⁰ Some researchers⁴⁻⁷ have shown that only anemic infants evidence deficits, but others⁸⁻¹⁰ have described alterations also in nonanemic iron-deficient infants. Thus, the precise level at which the effect becomes overt is still controversial. The reversibility of these changes after iron therapy has been discussed with contradictory results and remains uncertain.

It was shown in a recent review¹¹ that previous studies, although, in general, well designed and executed, have failed to provide conclusive solutions to the following questions: (1) the severity of iron deficit necessary to influence behavior, (2) the effect of the duration of iron deficiency, (3) the effect of a short-term iron trial (before correction of anemia), (4) the reversibility of changes after a long-term trial (sufficient to reverse anemia), (5) the specific areas of mental or motor processes most affected, and (6) the possible association of developmental deficits with behavioral patterns. To answer these questions, we describe a double-blind, placebo-control study in a cohort of 12-month-old infants from a homogeneous community who were carefully followed-up longitudinally since birth.

Answers to questions

has pose

ABBREVIATIONS. BSID, Bayley Scales of Infant Development; IBR, Infant Behavior Record; INTA, Instituto de Nutricion y Tecnologia de los Alimentos; MDI, Mental Developmental Index; PDI, Psychomotor Developmental Index.

Received for publication May 23, 1988; accepted Jul 19, 1988. Dedicated to the memory of Abraham Stekel, MD. Reprint requests to (T.W.) Instituto de Nutricion y Tecnologia de Los Alimentos, Casilla 15138, Santiago 11, Chile. PEDIATRICS (ISSN 0031 4005). Copyright © 1989 by the American Academy of Pediatrics.

Study Population and General Outline

This study was accomplished in concert with a field trial of iron-fortified foods conducted by the Hematology Unit of the Instituto de Nutricion y Tecnologia de los Alimentos (INTA) of the University of Chile from 1982 to 1985. The subjects were located in a geographically defined urban community of lower-middle socioeconomic level, homogeneous in ethnic background, with stable, definitive housing, running water, sewage, and electricity. It has been shown in previous studies that blood lead levels are low¹² and that anemia in Chilean infants is exclusively due to nutritional iron deficit.¹³ After informed consent was given by parents, infants were randomly allocated to four groups at 3 months of age. Those exclusively breast-fed were given a heme-iron fortified cereal or the solid food recommended by the health clinic. Those spontaneously weaned by 3 months of age were given either an iron-fortified formula or the routine nonfortified milk provided by the National Supplementary Food Program. All groups were advised to add fruit juices at 2 months of age, fruits at 3 months of age, a meat-vegetable soup and cereals at 4 months of age, eggs and legumes at 6 months of age, and table foods at 9 months of age. Details of this trial have been described elsewhere.^{13(pp42-46)} The infants were observed with monthly clinical and anthropometric evaluations by physicians from INTA at the Community Health Center and weekly at their homes by field nurses performing dietary and morbidity surveys. At 9 and 12 months of age, venipunctures were carried out for the measurements of hemoglobin, hematocrit, mean cell volume, serum iron, iron-binding capacity, free erythrocyte protoporphyrin, and serum ferritin.

Within a week after the 12-month visit and venipuncture, each infant was brought to INTA for psychologic testing with the Bayley Scales of Infant Development (BSID). Informed consent for this study was obtained and a double-blind control-placebo trial of oral iron treatment was conducted. Both mother and psychologist (C.G.P.) were unaware of the iron status or the preparation each infant received. After the first psychologic test, ferrous sulfate suspension (Fer-In-Sol, Mead Johnson, Evansville, IN) or placebo at a dose of 0.6 mL (15 mg of Fe²⁺) was given three times daily 1 hour before meals for the following 10 days. The field nurse visited the infant three or four times during this period, recording compliance by history corroborated by measuring the amount of remaining suspension. On day 11, a repeat BSID was performed. As confirmed by weekly house calls to measure

compliance, all infants, regardless of iron status, received the iron preparation thereafter at the same dose and schedule (as a therapeutic trial) until 15 months of age. At the end-of therapy, a clinic visit was made for evaluation, taking of hematologic measurements, and administration of a third BSID.

Hematologic Assessments

Samples were obtained by venipuncture at 9, 12, and 15 months after a 6-hour overnight fast. Hemoglobin, mean cell volume, and hematocrit were performed in a Coulter model ZBI (Coulter Electronics, Hialeah, FL), free erythrocyte protoporphyrin, in a hematofluorimeter (Aviv Biomedical Inc, Lakewood, NJ) serum iron and iron-binding capacity by a colorimetric method,¹⁴ and serum ferritin by radioimmunoassay (Gammadab, Division of Travenol, Cambridge, MA). After the venipuncture at 12 months of age, the infants were tentatively allocated to an iron status group by the hematologist (T.W.) on the basis of hemoglobin, mean cell volume, and free erythrocyte protoporphyrin values, results that were available within a day. To exclude all likelihood of mild iron deficiency, however, the infants were reclassified retrospectively after the therapeutic trial of iron. The trial was considered satisfactory if ≥ 1250 mg of iron was effectively taken. An increase in hemoglobin level of ≥ 1 g/dL was considered evidence of iron deficiency, superceding all biochemical measures. Thus, control infants were defined as those with all values in the normal range (hemoglobin ≥ 11.0 g/dL, mean cell volume ≥ 70 fL, Fe/iron-binding capacity $\geq 10\%$, free erythrocyte protoporphyrin < 100 μ g of zinc protoporphyrin per deciliter of RBC, serum ferritin ≥ 10 ng/mL) who had a lack of response to an adequate therapeutic trial of ferrous sulfate. The anemic infants were defined as those with hemoglobin levels < 11.0 g/dL and two or more abnormal biochemical measures. The non-anemic iron-deficient infants, that is, those with hemoglobin levels > 11.0 g/dL who were not eligible as control subjects, were a heterogeneous group that was subclassified in an attempt to grade the severity of iron deficit.

Subclassification of nonanemic iron-deficient infants in order of decreasing severity was as follows: grade 3 (nonanemic responder)—zero to four altered measures at 12 months of age with a therapeutic response of hemoglobin level ≥ 1 g/dL (considered the most severe grade); grade 2 (nonanemic-nonresponder)—one to four abnormal values but with a therapeutic response of hemoglobin level < 1.0 g/dL; grade 1 (iron depleted-nonanemic-nonresponder)—all measures normal except a serum ferritin level < 10 ng/mL.

Iron Status Classification
↓

Intervention
↓

Blinding

Dose

The venipuncture performed at 9 months of age provided the opportunity to evaluate the progression of iron status 3 months prior to the developmental tests. These values were classified as follows: anemic groups, hemoglobin <10.5 g/dL and two or more abnormal measurements, control group, hemoglobin ≥ 11.5 g/dL. Because we did not have a therapeutic trial to detect unequivocal controls, stricter hemoglobin levels were required as well as values of serum ferritin ≥ 12 ng/mL, free erythrocyte protoporphyrin <100 μ g/dL RBC, and mean cell volume ≥ 70 fL to reduce the likelihood of mild iron deficit.

Development Assessment

The BSID¹⁵ were used as a measure of each child's development. The inability of this test as well as others to predict later IQ at this age¹⁶ and the fact that its global nature precludes an indication of which aspect of behavior may be influenced by iron deficiency¹⁷ were not regarded as hindrances to its use. For evaluating infant behavior at 1 year of age, the BSID is considered the most reliable and comprehensive approach.¹⁸ A rapid progression of maturational events is occurring at this age, which fact is appropriately evaluated by this test. Furthermore, previous studies in this age group have been conducted using the BSID, which permits useful comparisons.

The BSID has three components, the Mental Scale, Motor Scale and the Infant Behavior record. The Mental Scale is designed to assess memory, learning, vocalization, and language communication; in general it determines the basis for abstract thinking and the conduct generally associated with "intelligence." The Motor Scale provides a measure for gross and fine coordination. These scales yield scores, the Mental Developmental Index (MDI) and the Psychomotor Developmental Index (PDI); respectively, that are age corrected so that the norm is 100 and the standard deviation 16, similar to that in most IQ tests. The third component, the Infant Behavior Record (IBR) consists of 30 items clinically rated by the tester and does not yield a score. The first BSID was administered 7 to 10 days after the 12-month venipuncture by the same psychologist (C.G.P.), who, again, was unaware of the infant's iron status or therapy group. The caretaker was always the mother. The raw scores obtained at the first and second BSID administrations were converted to indices based on the child's age at the first interview. Thus, the short-term iron therapy study was meant to compare the infant with himself as a control, not to establish the validity of the BSID for age. All infants were of similar age at the time of study; hence, individual items of mental

and motor skills as well as the IBR could be analyzed.

Intervening Variables

This large group of infants had been carefully characterized during the longitudinal follow-up study from birth. As a prerequisite to participate in the field study from which these infants were drawn, the following criteria had to be met: a birth weight >2500 g, no neonatal complications, no chronic or congenital disorders, and adequate growth and development. The infants were seen regularly by the same physician in the clinic and the same nurse at the weekly home visits. The following were regularly checked: complete monthly anthropometric measurements (height, weight, head and arm circumference, and skinfolds); dietary evaluation by 24-hour recall once a week; socioeconomic evaluation by the modified Graffar Scale¹⁹; breast-feeding duration and quality, and morbidity data, both at the clinic with consultation as desired and at home with a weekly questionnaire.

Data Analysis

Differences between the groups in terms of continuous variables were evaluated by ANOVA, and pairwise comparison was done by one-tailed Student's *t* test. Comparison of the same subjects in time was done with paired Student's *t* test. Differences in categorical variables were done by χ^2 test. All analyses were performed according to standard statistical methods²⁰ or with the SAS computer package.²¹

RESULTS

Hematologic Data

Complete hematologic evaluation was performed for 189 infants at 9 months of age, 196 infants at 12 months of age, and 196 infants at 15 months of age. Classification of the 39 anemic infants at 12 months of age was unequivocal based on hemoglobin level <11 g/dL (Table 1). The mean value was 10.0 g/dL (range 8.4 to 10.9), denoting that anemia was mild. Infants with hemoglobin levels >11.0 g/dL were tentatively preassigned depending on mean cell volume and free erythrocyte protoporphyrin values; however, definitive groups were demarcated retrospectively including Fe/iron-binding capacity and serum ferritin levels and response to iron. Only six infants who had all iron measures normal had a response to iron with hemoglobin levels >1 g/dL; thus, they were reclassified as nonanemic with iron deficiency. Consequently, the 30 infants definitively assigned to the control group complied with

the most stringent criteria available, ie, normal measures of iron status and lack of response to an adequate therapeutic trial. The lower range of hemoglobin values for control infants was 11.8 g/dL. The 127 remaining nonanemic iron-deficient infants, ie, those with hemoglobin levels ≥ 11.0 g/dL but with abnormal biochemical measures and/or a therapeutic response resulted in a heterogeneous group. The subclassification used to define varying degrees of severity of nonanemic iron deficiency was useful as judged by the gradual changes of the mean values (Table 1).

At 9 months of age, 21 infants had anemia (hemoglobin levels < 10.5 g/dL) and 34 infants were controls (hemoglobin levels ≥ 11.5 g/dL), serum ferritin levels ≥ 12 ng/mL, free erythrocyte protoporphyrin levels < 100 μ g of zinc protoporphyrin per deciliter of RBC, and mean cell volume ≥ 70 fL) and the remaining 134 were a heterogeneous group of nonanemic and iron-deficient infants.

Development Scores

A total of 576 BSID were administered. Using the age adjustment recommended in the manual, we found the mean MDI score was 102.4 ± 8.9 (means \pm SD) with a PDI score of 98.1 ± 11.0 . There was a significant difference between the means of mental and motor scores seen in this population as a whole studied at three age points. Both distributions were symmetric, with only six measurements (1.2%) < 2 SD in PDI and one infant > 2 SD in MDI.

Measures of Iron Status and Development

Anemic infants showed markedly lower MDI scores (mean \pm SEM) than control infants (96.4 ± 1.3 vs 102.1 ± 1.8 , $P < .001$) and nonanemic iron-deficient infants as a whole (103.4 ± 0.8 , $P < 10^{-5}$).

For PDI, anemic infants scored 90.0 ± 2.0 (mean \pm SEM) and differed from controls (101.2 ± 2.1 , $P < .0005$) and nonanemic iron-deficient infants (98.7 ± 1.0 , $P < .0001$). The one-way-ANOVA for MDI and PDI was highly significant ($P < 10^{-5}$). It must be noted, however, that all MDI and PDI scores are within 2 SD of the norm, so that even though anemic infants were at a disadvantage with respect to nonanemic infants, their scores were still within the normal range (Table 2). The subclassification of nonanemic iron-deficient infants demonstrated the pattern that only overt anemia and no other combination of biochemical markers of iron deficiency was the determinant for significant decreases in MDI and PDI scores (Fig 1).

Hemoglobin Concentration and Development Scores

We examined MDI and PDI scores at 12 months of age based only on hemoglobin level as the most commonly used indicator of iron status (Fig 2). A sigmoid distribution could be defined at hemoglobin level intervals of 0.5 g/dL, with leveling of developmental index values between the two groups of infants with hemoglobin levels < 10.5 g/dL, or among the three groups > 11.0 g/dL. An intermediate group was present at hemoglobin values of 10.5 to 10.9 g/dL.

Iron Status at Nine Months and Developmental Indices at 12 Months

To evaluate the effect of duration of anemia, infants who were anemic at both 9 and 12 months of age, ie, those whose anemia had a duration of 3 or more months ($n = 19$) were compared with those infants who were anemic at 12 but not at 9 months of age, ie, those whose anemia was presumed to have been present for less than 3 months ($n = 16$).

TABLE 1. Iron Status of Study Groups at 12 Months, Including Subclassification of Nonanemic Iron-Deficient

Iron Status Groups	Hemoglobin (g/dL)	Mean Cell Volume (fL)	Iron and Iron-Binding Capacity (%)	Free Erythrocyte Protoporphyrin (μ g Zinc Protoporphyrin/dL RBC)	Serum Ferritin (μ g/L)
Anemic (n = 39)	10.0 ± 0.9	62 ± 5	6.8 ± 2.9	195 ± 103	5.4 (3-9.8)
Nonanemic iron deficiency (n = 127)	12.1 ± 0.7	70 ± 4	12.2 ± 0.7	108 ± 33	11.9 (5.8-24)
Grade 1 (n = 6)	12.9 ± 0.6	74 ± 3	14.8 ± 3.6	81 ± 12	7.5 (6.7-8.2)
Grade 2 (n = 76)	12.2 ± 0.7	70 ± 4	12.3 ± 5.4	107 ± 30	15 (7.7-27)
Grade 3 (n = 45)	11.7 ± 0.5	69 ± 0.5	11.7 ± 6.8	115 ± 38	9 (4.1-19.6)
Control (n = 30)	12.7 ± 0.8	76 ± 3	16.7 ± 6.3	78 ± 13	19.8 (11.8-34)

* Results are given as means \pm SD, except serum ferritin results, which are given as geometric means with range of 1 SD.

† Grades are defined as follows: 1, iron depleted; 2, nonresponder; 3, responder.

TABLE 2. Developmental Index Scores of Infants According to Iron Status*

	Anemic	Nonanemic Iron Deficient	Control	P Value†
Initial values at 12 mo				
MDI	96.4 ± 1.3	103.4 ± 0.8	102.1 ± 1.8	10 ⁻⁸
PDI	90.0 ± 2.0	98.7 ± 1.0	101.2 ± 2.1	10 ⁻⁸
Effect of short-term trial‡				
Placebo				
MDI	6.7 ± 3.2	8.7 ± 3.2	8.3 ± 3.3	.26
PDI	5.1 ± 2.9	5.4 ± 3.5	4.4 ± 4.3	.73
Iron therapy				
MDI	8.6 ± 5.4	8.9 ± 3.4	8.7 ± 3.5	.96
PDI	6.7 ± 6.9	5.6 ± 2.9	5.6 ± 3.2	.66
Effect of long-term trial at 15 mo†				
MDI	-0.17 ± 5.1	0.26 ± 3.5	0.93 ± 3.7	.82
PDI	1.92 ± 6.2	0.16 ± 4.2	2.8 ± 6.9	.21

* Results for groups of infants are given as means ± SEM. Abbreviations: MDI, Mental Developmental Index; PDI, Psychomotor Developmental Index. The numbers of infants in the short-term trial group were as follows: anemic infants—placebo, 15; iron therapy, 24; nonanemic iron-deficient infants—placebo, 61; iron therapy, 66; control infants—placebo, 18; iron therapy, 12.

† According to one-way ANOVA. The analysis of variance for improvements in all iron status and iron-placebo groups is MDI ($F_{93, 190} = .46$) and PDI ($F_{5, 190} = .72$, $P = .61$). The highest, yet not significant, pair-wise comparison differences were for anemic infants + iron vs. anemic infants + placebo MDI ($P = .19$) and PDI ($P = .33$).

‡ Mean + SD increase in score from pretreatment development indices.

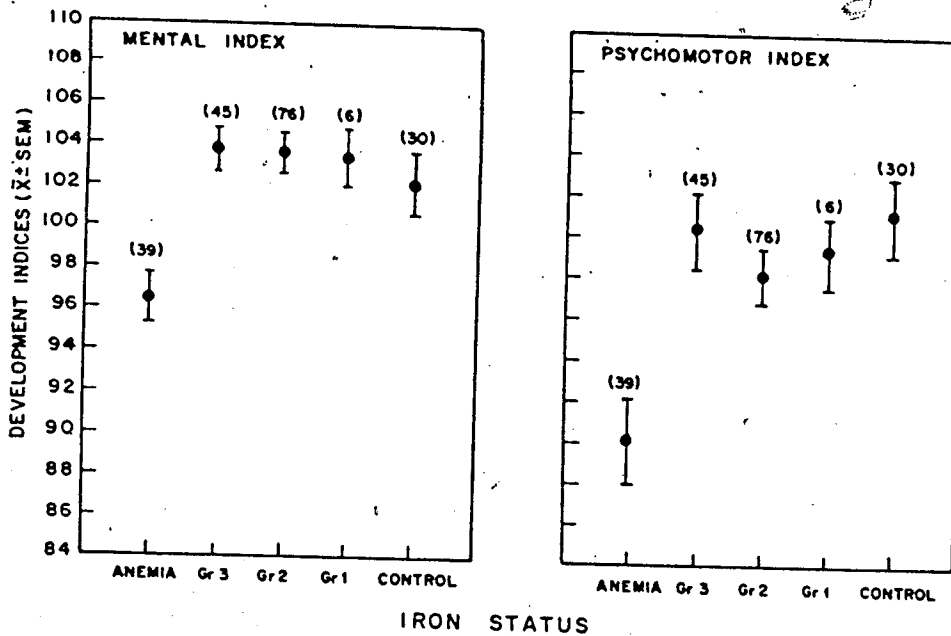


Fig 1. Pretreatment mental and psychomotor developmental indices (MDI and PDI) according to iron status at 12 months of age. Nonanemic iron-deficient infants are subclassified as follows: grade 1—nonanemic, iron depleted; grade 2—nonanemic nonresponder; grade 3—nonanemic responder. Values within parentheses indicate number of subjects per data point. The overall ANOVA for MDI ($F(4, 191) = 5.4$; $P < .002$) and PDI ($F(4, 191) = 5.4$; $P < .0001$). Pairwise comparisons of anemic infants vs every other group are significant ($P < .01$). Pairwise comparisons between all nonanemic infants are not significant. Only overt anemia determines significantly lower MDI and PDI scores.

Those infants who were anemic for more than 3 months had significantly lower developmental index scores than those infants who were anemic for less than 3 months (Table 3). Those infants who were nonanemic at 9 months of age were mainly those in the intermediate hemoglobin range of 10.5

to 10.9 g/dL at 12 months of age, at which time developmental index scores were less severely affected. Thus, infants who were not anemic at 9 months of age tended to have milder anemia at 12 months of age. Of the 17 infants in the intermediate hemoglobin group at 12 months of age, 14 were

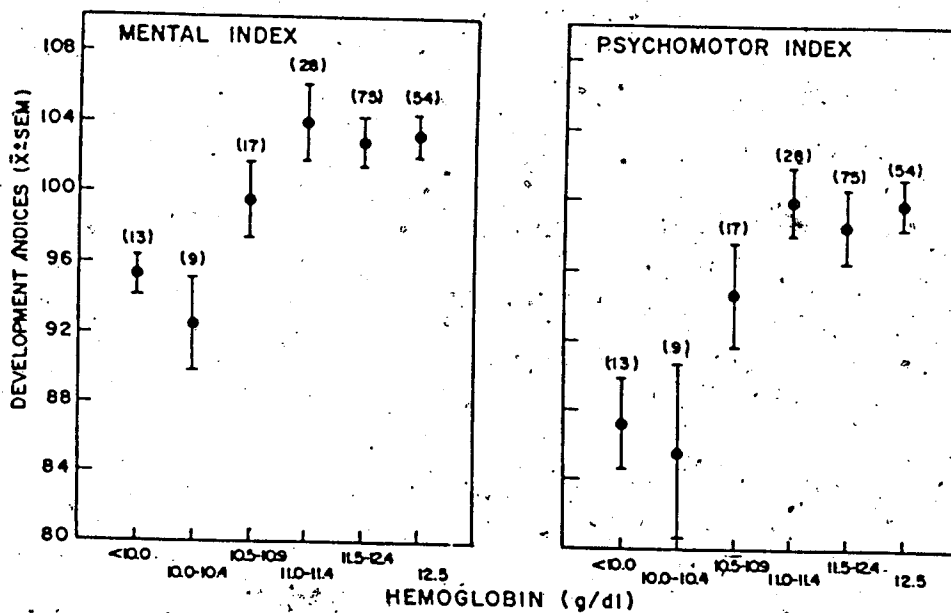


Fig 2. Pretreatment mental and psychomotor development indices (MDI and PDI) according to hemoglobin concentration in 0.5-g/dL intervals. Values within parentheses indicate number of subjects per data point. ANOVA for hemoglobin level < 10.5 g/dL (scores given as means \pm SEM; $n = 22$; MDI, 94 ± 1.3 ; PDI, 86 ± 2.5) vs hemoglobin level 10.5 -10.9 g/dL ($n = 17$; MDI, 99.5 ± 2.1 ; PDI, 94.6 ± 2.9) vs hemoglobin level >10.9 g/dL ($n = 157$, MDI, 103.2 ± 0.7 , PDI, 99.2 ± 2.9) is significant (MDI, $F(2, 193) = 6.90$; $P = 10^{-5}$; PDI, $F(2, 193) = 8.48$; $P = 10^{-5}$). For each point of MDI score, $F(5, 190) = 6.9$; $P < .001$; PDI, $F = 8.5$; $P < .0002$. Pairwise comparisons between each of three groups are significant ($P < .05$). Pairwise comparisons within groups with hemoglobin values <10.5 g/dL or ≥ 10.9 g/dL are not significant. For each point of MDI score, $F(5, 190) = 6.9$; $P < .001$; PDI, $F = 8.5$; $P < .0002$. The intermediate point (hemoglobin level 10.5 to 10.9 g/dL) is significantly different from both extremes ($P < .05$) in MDI and PDI scores.

TABLE 3. Duration of Anemia and Developmental Indices*

Developmental Index	Duration of Anemia	
	<3 Mo ($n = 16$)†	≥ 3 Mo ($n = 19$)‡
Mental	99.4 ± 2.5	94.1 ± 1.1
Psychomotor	93.7 ± 3.4	86.0 ± 2.6

* Results are given as means \pm SEM. $P < .05$ for <3-month group compared with ≥ 3 -month group for both indices.

† Infants with hemoglobin levels <11.0 g/dL at 12 months of age but ≥ 10.5 g/dL at 9 months of age.

‡ Infants with hemoglobin levels <11.0 g/dL at 12 months of age and <10.5 g/dL at 9 months of age.

evaluated at 9 months. Of these, 10 (71%) were not anemic at 9 months of age. In contrast, of the 21 infants who had hemoglobin values of <10.5 g/dL, only six (29%) were not anemic at 9 months of age ($P < .015$).

Iron Therapy

Infants given 10 days of oral ferrous sulfate were compared with those given a placebo. Developmental index scores of both groups significantly improved in the MDI and PDI to a similar extent,

regardless of prior iron status or therapy, with average improvements of 4.4 to 8.7 points (Table 2). After 3 months of therapy, even though anemia was reversed in all infants and hematologic measures of iron status were completely corrected in 11 of the formerly anemic infants, no significant improvement was detected between the scores at 12 and 15 months of age. The control and the non-anemic iron-deficient infants showed no significant change in their MDI or PDI scores. No significant changes from baseline BSID scores were seen either by paired Student's *t* test within groups (greatest $P = .18$ for controls) or by ANOVA of the differences of scores between groups (Table 2).

Specific Patterns of Failure

We evaluated the specific test items involved in the raw score that yield the developmental index when normalized for age. The MDI scores were encompassed representatively by items 99 to 117 (Table 4) and the corresponding PDI items were 40 to 52 (Table 5). If the Mental scale is reviewed, the items that required comprehension of language but did not involve a visual demonstration were passed significantly by fewer anemic infants than controls.

TABLE 4. Mental Development Index Results at 12 and 15 Months of Age

Item No. and Description	Infants With Passing Scores (%)		P Value*
	Anemic	Control	
99, pushes car along	56	77	NS
101, jabbbers expressively	92	93	NS
102, uncovers blue box	31	47	NS
103, turns pages of book	69	83	NS
104, pats whistle-doll, in imitation	44	63	NS
106, imitates words (mama, dada)			
at 12 mo	13	47	<.01
at 15 mo	75	100	<.07
108, places 1 peg repeatedly	28	27	NS
113, says 2 words (meaningfully)			
at 12 mo	0	7	NS
at 15 mo	42	93	<.005
117, shows own shoes, clothing, toy			
at 12 mo	0	18	NS
at 15 mo	25	60	<.07

* By χ^2 analysis of absolute numbers.

TABLE 5. Psychomotor Developmental Index Results at 12 and 15 Months of Age

Item No. and Description	Infants With Passing Scores (%)		P Value*
	Anemic	Control	
40, stepping movements	100	100	NS
41, pellet, fine prehension (neat pincer)	90	93	NS
42, walks with help	85	97	NS
43, sits down from standing	67	94	.01
44, pat-a-cake, midline skill	82	97	NS
45, stands alone	64	93	.02
46, walks alone	38	67	.05
47, stands up (from sitting)			
at 12 mo	3	7	NS
at 15 mo	42	80	.05
48, throws ball (forward fling)	59	70	NS
52, stands on left foot with help			
at 12 mo	0	10	NS
at 15 mo	8	40	.05

* By χ^2 analysis of absolute numbers.

On the BSID language development scores at 12 months of age, differences between anemic and control infants are most marked in item 106, "vocalization of bisyllabic words" ($P < .01$). The language item 113, "says two words (meaningfully)," was passed by too few infants at 12 months of age for significant results. However, at 15 months of age, the Mental scale appeared significant ($P = .005$) when item 117, "shows shoes or other clothing or own toy," also became suggestive ($P < .07$). Item 106 continued to show a tendency ($P < .07$), partly because most infants passed the item at 15 months of age.

In psychomotor milestones 40 to 52, those items relating to balance in the standing position and body control (sits from standing, stands alone, and walks alone) were done by significantly less anemic infants than controls. Differences for items 47, "stands up from sitting," and 52, "stands on left

foot with help," were not identified at 12 months of age but became significant at 15 months of age ($P < .05$).

Iron Status and IBR

Of the 24 items evaluated by the IBR at 12 months of age, nine were rated significantly ($P < .05$ or less by Fisher's exact test) better by controls compared with their anemic peers: responsiveness to examiner, responsiveness to mother, general emotional tone, goal directedness, attention span, activity, responsiveness to persons, vocalizations, and body motion. Further analysis of the IBR was performed associating behavior items related to "test affect" and "task orientation" as suggested by Matheny.²² The test affect combination rated significantly better in the control group vs the anemic group ($P < .04$). Abnormal "task orientation" in the anemic infants was markedly associated with

MDI scores less than the mean ($P < .01$); however, only a marginal tendency was seen for anemic infants with lower PDI scores ($P = .09$).

Intervening Variables

No significant differences were shown between the three iron status groups for mother's age, number of pregnancies, socioeconomic index, birth weight, weight, height, head circumference, or weight/height adequacy at 12 months of age (to the 50th percentile of National Center for Health Statistics tables) episodes of morbidity measured at home or clinic, and duration of exclusive or nonexclusive breast-feeding. Differences were found in sex, with preponderance of girls among the anemic infants. The food trial group also differed, showing, not surprisingly, that most of the anemic infants originated in the unfortified groups. Food trial assignment to the iron-fortified or noniron-fortified products was randomly determined and resulted as the strongest—and perhaps only—determinant of iron status of the infant. The educational level of the mother was significantly better in the anemic group. High school was completed by 38% of the mothers of the anemic group vs 29% of the mothers of the controls, and 21% of the mothers of the nonanemic iron-deficient group. Percentages of mothers who had completed primary grades were 50%, 42%, and 46%, respectively (overall $\chi^2 = .001$). The remainder of the mothers had educational levels that corresponded to incomplete primary education, but literacy was 100% because it was a requisite for acceptance into the food trial. In a multiple regression comparison using MDI (or PDI) as the dependent variable, forcing hemoglobin levels made all variables nonsignificant except height for age adequacy ($P = .014$). Hemoglobin level was significant at $P = .009$ and explained 4.3% of the variance.

DISCUSSION

Studies of cognitive development are often flawed by suboptimal control of intervening variables that influence behavior. Our population of infants was biased by design. We purposely excluded confounding influences in host and environment by selecting a longitudinally followed, homogeneous group of infants in an optimal state of health with the exception of iron nutritional status. The proximity of the development scores to the United States norm and the symmetry of their distribution was no doubt a consequence of this selection, with elimination of confounding performances usually present in disadvantaged populations.

Severity of Anemia and Developmental Scores

The results of the present study confirmed previous research showing that iron deficiency anemia has an adverse effect on psychomotor development in infancy. Iron deficits of a milder degree, those not reaching the level of anemia, have no measurable effect on BSID scores. Among anemic infants, hemoglobin concentration was correlated with performance in both mental and motor development, so that infants with moderate anemia (hemoglobin levels 8.4 to 10.4 g/dL) had scores that were significantly less than infants with milder degrees of anemia (hemoglobin levels 10.5 to 10.9 g/dL). These, in turn, had poorer performances than infants with normal hemoglobin levels (≥ 11 g/dL), with no graded improvement seen at higher levels of hemoglobin.

If the effect of iron deficiency on behavior is mediated by metabolic processes dependent on the presence of iron, it is understandable that overt anemia is necessary to disclose these effects. Siimes et al²³ showed in an animal model fed graded amounts of iron that tissue heme proteins were not affected until saturation of transferrin decreased significantly. Hemoglobin as well as tissue cytochrome c and myoglobin decreased steadily thereafter, showing that availability of iron to the erythroid marrow was limited concomitantly to other tissues. Changes in iron stores (liver nonheme iron) did not influence hemoglobin level or tissue heme iron proteins. In humans, the stage known as iron deficient erythropoiesis—when iron availability becomes a limiting factor for hemoglobin synthesis—corresponds to the moment when hemoglobin concentration begins to decrease along with other tissue iron proteins. However, overt anemia was reached only when hemoglobin values decreased to < 11.0 g/dL for the infants in this study. Anemia ensued, therefore, after a protracted and severe period of iron deficiency, ensuring significant depletion of tissue iron proteins. Milder iron deficit may be too little to cause sufficient tissue depletion to be reflected in behavior. It may also be the case that the psychologic tests available for this age group may be too crude for subtle deficits to be identified. These considerations help explain the absence of cognitive effect seen in the nonanemic responder population (subclassification of nonanemic iron-deficient group grade 3), which conceivably corresponds to the group of infants with limited hemoglobin synthesis soon to become anemic.

Duration of Anemia

Infants whose anemia had a duration of more than 3 months also had scores that were signifi-

cantly inferior to those of anemic infants whose hemoglobin levels were in the normal range 3 months earlier. Infants who were nonanemic, but were iron deficient at 9 months of age became those who at 12 months of age decreased to the intermediate group (hemoglobin levels 10.5 to 10.9 g/dL), having anemia of shorter duration and lesser severity that was associated with milder psychomotor derangements. It is reasonable to assume that if this group of infants were to continue with an iron-deficient diet, their anemia would increase in duration (and severity) and their psychomotor performance would deteriorate further. Therefore, infants with anemia of longer duration were also more likely to have anemia of greater severity. These two characteristics of anemia, duration and severity, could not be completely individualized with the current design.

Anemia and Mental vs Motor Developmental Indices

Throughout the study, absolute mental scores were consistently higher than motor scores. The explanation for these small, albeit significant, spreads is not clear; however, a similar distribution was also seen in our previous study⁸ and reports from Guatemala⁶ and Costa Rica.⁷ In our study, the lower motor performance scores were less marked in the control infants. Solomons²⁴ compared BSID norms from 1 to 12 months of age to those of infants from Yucatan, Mexico, and found that the scores of the latter were similar or slightly better than those of US infants until 10 months of age, when they crossed over and became inferior. The poorer performance of Mexican infants was more accentuated in the motor items. Iron status was not evaluated but it is conceivable that children from Yucatan had become anemic by 9 to 12 months of age and that these differences, mimicking those of our study, were, in fact, secondary to iron-deficiency anemia. The alternative explanation is that stimulation of motor milestones related to the onset of walking is culturally unheeded in Latin American societies.

Short-Term Iron Therapy

Short-term therapy, before reversing the anemia, was aimed at immediately correcting the availability of iron to processes of neurotransmitter metabolism that are hypothesized to mediate the behavioral derangements seen during iron deficiency.²⁵ In our previous study,⁶ we found significant improvement in the anemic infants after 10 days of oral iron, whereas no difference was noted in the nonanemic infants. However, doubts were raised in the

interpretation of those results that did not include placebo-treated infants, because neither regression to the mean nor "ceiling" effects could be excluded. In this double-blind study, the increase in scores among iron-treated anemic infants was not significantly greater than that found among placebo-treated anemic infants. The same was true for the other iron status groups. These findings show the importance of appropriate controls. Similar results were encountered recently by Lozoff et al⁷ supporting the assertion that short-term iron therapy does not exert a change in performance beyond what could be explained by a "practice effect."

Use of
placebos
controls
effect of
short-term
iron effect

Long-Term Iron Therapy

After 3 months of iron treatment the hematologic status of many infants had completely corrected, and in all it had improved markedly, reversing the anemia. This stage represents tissue replenishment of iron and ensures renewal of those iron enzymes that have turnover times similar to or shorter than those of hemoglobin. If these tissue iron proteins were responsible for developmental test performance, the scores of anemic infants should have approached those of their iron-replete peers. Because this did not occur, it is probable that either other reactions not directly dependent on iron availability are responsible for the derangements seen, a longer period of time may be needed for the correction of those iron-sensitive behavioral processes, or those processes are, in fact, irreversibly damaged. In the only comparable previous study in which the effect of correction of anemia was evaluated,⁷ anemic infants in whom all iron measures were corrected after 3 months of therapy, had scores that approached those of their nonanemic peers. However, those investigators,⁷ provided evidence suggesting that this effect could also be explained because complete correction with iron therapy tended to occur among anemic infants with less severe iron deficiency whose scores were less affected by iron deficiency. The nonanemic infants showed a decrease in scores that contributed to the leveling of their marks in relation to those of the fully corrected anemic group. We did not find improvements among those of our anemic infants whose iron deficiency was fully corrected (11 of 39), nor a decrease in the scores of the nonanemic infants. However, we also observed a difference in the effect of severity (and duration) of anemia on BSID performance. These considerations leave the issue of reversibility of cognitive deficits unresolved. Moreover, the only two studies in the literature, albeit retrospective, in which the late effects of iron deficit in early life are evaluated raise con-

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cerns regarding the persistence of deficits. Palti et al²⁶ described the results of a follow-up study of children who had iron-deficiency anemia in infancy. The hemoglobin levels of these infants at 9 months of age were correlated with IQ test scores obtained at 5 years of age. In another study described by Cantwell in abstract form,²⁷ infants without anemia were compared with infants who developed moderate to severe anemia at 6 to 18 months of age. At 6 to 7 years of age, children who had been anemic were less proficient at balancing on one foot, tandem walking, and repetitive hand or foot movements. These motor balance-coordination defects, although not followed by a full presentation, coincide with the areas affected in our anemic infant population. Additionally, animal studies designed to be analogous to our study in time scale with reference to brain growth and maturation show that brain iron deficiency induced early in life did not recover even after long-term iron administration,²⁸ probably because of slow iron turnover,²⁹ and that this model presented permanent electrophysiologic³⁰ and experimental behavior abnormalities.^{32,33} This evidence from experimental animal studies added to the human studies presented previously increased our concern regarding potential long-lasting effects of iron deficiency anemia in early infancy.

IBR and Patterns of Failure

The IBR findings in this study expand our previous findings⁸ and those of other investigators.^{4,5} There were no appreciable changes in the IBR after iron therapy, which correlated with the lack of change in developmental index scores. This fact lends support to the hypothesis that the unfavorable behavior pattern of these infants may be a mediator of the poorer BSID performance. If this contention were true, it should have probably affected individual items in a random fashion. However, in this study, item performance failures had a consistent pattern with preponderance of language in the mental items and body balance-coordination items in motor skills. The reason for this selective effect remains obscure. Moreover, these findings coincide closely with results from recent research in Costa Rica⁷ that, with a different study design, show similar selective failure patterns. This agreement is reassuring in that it ratifies our findings. It is disturbing, however, because no consistent improvement after short- or long-term iron therapy is shown.

In summary, developmental test performance in infancy has now been conclusively demonstrated to be impaired in children with anemia due to iron

deficiency. No abnormalities are detected in iron deficiency of lesser severity (or duration) not manifest as overt anemia. Among anemic infants, both severity and duration of anemia were associated with poorer performance. Short-term iron therapy, correction of anemia, or even complete reversal of iron status to normal values with long-term therapy did not yield improvement of cognitive abilities. The selected areas most clearly affected were those of language acquisition and proficiency in body balance and coordination development leading to the erect position and walking.

If extended term studies continue to show that children who were anemic as infants are disadvantaged in relation to their iron-sufficient peers, particular efforts should be made to prevent iron deficit in the vulnerable infant population, because iron therapy alone seems ineffectual in reversing developmental losses.

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ERRATA

In the article, "Cow's Milk Whey Protein Elicits Symptoms of Infantile Colic in Colicky Formula-Fed Infants: A Double-Blind Crossover Study," by Lothe and Lindberg (*Pediatrics* 1989;83:262-266), there was an error on page 264. The second sentence in "Results" should have read: Infants receiving cow's milk-based formula cried 3 h/d or more.

In the article, "Routine Chest Radiographs in Pediatric Intensive Care: A Prospective Study," by Hauser et al (*Pediatrics* 1989;83:465-470), there is an error in Table 3. The results in the second column should shift down one line (no No. or % corresponds to "Malposition").

