Folate Deficiency Associated with Drug Therapy for Tuberculosis

By Frederick A. Klipstein, Frederick G. Berlinger and L. Juden Reed

An association between megaloblastic anemia and therapy for tuberculosis has been described previously in five instances. In three patients megaloblastic erythropoiesis was associated with sideroblastic changes in the bone marrow, and in two the megaloblastic anemia was attributed to deficiency of vitamin B₁₂ resulting from malabsorption of this vitamin induced by therapy with para-aminosalicylic acid. Direct determination of serum vitamin concentrations was performed in only one of these five patients. The serum folate concentration was subnormal in this subject, who had a sideroblastic anemia.

We have recently had the opportunity to study two individuals who developed a megaloblastic anemia during therapy for pulmonary tuberculosis. Folate deficiency was demonstrated to be responsible for the megaloblastic erythropoiesis in both. This report presents observations made on these two patients, the results of assays for serum folate and vitamin B₁₂ concentrations which have been performed in 120 other patients with pulmonary tuberculosis, and the results of studies concerned with the in vitro effect of antituberculosis drugs on the microbiologic assay systems used to determine folate and vitamin B₁₂ concentrations.

Materials and Methods

In addition to the two patients described as case reports, serum folate and vitamin B₁₂ concentrations were determined in consecutively studied groups of 40 control subjects and...
120 patients with pulmonary tuberculosis. Control subjects were healthy, young adult medical personnel. Thirty-six patients with tuberculosis were studied prior to treatment. Twelve were poorly nourished alcoholics, the majority of whom were studied immediately subsequent to their admission to Bellevue Hospital. The other 24 were studied elsewhere, principally at the Bronx Municipal Hospital, and appeared to be well nourished. Eighty-four patients studied were receiving therapy for tuberculosis; 77 were seen at the Bronx Municipal Hospital and 7 at Bellevue Hospital. Fifty-two were ambulatory inpatients who had been hospitalized for periods of from 2 to 12 months prior to study, and the remaining 32 had been discharged from hospital and were attending the outpatient clinic. None of these 84 patients appeared to be overtly malnourished or phthisic.

Serum folate concentrations were assayed using *Lactobacillus casei*. In this laboratory, normal subjects are found to have values ranging from 7 to 25 μg./ml.; values of between 5 and 7 μg./ml. are in the indeterminate range; and values of less than 5 μg./ml. are considered to be subnormal. Folate activity of the serum in the absorption study was determined using *Streptococcus faecalis* which assays forms of folic acid that are not ordinarily found in the serum.

Serum concentrations of vitamin B₁₂ were assayed using *Lactobacillus leichmannii*. Normal values in this laboratory range from 150 to 900 μg./ml.; values of from 100 to 150 μg./ml. are considered indeterminate; and values of less than 100 μg./ml. are subnormal. The technics employed in the in vitro studies have been described previously.

Increasing concentrations of antituberculosis drugs, either singly or in combination, were added to known concentrations of crystalline folic acid, vitamin B₁₂, or to aliquots of serum with known vitamin activity and the growth of *L. casei* or *L. leichmannii* determined. A decrease in bacterial growth in excess of one-quarter of the value obtained prior to addition of any drug was considered to be evidence of depression of growth.

**Case Reports**

**Case 1**, a 49-year-old unemployed Negro male, was admitted to the chest service of Bellevue Hospital for the third time on March 7, 1965, with the complaints of productive cough, chest pain, anorexia, and weakness which had progressed in the 3 weeks since he had left the hospital. He was first treated for pulmonary tuberculosis in 1959 when he received triple therapy for 1 year at another hospital. During two previous admissions to Bellevue Hospital in 1964 and 1965, his resistant bilateral cavitary disease was treated with isoniazid, ethionamide, viomycin, para-aminosalicylic acid, and pyrazinamide. His hematocrit was 40 per cent and the erythrocytes were described as normochromic and normocytic in February 1965. All of these admissions were terminated by the patient who was discharged against medical advice. In the 3-week interval since discharge, his dietary intake had been poor and his consumption of alcohol excessive.

On admission, he was febrile and appeared underweight and chronically ill. Physical, radiologic, and bacteriologic findings of active, far-advanced pulmonary tuberculosis were noted. The hemoglobin was 9.2 Gm. per cent, the hematocrit was 31 per cent, the red blood cell count was 3.5 million, and the reticulocyte count was 1.7 per cent. The erythrocytes appeared moderately hypochromic. Serum iron concentration was 37 μg./100 ml. and the total iron binding capacity was 182 μg./100 ml. A sickle cell preparation was negative. The white blood cell count was 14,300/mm.³. Bone marrow aspiration, performed the day after admission, showed normal cellularity, a moderate degree of myeloid hyperplasia, and normoblastic erythropoiesis. Prussian blue stain of the marrow showed a reduced concentration of iron within the spicules; ringed sideroblasts were not seen. Liver function tests and other laboratory studies were all normal.

The patient's hematologic course and details of his antituberculosis and hematinic therapy are presented in Figure 1. His hematocrit rose following therapy with ferrous sulfate, but began to fall after cycloserine and ethionamide had been added because of persistently positive sputa. In early December, the hematocrit was 24 per cent, the hemoglobin concentration was 7.0 Gm. per 100 ml., the red blood cell count was 2.64 million, and the reticulo-
cytokine count was 0.7 per cent. The peripheral blood smear showed a dimorphic picture with hypochromic erythrocytes and macrocytes. Bone marrow examination revealed intense megaloblastic erythroid hyperplasia. Stainable iron was reduced in the spicules, but scattered iron granules were present in the cytoplasm of approximately one-quarter of the normoblasts; some of these cells were ringed sideroblasts. Serum folate concentration was 3.1 μg./ml.; serum vitamin B₁₂ concentration was 160 μg./ml.; serum iron concentration was 175 μg./100 ml. and the total iron binding capacity was 275 μg./100 ml. Treatment with a physiologic dose of folic acid, 50 μg. by mouth daily, was commenced on December 15. Therapy with cycloserine was inadvertently discontinued at this time for a period of 15 days. After 5 days the serum folate concentration rose to 8.4 μg./ml. and the reticulocyte count commenced to rise, reaching a peak of 11 per cent on the ninth day. Bone marrow examination at this time showed striking changes from the pretreatment specimen. Erythropoiesis was still extremely hyperactive, but the predominant cell was no longer the immature megaloblasts but rather smaller, intermediate megaloblasts and a few normoblasts were now present. There was a further reduction in the iron present in the spicules but no change was 175 μg./100 ml. and the total iron binding capacity was 275 μg./100 ml. Treatment with folic acid was discontinued following a 10-day course of treatment. A folic acid absorption study, conducted 2 weeks later, showed a peak serum folic acid level of 77 μg./ml. (normal subjects have peak serum concentrations of greater than 40 μg./ml. following an oral test dose). The administration of the pharmacologic "saturating" doses of folic acid given during this study was associated with a second reticulocyte response of 4.3 per cent. Six weeks after the initiation of folic acid therapy, the hematocrit was 46 per cent and the hemoglobin concentration was 14.5 Gm. per cent. Bone marrow examination showed normal cellularity with exclusively normoblastic erythropoiesis. There was reduced iron in the spicules; iron granules were present in only a few normoblasts and no ringed sideroblasts were seen. The 24-hour excretion of an oral test dose of μC₆B₁₂ was 8.6 per cent (normal subjects...}

Fig. 1.—Hematologic observations during therapy in case 1.
The patient, who had gained only 3 pounds during the first 9 months of hospitalization, gained 18 pounds in the 6 weeks after the initiation of folic acid therapy.

**Case 2**, a 25-year-old Puerto Rican housewife, was admitted to the Bronx Municipal Hospital for the first time on October 24, 1962, with the complaints of cough and weakness of one month's duration. From 1950 to April 1962 she had been treated for pulmonary tuberculosis with isoniazid, para-aminosalicylic acid, and streptomycin. One month prior to admission, she noted the recurrence of a nonproductive cough, weakness, anorexia, and lost 8 pounds during that month despite an allegedly good dietary intake.

On admission, she was febrile and appeared asthenic. Physical, radiologic, and bacteriologic examinations indicated the presence of far-advanced active pulmonary tuberculosis. The hemoglobin was 9.8 Gm. per 100 ml.; the hematocrit, 31 per cent; and the white blood cell count, 10,200 per mm.³ with a normal differential. The peripheral blood smear showed moderate hypochromia of the erythrocytes. Prothrombin time, serum carotene, calcium, oral glucose tolerance test, and hemoglobin electrophoresis, as well as routine chemistries, were normal.

The patient's hematologic course and antituberculosis regimen are depicted in Figure 2. In late December, she commenced to have episodes of fever, diarrhea, nausea, and vomiting, the etiology of which was not clarified by cultures of the blood, stool, and urine, agglutination tests, and complete radiologic studies of the intestinal tract and gallbladder. In mid-January, a course of tetracycline therapy, instituted because of productive cough, was associated with cessation of fever and these symptoms. In late January, the patient's hematocrit began to fall and on January 29 the hematocrit was 29 per cent, the reticulocyte count was 1.0 per cent, and the peripheral blood smear showed a dimorphic picture of hypochromic erythrocytes and macrocytes, occasional nucleated red blood cells, and some hypersegmented neutrophils. The white blood cell count was 10,000 per mm.³ and the...
Fig. 3.—Case 2. Bone marrow aspiration obtained February 22. Giant proerythroblasts are present but other forms of erythropoiesis are now absent.

Platelet count was 319,000 per mm.\(^3\). Bone marrow aspiration showed hypercellularity with immature and intermediate megaloblasts as well as giant metamyelocytes. Iron was markedly reduced in the marrow preparation but rare, ringed sideroblasts were present. Serum folate concentration was 2.7 \(\mu\)g./ml.; serum vitamin B\(_{12}\) concentration was 160 \(\mu\)g./ml.; serum iron concentration was 27 \(\mu\)g./100 ml., and the total iron binding capacity was 287 \(\mu\)g./100 ml.

Therapy with Imferon over a 6-day period was not associated with a reticulocytosis or rise in the hematocrit and repeat bone marrow examination on February 14 showed striking changes from the previous examination. The marrow was less cellular with a decrease in erythroid but increase in myeloid elements; immature megaloblasts were now absent and intermediate megaloblasts were reduced in number, although giant metamyelocytes were still present. Scattered throughout the marrow were large cells which had a diameter of 16 to 40 \(\mu\)m. and a large (diameter of 10 to 30 \(\mu\)m.) centrally located nucleus. The nuclei had a lightly basophilic, coarsely stippled chromatin and contained from 1 to 5 nucleoli, which were of variable size and shape, and occasional vacuoles. These cells were varied in size and appearance: the largest were the size of large megakaryocytes; the majority resembled giant proerythroblasts and the smallest resembled primitive megaloblasts. Iron was now present in normal quantity within the spicules. By February 22, the hematocrit had fallen to 21 per cent. The hemoglobin was 5.8 Gm. per 100 ml. and the red blood cell count was 1.66 million. No reticulocytes were present. Serum folate concentration was 4.0 \(\mu\)g./ml. and serum vitamin B\(_{12}\) concentration was 380 \(\mu\)g./ml. Bone marrow examination showed no change in cellularity from the examination conducted 8 days previously. There was complete maturation arrest of the erythroid series. Intermediate megaloblasts and giant metamyelocytes were no longer present; the proportion of giant proerythroblasts had increased and the larger forms now predominated (Fig. 3). Culture of the bone marrow did not grow acid-fast bacilli. At this time the patient's only complaint was of weakness; she had gained 5 pounds in weight during the first 3 weeks of February.

Therapy with folic acid, 15 mg. daily, was now given for an 8-day period. After 5 days, the reticulocyte count was only 0.4 per cent but bone marrow examination showed a striking
Table 1.—Serum Folate Concentrations in Control Subjects and in Patients with Tuberculosis

<table>
<thead>
<tr>
<th>Patient Material</th>
<th>No. of Subjects</th>
<th>Dietary Intake</th>
<th>Drugs Received</th>
<th>Number of Subjects with Serum Folate Levels in the:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal Range</td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
<td>Adequate</td>
<td>None</td>
<td>37</td>
</tr>
<tr>
<td>Tbc</td>
<td>12</td>
<td>Poor</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>Tbc</td>
<td>24</td>
<td>Adequate</td>
<td>None</td>
<td>15</td>
</tr>
<tr>
<td>Tbc</td>
<td>29</td>
<td>Adequate</td>
<td>INH</td>
<td>19</td>
</tr>
<tr>
<td>Tbc</td>
<td>26</td>
<td>Adequate</td>
<td>INH + PZA*</td>
<td>14</td>
</tr>
<tr>
<td>Tbc</td>
<td>29</td>
<td>Adequate</td>
<td>INH + Cyclo†</td>
<td>10</td>
</tr>
</tbody>
</table>

*PZA indicates pyrazinamide.
†Cyclo indicates cycloserine.

increase in cellularity with marked normoblastic erythroid hyperplasia. Giant proerythroblasts were still present but were decreased in size and number; basophilic erythroblasts predominated and mature normoblasts were still absent. The following day, the reticulocyte count was 3.3 per cent and 3 days later a reticulocyte peak of 16.1 per cent was reached. This reticulocyte response was accompanied by a rapid rise in the hematocrit. Bone marrow examination on March 15 showed normoblastic erythroid hyperplasia with a normal distribution of red cell precursors. Giant proerythroblasts were no longer present. Iron was not detectable on the marrow preparation and ringed sideroblasts were not seen. The serum iron was 55 µg./100 ml. and the total iron binding capacity was 226 µg./100 ml. The patient's hematocrit reached a peak of 47 per cent by the end of April. She had gained 7 pounds in the 2 months since the initiation of folic acid therapy. A Schilling test showed a 24-hour excretion of 12 per cent of the oral test dose of 60CoB12. During the remaining year that the patient remained in the hospital on antituberculosis drug therapy, she continued to receive folic acid and her hematocrit values remained in the 45 per cent range.

RESULTS

Survey of Patients with Tuberculosis

Serum Folate Determinations. The results of serum folate determinations performed in 40 control subjects and in 120 patients with pulmonary tuberculosis, all consecutively studied, are presented in Table 1. Serum folate concentrations were subnormal in 13 of 36 (36 per cent) patients with untreated pulmonary tuberculosis studied; levels were subnormal in 8 of 12 (80 per cent) malnourished patients but in only 5 of 24 (21 per cent) well-nourished patients. In 84 patients receiving antituberculosis drug therapy, serum folate concentrations were subnormal in 2 of 29 (7 per cent) patients receiving isoniazid and in 15 of 29 (52 per cent) of patients receiving combined therapy with isoniazid and cycloserine.

Serum folate concentrations were more frequently within the subnormal or indeterminate range in patients receiving therapy with cycloserine and isoniazid than in well-nourished patients with untreated tuberculosis. Levels were in these ranges in 38 per cent of well-nourished patients with untreated tuberculosis, in 34 per cent of patients receiving isoniazid, in 46 per cent of patients receiving pyrazinamide, and in 66 per cent of patients receiving cy-
closerine. The incidence of folate concentrations within the subnormal or indeterminate range was significantly higher (p \leq .018) in patients receiving cycloserine than in well-nourished untreated patients. The relationship of other antituberculosis drugs to serum folate concentrations was difficult to determine in view of the small number of patients who were taking these medications. Folate concentrations were subnormal or in the indeterminate range in 4 of 24 patients receiving para-aminosalicylic acid, in 5 of 15 patients receiving streptomycin, in 14 of 31 patients receiving ethanbutol, and in 3 of 6 patients taking ethionamide.

The data suggest that depression of serum folate concentrations in patients taking cycloserine was related to dosage rather than duration of time that drugs had been taken. Serum folate concentrations were subnormal in 6 of 7 (86 per cent) of patients taking 1000 mg. daily, in 7 of 14 (50 per cent) patients taking 750 mg. daily, and in 2 of 8 (25 per cent) patients taking 500 mg. daily. Folate levels were subnormal in 2 of 8 patients who had taken cycloserine for longer than a year and in 6 of 11 patients who had taken the drug for less than 3 months.

Serum Vitamin B₁₂ Determinations. Serum concentrations of Vitamin B₁₂ were in the indeterminate range in 2 control subjects, both of whom had normal absorption of ⁶⁰CoB₁₂, and normal in the other 38. Serum concentrations were normal in all 36 patients with untreated tuberculosis, in the indeterminate range in 6 patients receiving isoniazid, in one patient receiving pyrazinamide, and in 7 patients receiving cycloserine, and subnormal in one patient taking isoniazid and in 2 patients taking pyrazinamide. Of 24 patients taking para-aminosalicylic acid, vitamin B₁₂ concentrations were in the indeterminate range in 3 and subnormal in 1. Serum concentrations were also determined by the technic of radioisotope dilution using albumin-coated charcoal¹ in 10 patients who were found to have concentrations within the indeterminate or subnormal range by microbiologic assay. * Serum concentrations were subnormal when determined by the radioisotope technic in 5 of 6 patients taking isoniazid or pyrazinamide but in only 1 of 4 patients receiving cycloserine.

Hematologic Studies. Six of the 36 patients studied with untreated pulmonary tuberculosis had hematocrit values of less than 38 per cent. Five were poorly nourished alcoholics. Serum folate concentrations were subnormal in 4 of these individuals, in one of whom bone marrow aspiration was obtained which revealed megaloblastic erythropoiesis. Hematocrit values were less than 38 per cent in 6 of the 84 patients studied who were receiving drug therapy for tuberculosis. Two of these individuals had been anemic prior to the institution of antituberculosis medications and serum folate and vitamin B₁₂ concentrations were normal at the time of study in both. Four patients, all of whom were receiving isoniazid and cycloserine, developed a mild anemia after institution of therapy for tuberculosis. Serum folate concentrations were subnormal in 3 and in the indeterminate range in 1 and serum vitamin B₁₂

*The radioisotope dilution assays were kindly performed by Dr. Victor Herbert.
In Vitro Studies

Folate. The results of studies are presented in Figure 4. The addition of pyrazinamide* to the medium did not result in depression of the growth of *L. casei*. The addition of isoniazid resulted in depression of growth when added in a concentration of 500 μg./ml. (12 times the usual serum concentration

*Supplied through the courtesy of Dr. Richard T. Smith, Merck Sharp & Dohme Research Laboratories.
FOLATE DEFICIENCY AND DRUG THERAPY FOR TUBERCULOSIS

found in patients receiving therapeutic doses of this drug. The addition of cycloserine* depressed growth at a concentration of 16μg./ml. when added to medium containing crystalline folic acid and at a concentration of 166μg./ml. (threefold the usual serum concentration found in patients receiving therapeutic doses of this drug) when added to medium containing serum as a source of folate. The combination of isoniazid and cycloserine did not appear to have a synergistic effect in depressing growth.

The inhibitory effect manifested by either isoniazid or cycloserine on the growth of L. casei was less apparent when these drugs were added to medium containing crystalline folic acid to which had been added pyridoxine in a concentration of 10μg./ml. However, the inhibitory effect on growth of these drugs was not modified by the addition of pyridoxine to medium containing serum as a source of folate; further, the addition of pyridoxine to the assay system did not normalize the folate concentration of sera obtained from patients who were receiving isoniazid or cycloserine.

Vitamin B12 The addition of pyrazinamide or isoniazid, in concentrations up to 1000μg./ml., to the medium did not result in depression of the growth of L. leichmannii. Growth of this bacteria was depressed by the addition of cycloserine in a concentration of 10μg./ml. to medium containing crystalline vitamin B12 and by the addition of this drug in a concentration of 100μg./ml. to medium containing serum as a source of vitamin B12. Growth was proportionately further depressed by the addition of progressively increased concentrations of the drug. The inhibitory action of cycloserine on bacterial growth was not modified by the addition of pyridoxine to the medium in the in vitro studies and the addition of pyridoxine to the assay system did not normalize the vitamin B12 concentration of sera obtained from patients who were receiving antituberculosis drugs.

DISCUSSION

Two patients have been described in this report who were found to have a megaloblastic anemia during therapy with isoniazid and cycloserine for pulmonary tuberculosis. In both, the anemia was shown to be secondary to folate deficiency. The findings in these two patients and the observation that serum folate concentrations were subnormal in 17 and in the indeterminate range in 24 of 84 other patients who were receiving drug therapy for tuberculosis suggest that the administration of certain of these drugs, notably cycloserine, may result in the development of folate deficiency. However before attributing the abnormal folate determinations detected in these patients exclusively to a result of drug therapy, the role of inadequate dietary intake and the significance of the disease process itself in the etiology of folate deficiency must be considered.

Serum folate concentrations were also found to be subnormal in one-third of a group of 36 patients with untreated tuberculosis tested in the present study and were below the range found in control subjects in a similar percen-

* Supplied through the courtesy of Dr. L. D. Bechtoe, The Eli Lilly Laboratory for Clinical Research.
tage of patients with untreated tuberculosis recently described by Roberts and his associates in Great Britain. The data suggest that inadequate dietary intake, rather than the presence of the disease, was usually the principal factor responsible for abnormal folate determinations in patients with untreated tuberculosis described in the present report. Serum folate levels were subnormal in 80 per cent of poorly nourished, alcoholic patients with untreated tuberculosis seen at Bellevue Hospital, an incidence not appreciably greater than that which has been described in previous studies on the nontubercular alcoholic population both at Bellevue and elsewhere. It would seem probable that folate deficiency in these patients was principally on the basis of inadequate dietary intake. Both patients described as case reports had been on a hospital diet for at least 3 months and were gaining weight prior to the time that folate deficiency was detected, and in one patient normoblastic erythropoiesis was demonstrated by bone marrow examination prior to the institution of drug therapy for tuberculosis. Similarly, all 84 other patients studied who were on drug therapy had received a hospital diet for at least two months (the majority for periods of greater than 6 months) prior to study and appeared to be adequately nourished. It is possible, but seems unlikely, that inadequate dietary intake was responsible for the presence of folate deficiency in patients receiving drug therapy.

In contrast to the findings in poorly nourished alcoholic patients studied at Bellevue, serum folate concentrations were subnormal in only 21 per cent of patients with untreated tuberculosis who were studied at the Bronx Municipal and other hospitals; the majority of these patients came from a higher socioeconomic background and did not appear malnourished. The observations in this group of patients indicate that the presence of active pulmonary tuberculosis can be associated at times with folate deficiency even in adequately nourished subjects. The cause for this is unclear but it may be related to increased requirement for folate. We cannot exclude the possibility that the presence of pulmonary tuberculosis may have played a role in the etiology of the abnormal folate determinations which were observed in some patients who were receiving drug therapy, but it seems unlikely that this factor can account for the more than twofold higher incidence of subnormal serum folate concentrations found in patients receiving cycloserine than in well-nourished untreated patients.

The mechanism by which cycloserine can induce abnormal folate determinations has not been defined by this study. The administration of certain other drugs, principally the anticonvulsants, has also been found to be associated with the presence of subnormal serum folate concentrations in the absence of anemia as well as, in some instances, the development of a megaloblastic anemia which has responded to treatment with a physiologic dose of 25 µg. daily of folic acid. Although the locus of action of these drugs has not been determined, it is of interest that certain drugs used either as anticonvulsants or for the therapy of tuberculosis have a structural similarity to the folate molecule and that the two drugs, diphenylhydantoin and cycloserine, which have been found to be most commonly associated with abnormal folate
FOLATE DEFICIENCY AND DRUG THERAPY FOR TUBERCULOSIS

**Fig. 5.—Formulae for folic acid and certain drugs used in the treatment of convulsant disorders (upper) and tuberculosis (lower).**

Determinations both have a 5-membered nitrogen-containing ring which resembles the active N5\(^\circ\) 5-membered nitrogen-containing ring of folate (Fig. 5). In vitro studies have shown a dissimilarity between the anticonvulsant drugs and certain drugs included in this study. Unlike the anticonvulsant drugs,\(^7\) both isoniazid and cycloserine were found to depress the growth of *L. casei*, isoniazid at very high concentrations only and cycloserine at lower concentrations which approximated those achieved by pharmacologic doses of the drug. The finding that this inhibitory action was modified by the addition of pyridoxine to medium containing crystalline folic acid suggests that some of the abnormalities noted in the in vitro studies were related to a disturbance in pyridoxine metabolism and raises the possibility that a similar situation pertained in patients receiving cycloserine.

Both isoniazid and cycloserine have been identified as pyridoxine antagonists\(^{22-25}\) and the administration of a combination of these two drugs together with parenterally administered iron to experimental animals has been shown to result in the development of a hypochromic anemia and the appearance of
ringed sideroblasts in the bone marrow. Both patients described as case reports had received isoniazid, cycloserine, and iron prior to the precipitous development of anemia, and both were found to have, in addition to megaloblastic erythropoiesis, hypochromic erythrocytes in the peripheral blood smear and ringed sideroblasts in the bone marrow. These morphologic changes, which are characteristically found in sideroblastic anemia, have been described previously in 8 patients who were receiving therapy with isoniazid alone as well as in 6 patients who were receiving this drug in combination with either pyrazinamide or cycloserine or both. Treatment with pyridoxine or withdrawal of these drugs resulted in a hematologic remission in some of these patients. Megaloblastic changes in the bone marrow were also described in 3 of these patients, in one of whom the serum folate concentration was reported to be subnormal. The etiology of the folate deficiency in these patients, as well as in other patients with sideroblastic anemia secondary to a variety of other causes who have been found to have folate deficiency, is unknown. As has been the usual experience in patients with folate deficiency associated with sideroblastic anemia, treatment with folic acid resulted in only a partial hematologic remission in the 3 patients who were receiving antituberculosis drug therapy. This is in contrast to the complete hematologic remission achieved by such therapy in the 2 patients described in this report. At the present time it remains unknown as to whether the abnormal folate determinations observed in patients receiving isoniazid and cycloserine are related to an abnormality in pyridoxine metabolism.

Serum vitamin B12 concentrations were found to be in the indeterminate range in 14 and subnormal in 3 of the 84 patients in this study who were receiving drug therapy for tuberculosis. Heinivaara and Pavla recorded subnormal serum concentrations of this vitamin in 45 of 74 nonanemic patients who were receiving therapy with isoniazid and para-aminosalicylic acid and attributed the abnormal determinations in these patients, as well as the occurrence of a megaloblastic anemia in 2 other patients who were receiving triple therapy, to malabsorption of vitamin B12. A selective impairment of absorption of this vitamin has been described in from 43 to 90 per cent of patients receiving therapy for tuberculosis with isoniazid and para-aminosalicylic acid. The latter drug has been incriminated as the responsible agent since its withdrawal results in normalization of vitamin B12 absorption within 2 to 6 weeks. This absorptive defect has been attributed to an inhibition of enzymatic mechanisms involved in the passage of vitamin B12 across the ileal mucosa which may be related to a competitive inhibition of folate, since the concomitant administration of folic acid results in a return to normal of vitamin B12 absorption despite continued treatment with para-aminosalicylic acid. The results of the present study indicate that serum vitamin B12 concentrations can be subnormal in patients receiving antituberculosis drugs other than para-aminosalicylic acid since this drug was included in the medications of only 4 of the 17 patients who had serum concentrations that were below normal. Further, the results of both serum assays using the radioisotope dilution technic and the in vitro studies suggest that subnormal serum concentrations in
patients receiving cycloserine are not an indication of vitamin B₁₂ deficiency but, rather, reflect inhibition of the microbiologic assay system. A circumstance similar to this has been described in association with the use of other drugs.⁴²

The significance of the acute arrest of erythropoiesis which developed in case 2 is not clear. This arrest was characterized by the progressive disappearance of megaloblastic and other erythroid precursor forms from the bone marrow and the appearance of giant proerythroblasts in the marrow. The presence of these bizarre giant cells in the marrow, which are characteristically present during an acute aplastic crisis, has been described in a variety of hematologic disorders⁴³⁻⁴⁸ and in some instances has been attributed to a reaction to a variety of drugs including diphenylhydantoin.⁴⁷ The frequent association of episodes of acute arrest of erythropoiesis with antecedent infections⁴¹⁻⁴⁶⁻⁴⁸ has suggested a causal relationship between the two and it is possible that the acute arrest in case 2 was related to her prior febrile episode. Whether the sequential occurrence of these two hematologic abnormalities in this patient was related or purely coincidental is unknown. Megaloblastic changes have been described during the recovery phase of acute arrest of erythropoiesis⁴⁸⁻⁴⁹ but rarely preceding it.⁵⁰

**Summary**

Two patients have been described who were found to have a megaloblastic anemia due to folate deficiency while receiving treatment for pulmonary tuberculosis with isoniazid and cycloserine. In an additional 120 patients studied with tuberculosis, serum folate concentrations were subnormal in 7 of 12 inadequately nourished and in 5 of 24 well-nourished patients with untreated tuberculosis, in 15 of 29 patients taking isoniazid and cycloserine, but in only 2 of 55 patients receiving other combinations of drugs for tuberculosis. In vitro studies showed that isoniazid had a depressant effect on the growth of *L. casei* but only when added in a concentration that greatly exceeded the pharmacologic dose; cycloserine had a similar effect at a concentration that was only threefold greater than that usually found in patients receiving therapeutic doses of this drug.

Serum concentrations of vitamin B₁₂ were normal in both patients described with megaloblastic anemia as well as in all 36 patients with untreated tuberculosis and were in the indeterminate range in 14 and subnormal in 3 patients who were receiving drug therapy. The addition to medium in vitro of cycloserine, but not of isoniazid or pyrazinamide depressed the growth of *L. leichmannii*.

These results confirm previously reported observations by others which indicate that folate deficiency is a frequent occurrence in patients with untreated tuberculosis. They suggest that abnormal folate determinations in these patients are due principally to inadequate dietary intake. In addition, the significantly greater incidence of abnormal folate determinations that was observed in adequately nourished patients taking cycloserine and the results of the in vitro studies suggest that this drug can be responsible for folate deficiency in some instances. The mechanism for this is unknown.
SUMMARIO IN INTERLINGUA

Es describite duo patientes in qui anemia megaloblastic esseva demonstrate como effecto de carentia de folato associate con le administration de isoniazida e cycloserina in le tractamento de tuberculosis pulmonar. In 120 patientes tuberculotic additional, le concentrationes seral de folato esseva subnormal in (1) 7 de 12 inadequate nutriti subjectos, (2) 5 de 24 ben-nutrite subjectos sin pretractamento de tuberculosis, e (3) 15 de 29 patientes recipiente isoniazida e cycloserina sed in (4) solmente 2 de 55 patientes recipiente alte combinationes de pharmacos como tractamento de tuberculosis. Studios in vitro ha monstrate que isoniazida exerce un effecto depressori super le crescentia de Lactobacillus las casei sed solmente quando illo esseva addite in un concentration grandemente in excesso del dosage pharmacologic. Cycloserina exerceva un simile effecto in un concentration solmente tres vices plus grande que illo usualmente incontarat in patientes recipiente doses thera- peutic de iste pharmaco.

Le concentrationes seral de vitamina B₁₂ esseva normal in ambe le describite patientes con anemia megaloblastic e etiam in omne le 36 patientes con non-tractate tuberculosis. Illos esseva de un ordine de magnitude indetermine in 14 e subnormal in 3 patientes recipiente agents pharmatherapeutic. Le crescentia in vitro de Lactobacillus leichmannii esseva deprimite per le supplementation del medio con cycloserina sed non con isoniazida o pyrazinamida.

Iste resultatos confirma previamente reportate observationes per alte autores indicante que carentia de folato es un occurrence frequentemente in patientes con non-tractate tuberculosis. Illo suggestiona que anormal valores de folato in tal patientes es causate principalmente per un inadequate quota dietari de folato. In plus, le significativamente plus grande incidence de anormal valores de folato observate in adequately nutriti patientes recipiente cycloserina, insimil con le resultatos del studios in vitro, suggestiona que iste pharmaco pote esser responsable pro carentia de folato in certe casos. Le correspondente mechanismo non es cognoscite.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Helen Ranney for calling their attention to case 2, Drs. Henrietta Marcus and Simon Schwartz for assistance in obtaining patient material, and Dr. Restituto Ruiz and Mrs. Patricia Weitzner for performing the microbiologic assays.

REFERENCES

10. Lau, K-S., Gottlieb, C., Wasserman, L. R., and Herbert, V.: Measurement of serum vitamin B₁₂ level using ra-
FOLATE DEFICIENCY AND DRUG THERAPY FOR TUBERCULOSIS

911


Folate Deficiency Associated with Drug Therapy for Tuberculosis

FREDERICK A. KLIPSTEIN, FREDERICK G. BERLINGER and L. JUDEN REED