Vitamin B₁₂ and Folic Acid Metabolism in Hookworm-Infected Patients

By Miguel Layrisse, Norma Blumenfeld, Iris Dugarte and Marcel Roche

Hookworm infection is the principal cause of anemia in the general population of Venezuela. In previous papers it has been shown that individuals infected by Necator americanus and Ancylostoma duodenale, whose erythrocytes have been tagged with Cr, lost considerable quantities of blood, ranging approximately from 2 to 100 ml per day, the loss being in rough proportion to the severity of the infection. It has also been shown that a significant proportion of the iron contained in the lost blood is reabsorbed from the intestinal tract (average 44 per cent); however, sufficient blood may be lost to upset iron balance.

As a part of the study of the antianemic factors in these patients, some studies on folic acid and vitamin B₁₂ metabolism were carried out. In the present report, it is shown that in addition to the iron deficiency present in patients with severe hookworm infection, malabsorption of folic acid and diminution of serum vitamin B₁₂ concentration is also present in many of them.

Material and Methods

The clinical material is represented by five groups of individuals: men and non-pregnant women; groups A and B were patients with severe anemia and heavy hookworm infection who were hospitalized in the Medical wards of Hospital Vargas and Hospital Universitario in Caracas. They came from small villages and farms where they worked in agriculture. They usually walked barefoot. Their food consisted mainly of corn, bread, rice, black beans and plantain, and they ate little or no meat and fat for years. Most of them complained of fatigue, weakness, pallor and diminution of work capacity occurring over the last six to 12 months. Apart from the severe anemia and a certain degree of malnutrition the patients had no other complications.

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Group A is represented by eight patients in which only the following tests were performed: morphology of the peripheral blood; estimation of intestinal blood loss, according to the method of Roche et al.; serum vitamin \( B_{12} \) concentration by Rosenthal and Sarett's method; and hookworm ova counting in the feces by the method of Caldwell and Caldwell.

The counting of parasites in the feces after deworming is the best method of estimating the degree of infection. However, the counting of hookworm eggs per gram of feces, although less exact than the parasite count, gives a rough idea about the intestinal blood loss, which is most useful clinically. In a previous paper, a comparison was established between the number of eggs, the amount of intestinal blood loss and the hemoglobin values. It was found that a patient lost daily an average of 2.74 ml. per thousand ova per gram of stool with standard deviation of ±1.50. It was also found that patients who exhibited less than 4,000 eggs per gram of feces and consequently lost small amounts of blood showed hemoglobin values of over 11 Gm. per 100 ml.

In group B, made up of 13 patients, laboratory tests were especially directed toward the study of \( B_{12} \) and folic acid metabolism because of the low values of vitamin \( B_{12} \), which had been found in the serum of some patients of the first group. In addition to morphology of the peripheral blood, serum vitamin \( B_{12} \) concentration tests and counts of hookworm eggs, the following laboratory tests were performed: morphology of the sternal bone marrow and evaluation of hemosiderin storage according to Finch and Rath's method; \( B_{12}\text{Co}^{60} \) urinary excretion by the Schilling technic; plasma iron by the Bathophenanthroline method of Peter et al.; and folic acid tissue storage and folic acid intestinal absorption according to Girdwood's method. When indicated, the subcutaneous test was performed once and the oral test twice. The urine was collected in 2.5 liter bottles containing 50 ml. of 1/5 molar phosphate buffer, pH 7.2 and 10 ml. of toluol.

In 10 patients of this group the following protocol was observed: (1) After the laboratory tests, including vitamin \( B_{12} \) and folic acid, the patients were only treated with iron, seven cases by intramuscular injections of Imferon and three cases by oral doses of ferrous sulphate. In cases 5, 6, 9, 11 and 12, the \( B_{12} \) urinary excretion tests were performed when the hemoglobin of the patients was above 9 Gm. per cent. Also, in cases 12 and 13 folic acid tests were performed after the patient's hemoglobin rose to 10 Gm. per cent. (2) When the patient's hemoglobin reached the vicinity of 10 Gm. per cent subcutaneous and oral folic acid tests were made again, and saturation with the vitamin by oral or subcutaneous doses of 15 mg. daily for 10 days was given. This saturation was enough in all cases but one to bring to normal the folic acid storage. The only exception was case 7, in which a second course of saturation for 7 days was required. (3) Two or three treatments with tetrachlorethylene were given in the patients in order to remove the parasites, and another oral PGA determination was performed afterwards.

Group C is also represented by hookworm-infected subjects. However, the number of hookworm ova per gram of feces did not exceed 2,000. The patients had been living in the city for a few years, but their diet was practically the same as that of group A and B before and during their stay in the city. They were hospitalized for symptoms distinct from anemia.

Five noninfected patients living in the city with various degrees of iron deficiency anemia formed group D, and the last group (E) was a normal group formed by individuals working in our laboratory on whom only serum vitamin \( B_{12} \) and folic acid tests were performed.

**RESULTS**

As is expected in anemia due to heavy hookworm infection, the peripheral blood of the patients of group A and B showed hypochromic microcytic anemia with low MCV and MCHC values (table 1). Lack of bone marrow hemosiderin and very low values of plasma iron in the cases
tested confirmed the diagnosis of iron deficiency anemia. The bone marrow showed either slight or moderate erythoblastic hyperplasia. No essential abnormality was seen in the granulocytic and megakaryocytic series.

The comparison between the number of hookworm eggs in the stool, the intestinal blood lost and the hemoglobin values of the infected patients presented here agreed closely with previous reports. In group A, which showed an average of 22,025 ova per gram of feces, intestinal blood loss was about 45 ml per day, and all the patients had a severe iron deficiency
anemia. In group B, although intestinal bleeding was not estimated, a large amount of blood loss could be expected since the number of eggs was also elevated (mean: 13,000 per gram of feces). That this was actually so is made more probable by the low hemoglobin values found in patients of this group.

On the other hand, in group C, hemoglobin was generally high and the number of ova low. In four cases the hemoglobin values were over 10 Gm. per 100 ml. and the only severely anemic case was a 15 year old girl showing 1.5 million red cells, a hemoglobin concentration of 3.2 Gm. per cent, hypoplasia of the erythroblastic series in the bone marrow, the M/E ratio being 5/1, and 1,500 hookworm eggs per gram of feces. This patient, in whom iron deficiency anemia and erythroblastic hypoplasia of the bone marrow were combined, recovered very quickly with iron treatment only.

**Serum Level and Intestinal Absorption of Vitamin B₁₂**

The level of serum B₁₂ in the normal individuals tested in our laboratory (fig. 1) by Rosenthal and Sarett’s method corresponds in general to the

![Graph](image_url)

**Fig. 1.—Serum vitamin B₁₂ concentration in patients with severe hookworm infection (groups A and B), patients with light hookworm infection (group C), noninfected patients with iron deficiency anemia (group D) and normal subjects (group E).**

- ○ = B₁₂ urinary excretion was not estimated; ● = B₁₂ Co⁶⁰ urinary excretion was estimated, with normal values being found.
VITAMIN B₁₂ AND FOLIC ACID IN HOOKWORM INFECTION

It is important to note the diminution, as compared to the normal groups, of the mean vitamin B₁₂ value found in patients with heavy hookworm infection associated with severe anemia. In group A, three of eight cases, and in group B, five of 10, exhibited values below 100 μg/ml of serum; in two of them it was as low as it is found in pernicious anemia during relapse. On the other hand, the subjects with light hookworm infection or those who were not infected but had iron deficiency anemia showed no significant diminution of this vitamin. It was observed that the low levels of the vitamin found in patients of group A and B did not induce morphologic changes in the bone marrow, and that they responded fully to the iron treatment.

In the second group (B) the studies of serum B₁₂ were complemented by the estimation of B₁₂CO₃ intestinal absorption, which showed normal levels in all cases performed. In order that the flushing with 1,000 μg. vitamin B₁₂ used in Schilling's test did not correct the vitamin deficiency in those patients with levels below 100 μg., this test was performed during the iron treatment when the hemoglobin values rose above 9 Gm. per 100 ml.

Folic Acid Tissue Storage and Intestinal Absorption

In figure 2 the results in the groups studied can be seen. Both the subcutaneous and oral tests showed a level above 1,500 μg. of urinary folic acid activity in normal subjects and noninfected patients with iron deficiency anemia. There was only one normal subject which showed an oral test just below the normal limit (1,475 μg.).

The patients of group B who had severe hookworm infection demonstrated significantly low levels with the subcutaneous test in five cases, and in all but one with the oral test. According to these results, six patients had impairment of the absorption of PGA; in five cases in which both the subcutaneous and the oral test were low, indicating diminution of the storage of the vitamin, malabsorption was demonstrated after folic acid saturation; one patient showed slight diminution of intestinal absorption and only one behaved normally.

Group C with light hookworm infection exhibited no significant differences from the results of the normal group. In one of five patients, folic acid absorption was impaired, although this patient showed normal hematologic values and only 300 hookworm eggs per gram of feces.

The 10 cases of group B treated with iron responded favorably, showing increase of the reticulocyte and hemoglobin values. Table 2 shows the results obtained in seven cases treated with Imferon. It may be seen that the patient's hemoglobin increased rapidly in the week following the start of treatment. In a period of about 44 days the hemoglobin value as well as the plasma iron concentration and the amount of hemosiderin in the bone marrow were within normal limits, thus indicating full recovery from the iron deficiency.
Table 2—Hookworm-Infected Patients Treated with Imferon

<table>
<thead>
<tr>
<th>Case</th>
<th>Plasma iron  ( \mu g )</th>
<th>Bone marrow</th>
<th>Total dose iron (mg)</th>
<th>Hemoglobin (Gm. per 100 ml)</th>
<th>Days after starting treatment</th>
<th>Hgb. (Gm.%)</th>
<th>Plasma iron (µg%)</th>
<th>Bone marrow hematocrit</th>
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<tr>
<td>1</td>
<td>35</td>
<td>2.700</td>
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<td>8.0</td>
<td>8.6</td>
<td>11.6</td>
<td>13.6</td>
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<tr>
<td>2</td>
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<td>4.9</td>
<td>8.4</td>
<td>10.3</td>
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<td>12.9</td>
<td>54</td>
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<td>4</td>
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<td>2.100</td>
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<td>7</td>
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<td>3.600</td>
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<td>11.5</td>
<td>13.9</td>
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<tr>
<td>10</td>
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<td>2.400</td>
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<tr>
<td>Mean</td>
<td>30.3</td>
<td>4.2</td>
<td>7.1</td>
<td>9.5</td>
<td>11.2</td>
<td>11.6</td>
<td>13.1</td>
<td>99.5</td>
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</table>

The rapid response to iron therapy observed in the patients of group B is in accordance with what is seen in clinical practice when patients of the same type recover quickly from the anemia with iron by mouth or parenterally. This finding was also confirmed by Pérez-Giménez, Layrisse and
Folic Acid Test

Imferon 2800 mg

Fig. 3.—Patient 10. This subject had an initial hemoglobin value of 4.3 Gm. per 100 ml. of blood and 9,200 hookworm eggs per gram of feces. The folic acid absorption was partially corrected after the saturation with folic acid, but continued to be below normal levels in the following 90 days in spite of the patient's adequate diet and the removal of the parasites.

Roche, who demonstrated that this type of patient has a rapid Fe\(^{59}\) plasma clearance.

The folic acid tests performed when the hemoglobin values of the patients rose to the neighborhood of 10 Gm. showed practically the same results. The saturation with folic acid corrected the subcutaneous tests but oral tests still showed abnormality (figs. 3 and 4). New tests performed after the second or the third vermifuge, when the stool examination showed less than 500 eggs per gram, revealed a correction of the malabsorption in two of 10 patients tested.

**Discussion**

The serum level of vitamin B\(_{12}\) is under most circumstances an accurate index of tissue saturation. Low serum levels could reflect impairment of intestinal absorption, as in pernicious anemia, sprue, idiopathic steatorrhea, etc., depletion of exogenous B\(_{12}\) by parasites (*Diphyllobothrium latum*)\(^{10}\) or micro-organisms, dietary deprivation of the vitamin as in vegans,\(^{20}\) or increase in demands occurring in pregnant women.\(^{15,21}\) On the other hand, the low serum values of this vitamin are not necessarily associated with the development of megaloblastic anemia.\(^{17,20}\)

The patients of groups A and B harboring severe hookworm infection showed significantly low vitamin B\(_{12}\) levels, as compared with normal in-
Subcut. Oral

Subcut. Folic Acid Saturation

DAYS- 0 7 (4 28 35 40

LAYRISSE, BLUMENFELD, DUGARTE AND ROCHE

Folic Acid Tests

Subcut Oral

2000 µg

1000 µg

Folic Acid Saturation

Vermifuges

Fig. 4.—Patient 11. This subject had a hemoglobin value of 3.2 Gm. per 100 ml. of blood and 9,500 hookworm ova per gram of stool. Folic acid tissue storage and absorption were initially low, but after saturation with the vitamin the subcutaneous test became normal and 15 days after the vermifuges, the intestinal absorption also became normal.

dividuals. Apparently their intestinal absorption of the vitamin was adequate, since Schilling's test performed in nine patients of group B, in which five exhibited values of serum B12 below 100 µg./1 ml., demonstrated normal absorption. It may be presumed that these low values could be due to a dietary deficiency in animal proteins as deduced from nutritional history; however, no studies have been carried out to demonstrate if the hookworm might utilize part of the vitamin B12 contained in the foods.

Folic acid tests were found to be abnormal, especially the oral test, in the so-called folic acid deficiency anemias, such as steatorrhea, sprue, megaloblastic anemia of pregnancy, etc. There are some exceptions in which normal absorption was observed in cases of megaloblastic anemia of pregnancy, and conversely impairment of absorption in normal, pregnant women.

We have also observed folic acid malabsorption in three pregnant women with normal peripheral blood and bone marrow morphology.

The response to iron treatment alone in cases of group B showing folic acid malabsorption may be explained in different ways:

A. The amount of folic acid (15 to 20 mg.) given for the tests prior to iron treatment saturated the marrow, so that it can respond rapidly as
soon as it receives the iron. This interpretation might be correct. However, in case 12, in which malabsorption and diminution of storage was observed, and in case 13, which showed only malabsorption, iron treatment induced a rapid remission of anemia, although the folic acid tests were performed after the hemoglobin rose to values of about 9 Gm. per cent.

B. In the hospital the patients received a diet which is relatively richer in proteins and better balanced than their own diet at home. But it seems unlikely that the minimal amounts of folic acid contained in the hospital diet could correct the folic acid deficiency so rapidly, especially since the patients suffered from folic acid malabsorption.

C. The folic acid tests performed were not adequate. This statement may give a general explanation of our results. Possibly, folic acid tests used clinically, including Girdwood's technic, are not always reliable if the folic deficiency is impairing erythropoiesis, provoking megaloblastic proliferation. Folic acid malabsorption seems to be a condition associated in most cases with megaloblastic anemia due to the deficiency of the vitamin, though malabsorption may exist in cases without megaloblastic anemia.20,21

Folic acid tests have not been used often in the estimation of intestinal absorption. $\text{B}_{12}\text{Co}_{60}$, fat, vitamin A and $\text{D}-\text{xylene}$ have been used in most cases reported in the last five years or so to estimate the intestinal absorption, and folic acid deficiency has been suspected only when megaloblastic proliferation coexists with normal $\text{B}_{12}\text{Co}_{60}$ absorption. It is important to point out that in some of the cases in which the malabsorption was fully demonstrated normal hematologic values were found.17,22

The fact that four of five patients with light infection showed normal absorption of PGA, that 12 of 13 patients with severe hookworm infection showed malabsorption and that in two of them it was corrected after de-worming apparently indicate that the presence of the hookworm in the intestine in certain amounts may interfere with the absorption of the vitamin. The lack of correction of the malabsorption after de-worming, in most cases, may be due to a certain condition in the intestinal wall or in the intestinal microflora, or in both, which remains after the parasites are removed.

Another question which arises from the material presented here is whether the effect of poor diet may be responsible alone or together with the presence of heavy hookworm infection for the deficient absorptive capacity of the patients, since people with inadequate diet for a long period may have a propensity to develop malabsorption.18 This question has been answered almost completely when different patterns of folic acid absorption were shown in patients of group B and C, who had the same poor diet but a different rate of parasite infection. Furthermore, this problem can be solved by testing noninfected, normal, control individuals from the patients' own village; however, it is very difficult to obtain noninfected individuals living in the same villages and under the same economic and dietary conditions.

Another way of answering the problem of dietary effect would be to maintain patients with severe infection for a long time on a good diet and de...
termining if the malabsorption is corrected in spite of their still harboring the parasite. We were unable to follow this protocol, since our patients did not live in the city and it was very difficult for them to stay for a long period in the hospital, and so the interval between the folic acid saturation and the deworming was only about 10 days. There was only one patient (fig. 3) who was kept in the hospital for two months after the hemoglobin had risen to normal levels and who had adequate saturation. This patient, in spite of a long stay in the hospital on a rich diet, still had malabsorption afterwards.

Although the data on vitamin B₁₂ and folic acid presented here are not sufficient to permit a firm conclusion, they nevertheless suggest that in addition to the blood loss some other nutritional factors may be involved in hookworm-infected patients.

**SUMMARY**

Studies on the metabolism of B₁₂ and folic acid were performed in patients with heavy hookworm infection and severe iron deficiency anemia, and in patients with light infection, noninfected patients and normal subjects.

Patients with heavy hookworm infection showed a marked decrease of the serum B₁₂ as compared with normal subjects. Eight of 21 cases studied showed values of serum B₁₂ below 100 μg./ml.

Twelve of 13 patients with severe hookworm infection showed impairment of the pteroylglutamic acid intestinal absorption; however, none of them exhibited megaloblastic proliferation in the bone marrow. They all recovered with iron therapy alone. The patients with light infection and the noninfected patients with iron deficiency anemia did not demonstrate significant differences from the normal subjects studied.

**SUMMARIO IN INTERLINGUA**

Studios del metabolismo de vitamina B₁₂ e de acido folic esseva effectuate in pacientes severmente inficite per ankylostoma e severmente anemic per carentia de ferro, in pacientes con leve grados de ille infection, e in subjectos normal.

Patientes con sever infection per ankylostoma exhibiva un marcate reduction del valores seral pro vitamina B₁₂ in comparation con subjectos normal. Octo inter 21 tal casos studiate monstrava valores seral pro B₁₂ de minus que 100 μg./ml.

Dece-duo inter 13 pacientes con sever infection per ankylostoma monstrava un defective absorption intestinal de acido pteroylglutamic. Tamen, nulle de iste casos exhibiva proliferation megaloblastic in le medulla osse. Omne iste patientes se restabliva per therapia a ferro sol. Le patientes con leve grados de infection per ankylostoma e le patientes sin ille typo de infection sed con anemia per carentia de ferro non exhibiva differentias significative in comparation con le normal subjectos de controlo.

**REFERENCES**


Vitamin $B_{12}$ and Folic Acid Metabolism in Hookworm-Infected Patients

MIGUEL LAYRISSE, NORMA BLUMENFELD, IRIS DUGARTE and MARCEL ROCHE