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Anemia
folic acid
iron deficiency
platelets
vitamin B_{12}
megaloblastosis

Evaluation of Parameters of Folic Acid and Vitamin B_{12} Deficiency in Patients with Iron Deficiency Anemia

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Extract

Neutrophil lobe averages and hypersegmentation, bone marrow examinations, serum folate and vitamin B_{12} levels, and formiminoglutamic acid (FIGLU) excretion studies were performed in a group of 55 infants and children with iron deficiency anemia, some with and some without thrombocytopenia. Increase lobe averages (> 3.71) and hypersegmentation were found in 13 and 32 patients, respectively. Twenty-five patients demonstrated some megaloblastic changes in their marrow. None had decreased serum levels of vitamin B_{12}; two were considered folic acid deficient, based on serum folate assays (< 3 ng/ml). Three had elevated urinary levels of FIGLU (> 6.6 μmol/h). In only five instances could a correlation be established between the morphologic and biochemical findings. The presence or absence of thrombocytopenia did not appear to be related to megaloblastic morphologic changes in the blood and bone marrow or to the number of bone marrow megakaryocytes.

Speculation

It is postulated that some of the changes in peripheral blood and bone marrow found in iron deficiency states are due to a relative lack of folate or vitamin B_{12} at the tissue level resulting from hyperplasia. The exact mechanism for the thrombocytopenia is not clear.

Introduction

Thrombocytopenia, an unexplained complication of iron deficiency anemia, is usually responsive to therapy with iron [6, 14, 22]. Megaloblastoid bone marrow changes and increased urinary excretion of formiminoglutamic acid (FIGLU) have also been reported in some of these cases [6]. In some patients with megaloblastoid bone marrows, the initial platelet response following iron therapy undergoes a rapid drop succeeded by a secondary rise when therapy with folate or vitamin B_{12} is instituted. NAIMAN *et al.* [16] have found a high incidence of both functional and histologic abnormalities in the upper intestinal tract in infants and children with nutritional iron deficiency anemia. Malabsorption can impair the absorption of folic acid and/or vitamin B_{12}. These facts led us to study folate and vitamin B_{12} metabolism in patients with iron deficiency anemia, both with and without thrombocytopenia.

Methods

Red blood cell counts, hemoglobins, hematocrits and reticulocyte and phase microscopy platelet counts and serum iron and iron binding capacities were done by

standard methods [2, 18, 25]. Bone marrow particles were stained with Wright's stain. Bone marrow hemosiderin was estimated by scanning potassium ferrocyanide-stained smears [19]. Serum folate was determined by the aseptic addition method [7] using *Lactobacillus casei*, the test being performed on blood specimens obtained prior to administration of any antibiotics. Levels of vitamin B_{12} in serum were assayed by the radioisotope dilution technique [11] using cobalt-57 B_{12}. Urine for FIGLU determination was collected for a period of five hours, starting three hours after a histidine loading dose (200 mg/kg body weight) [3]. The FIGLU was assayed according to the method of TABOR and WYNGARDEN [24] and expressed as micromoles excreted per hour. One observer (P.V.) examined the nuclear lobes in 100 neutrophils in each blood smear. The lobe average was determined by dividing the total number of lobes found by 100 [1, 8, 9]. The coefficient of variation on repeated counts of the same blood smear was 0.9 %. Hypersegmentation was considered present when more than three five-lobed neutrophils were found per 100 cells. The bone marrow was examined for 'open' nuclei in the erythroid elements, Howell-Jolly bodies, nuclear hyperfragmentation or large metamyelocytes. Megakaryocyte numbers were evaluated by scanning the bone marrow smear under low power. The marrows were examined independently by both authors and only those changes which were noted by both were accepted. A marrow was classified megaloblastoid if both myeloid and erythroid abnormalities were found.

Materials

Prior to transfusion or iron therapy, 55 patients who were admitted to the Children's Hospital of the District of Columbia with the diagnosis of iron deficiency were studied by obtaining red cell indices, reticulocyte and platelet counts, serum iron determinations, total iron binding capacities, bone marrow examinations, granulocyte lobe counts, FIGLU excretion, and assays of folate and vitamin B_{12} in serum. The patients were divided into four groups depending on the presence or absence of thrombocytopenia and acute infection as follows: Group I, iron deficiency without thrombocytopenia or infection; Group II, iron deficiency without thrombocytopenia but with infection; Group III, iron deficiency with thrombocytopenia but without infection; and Group IV, iron deficiency with thrombocytopenia and infection (see tables I and II).

Granulocytic lobe counts, urinary FIGLU determinations, and assays of folate and vitamin B_{12} in serum were also performed on groups of normal children ranging in age from 6 weeks to 17 years with a socioeconomic background similar to that of the patients. These controls were obtained from a well-baby clinic and the elective surgical ward of the hospital. All had normal hemoglobin levels for their ages and were free of obvious disease.

Results

Normal Children

Table I shows the results of the neutrophil lobe studies performed on normal children. Figures 1–4 show the individual results of the granulocytic lobe averages, serum folate, serum vitamin B_{12}, and FIGLU determinations.

The mean lobe average for the normal children was 3.16 ± 0.32. This value is similar to that found by HERBERT [13, 14] and by us [12] in normal adults. Ten of the 56 normal children exhibited hypersegmentation.

The mean serum folate of the normal children was 10.38 ng/ml and the range was from 4.5 to 31.75 ng/ml. In the adult, levels less than 5.0 ng/ml are suggestive of folate deficiency, and levels less than 3.0 are indicative of the deficiency state [8]. The results obtained by us are slightly different from those obtained by SHOJANIA and GROSS [23], who reported a mean of 11.37 and a range of 4.12 to 21.25 ng/ml in a group of 24 children from 1 to 6 years of age.

The mean serum vitamin B_{12} level was 773 pg/ml. The range obtained by us for normal children is somewhat higher than that reported by LAU *et al.* [11] for adults. This difference, however, is consistent with the observations of KILLANDER [10] and ROGER *et al.* [21] which indicate that levels tend to be higher in the young. Determinations of FIGLU excretion in 12 normal children ranged from 0.0 to 6.61 μmole/h with a mean of 1.85 μmole/h.

Iron Deficiency Anemia Patients

The children with iron deficiency anemia ranged in age from 6 months to 15 years. Forty-two were Negroes and 13 were Caucasians. Thirty-five children were male and 20 were female; the majority were infants (<2 years of age). Most of them were from a low socioeconomic group. Twenty had birth weights below five pounds and eight ounces. Of the entire group, four demonstrated subnormal growth based on their admission weight. Four, eleven, five and eight patients in Groups I, II, III, and IV, respectively, had inadequate dietary histories in that they ingested little iron-containing food. Three had evidence of neuromuscular retardation. The stools of 13 patients were guaiac positive. In five infants and children, a definite bleeding lesion was found by x-ray and/or laparotomy. Chronic bleeding was the only explanation for the anemia in the four adolescents in this study and was due to esophagi-

tis secondary to hiatal hernia, duodenal ulcer, pancreatic tumor and multiple polyposis. Petechiae were not seen in any of the patients. The spleen was found to be palpable in five patients in Group I, four in Group II, five in Group III, and four in Group IV, and measured from 1 to 3 cm below the left costal margin. There was no difference in the magnitude of splenic enlargement among the various groups.

In Groups II and IV, acute infections encountered were pneumonia, otitis media, gastroenteritis, tonsillitis, urinary tract infections, and viral exanthemata. On investigation, a specific bacterial or viral agent was present in most cases.

The results of the laboratory studies in these groups are shown in tables I, II, and III and figures 1–4. In all groups, the children with iron deficiency anemia had hypochromic microcytic erythrocyte morphology and indices, decreased serum iron and bone marrow iron stores, and increased total iron binding capacity. No significant differences were noted among the various groups in reference to these parameters. The mean hemoglobin level of the combined nonthrombocytopenic groups was somewhat higher than the mean for the combined thrombocytopenic group (5.1 g % *vs* 4.3 g %), but the difference was not statistically significant ($p > 0.05$).

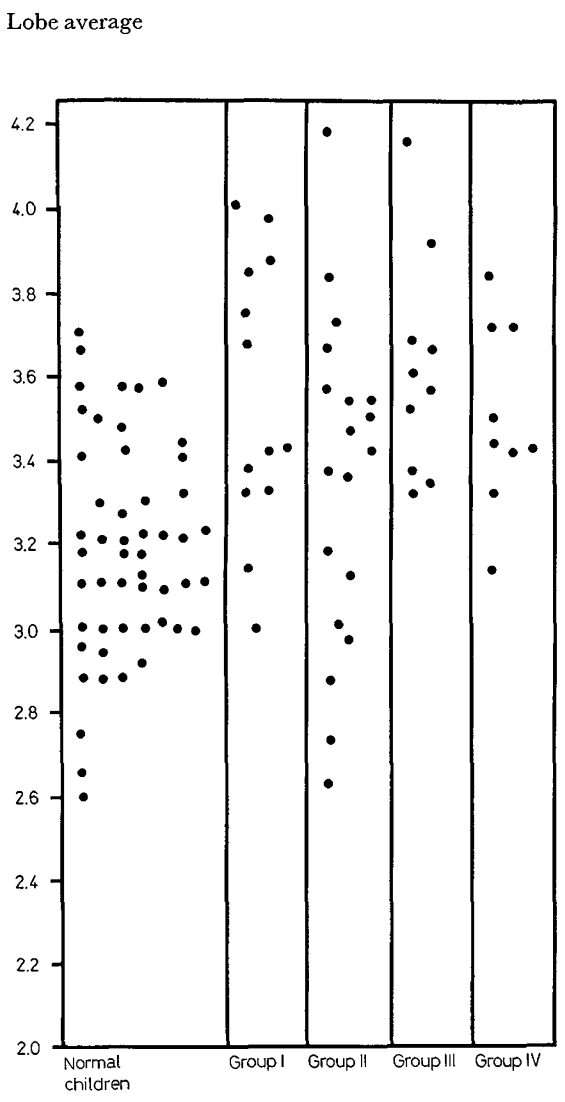

Fig. 1. Granulocyte lobe averages of normal children and groups of iron-deficient patients.

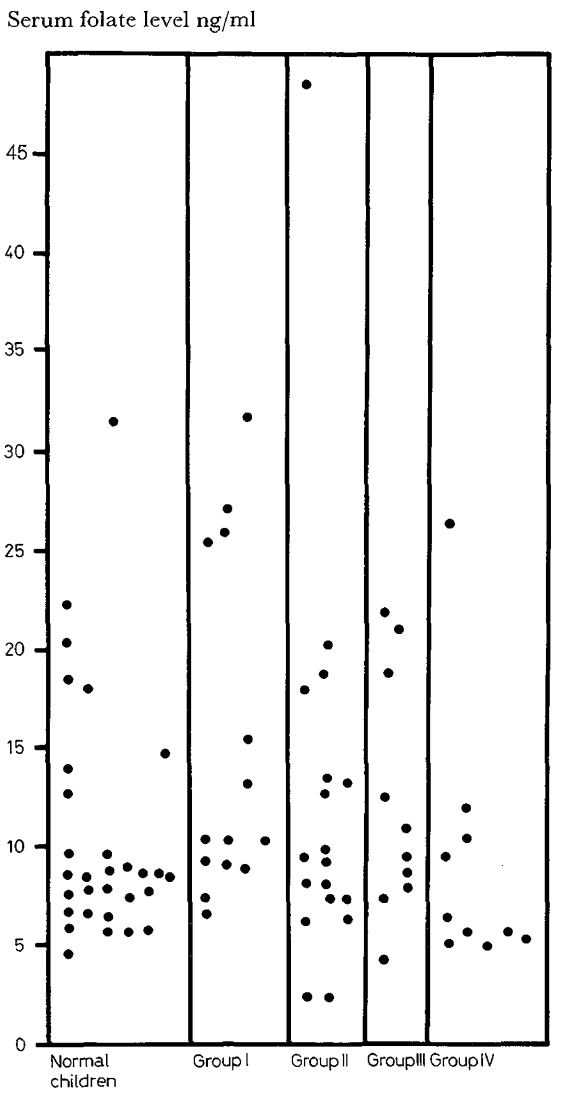

Fig. 2. Levels of folate in serum of normal children and groups of iron-deficient patients.

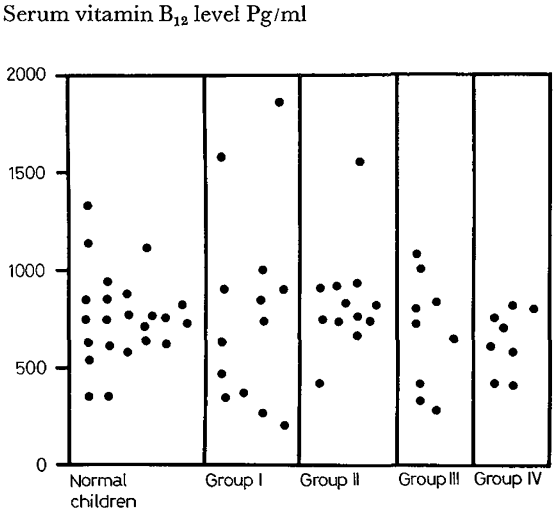

Fig. 3. Levels of vitamin B_{12} in serum of normal children and groups of iron-deficient patients.

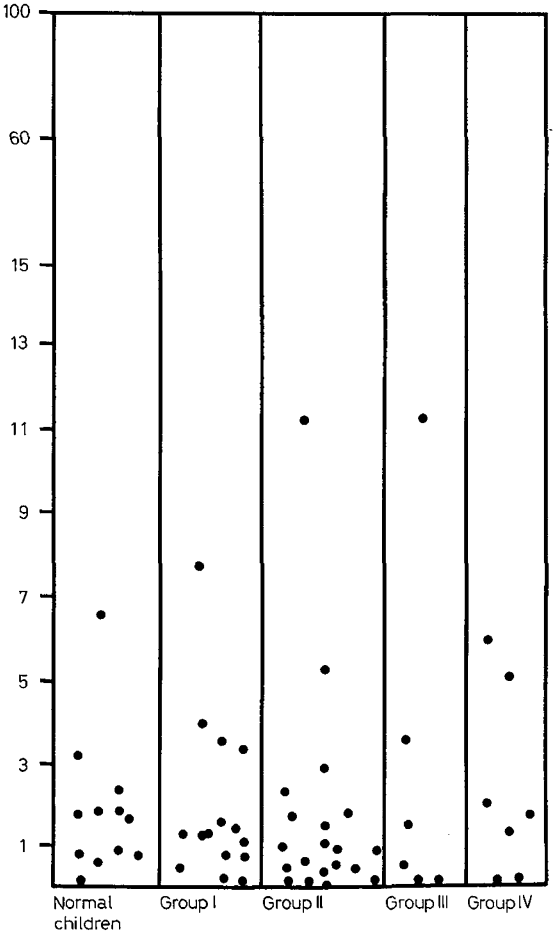

Fig. 4. Urinary formiminoglutamic acid (FIGLU) excretion of normal children and groups of iron-deficient anemic patients.

Thirteen anemic patients exhibited lobe averages greater than the upper limits of the normal children. The lobe averages of the normal children and Groups I and III differed significantly ($p < 0.025$ and < 0.005), but this was not the case between the normal children and Groups II and IV. Significant differences, however, were present between normal children and all four iron-deficient groups in reference to the frequency of hypersegmentation ($p < 0.025$, < 0.005, < 0.005, < 0.025 for each group respectively).

The bone marrows of 50 of the 55 patients exhibited erythroid hyperplasia. Table III shows the results of evaluation of the megaloblastoid changes and megakaryocytes in these bone marrows. Twenty-five of the 55 patients had mild myeloid and erythroid megaloblastoid marrow aberrations. Although the incidence of megaloblastoid changes was greater in the combined thrombocytopenia groups (Groups III and IV) than in the nonthrombocytopenia groups (Groups I and II), the difference was not statistically significant. Decreased megakaryocytes, found in twelve instances, were present in both thrombocytopenic and nonthrombocytopenic children.

The mean folate and vitamin B_{12} levels in serum of the normal subjects and patients with anemia did not differ significantly. Two patients in Group II had serum folate levels of less than 3 ng/ml. Three patients with iron deficiency anemia had increased FIGLU excretion, one each in Groups I, II and III, respectively.

Follow-up Observations

Serial platelet levels were performed on several of the patients with thrombocytopenia.

(a) Three patients received iron medication by mouth from the time of hospital admission in a dose of 2 mg/kg three times a day. The mean platelet count in this group rose from 89,000/mm^3 to a mean of 570,000/mm^3 on the sixth hospital day. This was associated with a steady rise in the mean reticulocyte count which reached a peak (13 %) on the fifth hospital day.

(b) In three other patients, iron therapy was withheld and the children were maintained on a regular hospital diet. The platelet count rose from thrombocytopenic levels at the time of admission to normal levels of greater than 150,000/mm^3 on the fourth hospital day in one patient and on the seventh hospital day in two patients, despite no significant change in the reticulocyte count. Three other patients received a single transfusion of sedimented red cells on admission. Their initial platelet levels, which had been less than 50,000/

Table I. Neutrophil lobe studies, serum folate, serum vitamin B_{12} and urinary FIGLU excretion in normal children and various groups of children with iron deficiency anemia

		Normal children	Group I	Group II	Group III	Group IV
Number		52	14	20	11	10
Lobe average	mean	3.16	3.54	3.36	3.53	3.40
	SD	0.28	0.09	0.08	0.10	0.13
Number with hyper-segmented neutrophils		10	7	11	8	6
Number		30	14	19	9	10
Serum folate (ng/ml)	mean	10.64	15.3	14.1	11.2	9.3
	SD	6.13	8.56	13.29	5.49	6.20
Number		23	14	18	9	8
Vitamin B_{12} (pg/ml)	mean	774	820	836	689	646
	SD	239.7	501	284	280	154
Number		12	14	19	5	7
FIGLU (μmole/h)	mean	1.87	1.88	1.64	3.39	1.63
	SD	1.72	2.09	2.64	4.06	1.89

Table II. Hematologic data on various groups of children with iron deficiency anemia

Groups		Group I	Group II	Group III	Group IV
Number		14	20	11	10
Hemoglobin (g/100 ml)	mean	4.3	5.6	3.9	4.6
	SD	1.5	1.2	1.7	1.2
Serum iron (μg/100 ml)	mean	24.92	32.16	33.89	28.67
	SD	16.2	12.5	21.4	17.0
Total iron-binding capacity	mean	521.15	439	576.87	431.67
(μg/100 ml)	SD	96.7	74.3	100.0	37.2
Platelets (10^3/mm^3)	mean	419	363	75	59
	SD	150	145	47	37

Table III. Bone marrow findings in the various groups of patients with iron deficiency anemia

	Number of patients	Number of patients with decreased megakaryocytes	Number of patients with megaloblastoid myelopoiesis and erythropoiesis
Group I	14	4	5
Group II	20	1	9
Group III	11	4	6
Group IV	10	3	5

mm^3 in each instance, rose, reaching normal levels by the seventh hospital day. During this period, no significant change in reticulocyte levels occurred.

Patients with and without infection were present in both of the above groups. It is difficult to make any generalizations concerning platelet response in the other thrombocytopenic patients because of the timing and combinations of therapy in these children. Serial platelet counts were not performed in any of the non-thrombocytopenic patients.

Discussion

There are several possible mechanisms which might explain thrombocytopenia occurring in iron deficiency. These include insufficient production of platelets due to the lack of a factor(s) involved in thrombopoiesis or increased utilization or destruction of platelets. Because of past observations of megaloblastoid changes in bone marrows of patients with iron deficiency, the poor dietary history associated with iron deficiency, and the report of malabsorption in this anemia, it was decided to investigate future cases of iron deficiency with and without thrombocytopenia for evidence of folate and/or vitamin B_{12} deficiency or metabolic impairment.

The most striking observations in this study were the presence of increased lobe averages in the peripheral blood smears of a number of the children with iron deficiency anemia and an incidence of individuals with hypersegmented granulocytes in the four iron-deficient groups greater than that found in normals. According to HERBERT [8, 9], the finding of an increased neutrophil lobe average and hypersegmented neutrophils is suggestive of megaloblastosis. Twenty-five patients had some megaloblastoid changes in their bone marrows. Based on lobe average and bone marrow examination, 33 patients had some evidence of megaloblastoid dysplasia. Similar findings have been reported [5, 26] in iron-deficient anemic adults.

These morphologic findings would suggest that a folate or vitamin B_{12} deficiency state or impairment of folate or vitamin B_{12} metabolism exists in some of these patients with iron deficiency anemia. The absence of decreased serum levels of vitamin B_{12} in any of the anemic patients would rule out the possibility of a deficiency of this vitamin. Two patients in Group II had decreased serum folate levels. Both patients had megaloblastoid morphologic changes in their bone marrow. Thus, the bone marrow findings in these two patients can be explained on the basis of folate deficiency.

VITALE *et al.* [27] have shown that rats made iron deficient have a defect in folate metabolism with an increase in urinary FIGLU excretion. Three patients in the present study had elevated FIGLU excretion, one each in Groups I, II and III. Two of them exhibited hypersegmentation and one of the two demonstrated a megaloblastoid bone marrow. Therefore, the morphologic changes found along with the elevated FIGLU excretion might be explained on the basis of folate deficiency. However, this leaves the large majority of the patients without folate or vitamin B_{12} deficiency, or a metabolic block, to explain the morphologic abnormalities observed in their blood and bone marrow. A possible explanation for the morphologic changes in the blood and bone marrow of these patients is that the iron deficiency anemia may produce a functional deficiency of folate or vitamin B_{12} at the cellular level in the marrow. This relative insufficiency might be produced by an increased demand for these metabolites in conjunction with marked erythroid hyperplasia. Increased folic acid clearance after intravenous injection has been reported in patients with iron deficiency anemia [4]. Megaloblastoid changes in the bone marrow are frequently seen in chronic hemolytic disease associated with erythroid hyperplasia with conversion to a normoblastic state after folic acid therapy [13, 15, 17].

A functional deficiency of folic acid or vitamin B_{12} at the bone marrow level with subsequent megaloblastoid hematopoiesis might result in thrombocytopenia. However, the incidence and degree of bone marrow and neutrophil abnormalities in the thrombocytopenic and nonthrombocytopenic groups did not differ. These observations raise a question concerning the exact role of megaloblastoid hematopoiesis in the etiology of thrombocytopenia in patients with iron deficiency anemia and indicates that although this may be one factor, other factors may play important roles. As megaloblastoid aberrations have been reported in conditions other than folate and vitamin B_{12} deficiency states [20, 28], it is also possible that some factor(s) other than folate or vitamin B_{12} is involved in the development of these morphologic changes.

Thrombocytopenia may be caused by insufficient platelet production due to decreased megakaryocytopoiesis. In sixteen children with iron deficiency and thrombocytopenia reported by GROSS *et al.* [6], four had decreased megakaryocytes in the bone marrow. Similar findings were noted by us in 7 of 21 children with thrombocytopenia; however, 5 of 34 children in the present series without thrombocytopenia also exhibited decreased megakaryocytes. Therefore, the role of decreased megakaryocytopoiesis as a cause of thrombocytopenia associated with iron deficiency anemia remains unclear.

The small number of patients in the groups followed with serial studies makes the results of platelet response inconclusive. It is interesting to note, however, that the delay in the rise of platelet count in patients given medication orally reported by GROSS *et al.* [6] was not seen in any of the three patients which we treated in this manner. In those not receiving iron therapy with and without red cell transfusions, a platelet rise also occurred. The cause for the rise of the platelet count in these latter patients is not known. It is suspected that it may be due to either folate, iron, or some other nutrient provided in our standard hospital diet. Because of this unexplained rise in platelet levels after hospitalization, one must be careful in the conclusions drawn with specific therapies unless appropriate control patients are included and the diet is rigidly controlled.

In order to determine the role that acute infection has on folate or vitamin B_{12} metabolism (as related to the thrombocytopenia of iron deficiency anemia), the patients were placed in separate categories based on the presence or absence of infection. No differences in mean serum folate, vitamin B_{12}, FIGLU excretion, neutrophil lobe average, and bone marrow megaloblastoid changes were apparent among these groups. Two patients in the group with infection but without thrombocytopenia did have decreased serum folate levels. These facts would indicate that only in isolated instances does acute infection influence folate metabolism.

The incidence of thrombocytopenia (38 %) in this study is not dissimilar from that reported to occur in the group of patients with comparable hemoglobin levels (< 7 g %) reported by Gross *et al.* [6]. The overall incidence of thrombocytopenia in iron deficiency anemia which we have found may be somewhat misleading, because all of the cases of nonthrombocytopenic iron deficiency seen in the patients at our institution were not admitted to the hospital for study.

Summary

Although megaloblastic changes could be found in the blood and bone marrow of 33 iron-deficient children with and without thrombocytopenia, evidence of folic acid or vitamin B_{12} deficiency could be documented in only five of them. It is postulated that a relative deficiency of folic acid or vitamin B_{12} at the bone marrow level may have produced the morphologic changes; however, the relation of the morphologic changes and thrombocytopenia found in iron deficiency is not apparent.

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