Serum Vitamin B₁₂ in Leukemias and Malignant Lymphomas

By M. Rachmilewitz, G. Izak, A. Hochman, J. Aronovitch and N. Grossowicz

RECENTLY developed microbiologic methods for the determination of vitamin B₁₂ concentrations in body fluids² have established that the vitamin B₁₂ levels in the serum are low in patients with pernicious anemia in relapse and other macrocytic anemias.¹ Normal blood levels of vitamin B₁₂ were found in a wide variety of diseases including the sera of patients with lymphatic leukemia, undifferentiated stem cell leukemia and some types of malignant lymphomas⁴ while extremely high vitamin B₁₂ values were reported in the sera of patients with myeloid leukemia.⁴⁻⁷

In this communication our findings on B₁₂ concentrations in various types of leukemias and malignant lymphomas are reported. An attempt has been made to use the serum B₁₂ concentrations as additional means of differentiation between the various types of malignant conditions of the blood forming organs. In addition the effect of treatment in chronic leukemia on serum vitamin B₁₂ concentrations was studied.

METHODS AND MATERIAL

The modified E. coli assay described previously⁴ was employed. Serum vitamin B₁₂ values ranged from 200–500 μg/ml with a mean of 340 μg/ml in 25 healthy individuals. The serum samples were diluted 1:8 with distilled water. For estimation of the total vitamin B₁₂ content the diluted serum (1:8) was heated in a boiling water bath for 30 minutes and added aseptically to the medium. In an alternative procedure the vitamin B₁₂ was liberated from the serum proteins by precipitation with acetate buffer (pH 5.5), according to Spray;⁸ nearly the same results were obtained as without precipitation. The free vitamin B₁₂ was determined in the unheated sample. The bound vitamin was calculated as the difference between the total and free values.

To determine the maximum binding capacity of serum for B₁₂ (M.B.C.) 1 ml. of serum was mixed with 0.5 ml. of distilled water containing known amounts of crystalline vitamin B₁₂ and incubated at 4 C. for 24 hours. The serum was then re-assayed for bound and free vitamin B₁₂. The M.B.C. was determined using at least two different concentrations of vitamin B₁₂, usually 1,500 and 3,000 μg/ml. In 10 normal individuals in whom the total B₁₂ ranged from 200–500 μg/ml., the M.B.C. was in the range of 1,200–2,000 μg/ml.; the recovery was 80–110 per cent of the added vitamin. When the serum with added B₁₂ was incubated for one hour instead of twenty-four hours, the M.B.C. was found to be lower, approximately 1,000 μg/ml.

Since the E. coli responds to methionine as well as to vitamin B₁₂, control assays were done on serum treated with sodium hydroxide (final concentration 0.4N), to destroy the B₁₂. Thereafter the samples were steamed for one hour, neutralized and re-assayed. Practically no growth promoting activity was found in the sera after this treatment.

Sixty-seven patients are included in this study.

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Patients

- Acute, subacute and chronic myeloid leukemia: 20
- Chronic lymphatic leukemia: 8
- Undifferentiated leukemia: 9
- Hodgkin’s disease: 11
- Generalized and localized lymphosarcoma: 6
- Myelosclerosis: 3
- Primary polycythemia: 8
- Multiple myeloma: 2

In some of the patients with myeloid leukemia who showed elevated serum levels of vitamin B₁₂, repeated determinations were performed after various therapeutic procedures.

Results

Table 1 shows the vitamin B₁₂ concentrations in patients with myeloid leukemia. It may be seen that all but one patient had very high serum vitamin B₁₂ values ranging from 1,100 to 25,000 μg/ml. This group includes one patient with subacute (case 1, table 1) and another with acute (case 6, table 1) myeloid leukemia, with myeloid differentiation; in both these cases the serum B₁₂ was high.

In the one patient with chronic myeloid leukemia with normal W.B.C. counts the assay was performed shortly before death at the time when the disease was complicated by erysipelas and septicemia for which large amounts of antibiotics had been administered.

Generally, there was no correlation between the total W.B.C. count and the serum vitamin B₁₂ concentrations; the highest B₁₂ value of about 25,000 μg/ml. was found in a case where at the time the W.B.C. was 14,000/cu.mm. On the other hand, the serum of a patient with 166,000 W.B.C./cu.mm. had a serum B₁₂ level of 2,500 μg/ml.

There also appeared to be no correlation between the duration of the illness, the extent of hepato-splenomegaly and the serum vitamin B₁₂ concentrations.

Serial examinations of B₁₂ in 4 cases (1, 2, 5, 19, table 1) of myeloid leukemia illustrate the effect of treatment on the B₁₂ level. In case no. 2 the initial B₁₂ level of 3,350 μg/ml. fell to 2,750 μg/ml. within 10 days following x-ray therapy and then dropped to 720 μg/ml. 4 weeks later. At the same time the leukocyte count had dropped gradually from 135,000/cu.mm. to 68,000/cu.mm. after 10 days and down to 5,900/cu.mm. after 4 weeks. Two months later, the B₁₂ rose slightly to a level of 1,120 μg/ml., while the leukocyte count was 23,400/cu.mm. At this time x-ray therapy was reinstituted, following which the B₁₂ level dropped to 680 μg/ml. in spite of a high leukocyte count (45,300) (fig. 1).

In another patient (case 1, table 1) a woman aged 32, with subacute myeloid leukemia and a W.B.C. count of 3,350/cu.mm., the B₁₂ value was found to be 2,700 μg/ml. After cortisone therapy and several blood transfusions the patient’s condition improved, the vitamin B₁₂ concentrations fell to 950 μg/ml. 2 weeks later, but it soon rose to the pre-treatment level in spite of continuous therapy (fig. 2). In 2 other cases of chronic myeloid leukemia (cases 5 and 19) the drop in white cell count following x-ray therapy preceded the decrease in the serum B₁₂ concentration (fig. 3).

In a case of myeloid leukemia which developed in the course of a long-standing
polycythemia (case 7, table 3) x-ray treatment had no effect on the W.B.C. and on the size of liver and spleen; the B₁₂ level was very high (6,250 μg/ml.) and remained so despite treatment.

**Acute and Chronic Undifferentiated Leukemia**

This group includes 9 patients of whom 7 were suffering from acute stem cell leukemia, 1 from chronic stem cell leukemia and 1 from chronic reticulum cell leukemia. The diagnosis in these cases was established on the basis of the morphology of the peripheral blood and bone marrow. In 4 of the cases post mortem...
examinations confirmed the clinical diagnosis. In all of these patients the serum vitamin B₁₂ levels were normal and ranged from 130 to 600 μg/ml. (table 2.)

In 8 patients with chronic lymphatic leukemia the serum vitamin B₁₂ fell within the normal range (from 50 to 650 μg/ml.).

**Hodgkin’s Disease**

In 11 cases the serum vitamin B₁₂ examined at different stages of the disease showed normal values (from 120 to 650 μg/ml.) (table 2).

**Lymphosarcoma**

Four cases with generalized lymphosarcoma with systemic lymph node involvement, without abnormal cells in the peripheral blood and bone marrow were studied. The diagnosis in these cases was established by biopsy. In 2 of them the serum B₁₂ was normal (380, 450 μg/ml.) while in the other 2 cases the serum B₁₂ was increased (790 and 1,450 μg/ml.). Two cases with localized lymphosarcoma were examined. In one case with round cell sarcoma involving the spleen and abdominal lymph nodes, which was verified at autopsy, the serum B₁₂ value was moderately increased (780 μg/ml.). In the sixth case with lymphosarcoma of the spleen infiltrating the surrounding tissue, the B₁₂ was normal (table 2).

**Myeloproliferative Disorders: Polycythemia Vera**

Eight patients were examined and the results are given in table 3. One patient was found to have very high B₁₂ serum levels (case 7, table 3) at the time when she developed clinical and hematologic signs of myeloid leukemia. In this patient
SERUM VITAMIN B₁₂ IN LEUKEMIAS AND MALIGNANT LYMPHOMAS

**Fig. 2**

**Fig. 3**
the serum B₁₂ was found to be high on three occasions during x-ray therapy and remained high following the treatment. The remaining 7 patients had serum vitamin B₁₂ concentrations within the normal range with leukocyte counts as high as 60,000/cu.mm., but with a normal differential count.

In another patient (case 8, table 3) with polycythemia of 5 years duration which had been treated by venesection, at the time of the serum vitamin B₁₂ examination, the leukocyte count was 36,000 with a differential count of 1 per cent blasts, 3 per cent myelocytes, 29 per cent metamyelocytes, 54 per cent polymorphonuclears, thus suggesting myeloid leukemia. The serum vitamin B₁₂ was in the upper range of normal.

**Myelosclerosis**

Three patients were examined. In one case of myelosclerosis (case 1, table 4) with myeloid infiltration of kidneys, liver and spleen, which was later verified at autopsy, the total serum vitamin B₁₂ was at first slightly increased (970 μg/ml). The patient had marked leukopenia without abnormal cells in the peripheral blood. Repeated blood transfusions were followed by the lowering of the serum vitamin B₁₂ level (480 and 300 μg/ml). Shortly before death the serum vitamin

### Table 2

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of cases</th>
<th>Serum B₁₂ (μg/ml)</th>
<th>Minimum</th>
<th>Maximum</th>
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<tr>
<td>Lymphosarcoma</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(a) Generalized</td>
<td>4</td>
<td></td>
<td>340</td>
<td>1,450</td>
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<tr>
<td>(b) Localized</td>
<td>2</td>
<td></td>
<td>360</td>
<td>780</td>
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<tr>
<td>Multiple myeloma</td>
<td>2</td>
<td></td>
<td>250</td>
<td>280</td>
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<tr>
<td>Stem cell leukemia</td>
<td>9</td>
<td></td>
<td>130</td>
<td>600</td>
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<tr>
<td>Lymphatic leukemia</td>
<td>8</td>
<td></td>
<td>50</td>
<td>650</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>11</td>
<td></td>
<td>120</td>
<td>650</td>
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</table>

### Table 3.—Polycythemia Vera

<table>
<thead>
<tr>
<th>No.</th>
<th>Date</th>
<th>Hb Gm%</th>
<th>R.B.C./cu.mm. (million)</th>
<th>W.B.C./cu.mm.</th>
<th>Thromb./cu.mm.</th>
<th>Serum B₁₂ μg/ml</th>
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<tr>
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<td>3.6.55</td>
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<td>7.5</td>
<td>60,000</td>
<td>280,000</td>
<td>260</td>
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<td>16.8</td>
<td>5.6</td>
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<td>288,000</td>
<td>400</td>
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<tr>
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<td>16.7</td>
<td>5.6</td>
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<td>350,000</td>
<td>190</td>
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<td>17.0</td>
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<td>14,000</td>
<td>280,000</td>
<td>350</td>
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<tr>
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<td>5.9.55</td>
<td>21.7</td>
<td>7.1</td>
<td>10,300</td>
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<td>520</td>
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<td>7</td>
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<td>9.5</td>
<td>3.2</td>
<td>10,500</td>
<td>160,000</td>
<td>3,000</td>
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<td>3</td>
<td>4.56</td>
<td>9.5</td>
<td>3.36</td>
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<td>3,000</td>
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<td>120,000</td>
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<tr>
<td>8</td>
<td>4.26.56</td>
<td>17.2</td>
<td>7.65</td>
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<td>600</td>
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</table>
Table 4.—Myelosclerosis

<table>
<thead>
<tr>
<th>No.</th>
<th>Date</th>
<th>Hb Gm%</th>
<th>R.B.C., cu.mm. (million)</th>
<th>W.B.C., cu.mm.</th>
<th>Serum B₁₂ µg/ml</th>
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<td>800</td>
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<td>9.0</td>
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<td>3,600</td>
<td>900</td>
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<td></td>
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<td>2.1</td>
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<td>400</td>
</tr>
<tr>
<td></td>
<td>5.2.55</td>
<td>4.8</td>
<td>1.6</td>
<td>3,300</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>5.22.55</td>
<td>5.4</td>
<td>—</td>
<td>21,000</td>
<td>2,000</td>
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<tr>
<td>2</td>
<td>5.31.55</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>700</td>
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<td></td>
<td>6.6.55</td>
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<td>2.2</td>
<td>3,100</td>
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<tr>
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<td>11.11.55</td>
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<td>3</td>
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<td>—</td>
<td>—</td>
<td>16,500</td>
<td>300</td>
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<td></td>
<td>7.24.56</td>
<td>—</td>
<td>—</td>
<td>15,400</td>
<td>400</td>
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</tbody>
</table>

**Fig. 4**

Graph showing changes in B₁₂ µg/ml and W.B.C./cu.mm over time.
B_{12} concentration suddenly rose to 2,000 \mu g/ml., together with the appearance of leukocytosis and young myeloid cells in the peripheral blood (fig. 4).

In the second case, which ran a chronic course, the vitamin B_{12} concentration in the serum was slightly elevated on two occasions (700, 900 \mu g/ml.). Seven months later, the patient developed a full-blown picture of myeloid leukemia and died shortly afterwards. In the third case the serum vitamin B_{12} was normal.

In 2 cases of multiple myeloma the serum vitamin B_{12} concentrations were normal (table 2).

**Serum Binding Capacity of Vitamin B_{12}**

In 6 patients with chronic myeloid leukemia with high serum vitamin B_{12} concentrations, free and total B_{12} were examined. In 5 of them practically no free B_{12} was present and all the B_{12} was bound to the proteins of the serum. In the sixth patient, free vitamin B_{12} constituted 20 per cent of the total. The B_{12} binding capacity was examined in 5 patients of this group. The values found were rather high: 3,500, 4,300, 5,000, 5000 and 8,000 \mu g/ml. respectively.

In 6 patients with Hodgkin's disease, with normal vitamin B_{12} concentrations, the binding capacity was within the normal range (900–1,400 \mu g/ml.).

In 2 cases with acute undifferentiated leukemia and in 1 case of lymphosarcoma, the binding capacity was normal (750, 750, and 1,050 \mu g/ml. respectively).

In 1 case of myelosclerosis, with normal serum vitamin B_{12}, the binding capacity of the serum was increased (1,900 and 3,100 \mu g/ml.). When the serum vitamin B_{12} increased to 2,000 \mu g/ml. the binding capacity of the serum rose to 6,000 \mu g/ml.

**DISCUSSION**

Our findings of high serum vitamin B_{12} concentrations in chronic myeloid leukemia are in agreement with those reported by Beard et al.\textsuperscript{5, 6} Killander\textsuperscript{7} and Mollin and Ross.\textsuperscript{4} In 2 cases of acute myeloid leukemia with differentiation beyond the myeloblast stage, high serum vitamin B_{12} values were found. On the other hand, acute and chronic stem cell leukemia and lymphatic leukemia were associated with normal serum vitamin B_{12} levels. These results indicate that the serum vitamin B_{12} determinations may be used as an additional means of differentiation between the various types of leukemia, particularly in those cases where the classification on a morphologic basis is difficult or impossible.

It seems that the myeloid proliferation is responsible for the elevation of serum vitamin B_{12}. There is, however, no correlation between the number of leukocytes in the peripheral blood and serum vitamin B_{12} concentrations. It was also impossible to correlate the differences in the serum vitamin B_{12} concentration with differences in the numbers of mature or immature cells in the peripheral blood and bone marrow, an observation made also by Mollin and Ross.\textsuperscript{4}

It might be concluded, therefore that the degree of increase of serum vitamin B_{12} is dependent upon the extent of the myeloid proliferation in the bone marrow and in the extramedullary sites of hematopoiesis, and not on the degree of release of myeloid cells into the peripheral blood. This point is also borne out by the observation in myelosclerosis, where the serum B_{12} level seems to rise with
myeloid proliferation in the extramedullary organs, in the absence of abnormal cells in the peripheral blood.

The cause of the increase in serum vitamin B₁₂ is not known. The increased binding capacity of serum protein for vitamin B₁₂ reported,⁶ and confirmed in this study, suggests that in the process of myeloid proliferation vitamin B₁₂ binding proteins are liberated in large amounts. In view of our findings of increased serum vitamin B₁₂ values in liver disease as well as in experimental liver damage,¹⁰ the possibility of liver cell disintegration in myeloid leukemia as the cause of the high serum vitamin B₁₂ concentrations must be considered, more so since in liver cell necrosis the binding capacity of serum for vitamin B₁₂ is also increased, together with elevation of serum vitamin B₁₂. However, it is questionable whether the extent of liver cell injury in myeloid leukemia could account for the increase of serum vitamin B₁₂.

It is possible that the moderate elevation of serum vitamin B₁₂ in 2 cases of generalized lymphosarcoma was due to invasion of the liver. This assumption gains support by the findings of high B₁₂ values in the sera of patients with malignant tumors with extensive metastases to the liver.¹¹ The role of the liver as a possible cause of elevation of serum vitamin B₁₂ in myeloproliferative diseases needs further elucidation.

Our findings in polycythemia suggest that leukocytosis alone is not associated with elevated serum vitamin B₁₂. In 1 case with a leukocyte count as high as 60,000 the serum vitamin B₁₂ concentration was found to be normal. In another case, however, in which the full-blown clinical and hematologic picture of leukemia developed in spite of only a moderate leukocytosis, a very high B₁₂ concentration was present and the values increased steadily despite x-ray therapy. Thus the serum vitamin B₁₂ determination may possibly help to differentiate between leukemoid reactions and real leukemia in the course of polycythemia.

It is also possible that the serum vitamin B₁₂ may be used in evaluating the extent of myeloid metaplasia in myelosclerosis.

**Summary**

High serum vitamin B₁₂ levels were found in chronic myeloid leukemia and in acute leukemia with myeloid differentiation. Following x-ray therapy and repeated blood transfusions, a drop of serum vitamin B₁₂ was found.

In chronic lymphatic leukemia, undifferentiated stem cell leukemia, Hodgkin’s disease, and multiple myeloma, the serum vitamin B₁₂ concentrations were normal.

In polycythemia with marked leukocytosis the serum B₁₂ was normal.

In myelosclerosis high B₁₂ values may be found.

The serum vitamin B₁₂ in chronic myeloid leukemia is in a bound form and the binding capacity for added B₁₂ is increased.

Serum vitamin B₁₂ determination may be of some value in differentiating various types of leukemia and other myelo-proliferative disorders.

**Summary in Interlingua**

Alte nivellos seral de vitamina B₁₂ esseva trovate in chronic leucemia myeloide e in acute leucemia con differentiation myeloide. Post roentgenotherapia e
repetite transfusiones de sanguine, un reduction del nivello seral de vitamina B$_{12}$ esseva constatate.

In chronic leucemia lymphatic, leucemia a cellulas indifferentiae, morbo de Hodgkin, e myeloma multiple, le concentrationes seral de vitamina B$_{12}$ esseva normal.

In polycythemia con marcate grados de leucocytosis le nivello seral de vitamina B$_{12}$ esseva normal.

In myelosclerosis, alte nivelllos seral de vitamina B$_{12}$ esseva trovate.

In leucemia myeloide, le vitamina B$_{12}$ del sero es presente in forma ligate, e le capacitate ligatori pro B$_{12}$ additional es augmentate.

Determinationes del nivelllos seral de vitamina B$_{12}$ va possibilemente esser de valor in le differentiation de varie tipos de leucemia e de altere disordines myelo-proliferative.

REFERENCES
Serum Vitamin B$_{12}$ in Leukemias and Malignant Lymphomas

M. RACHMILEWITZ, G. IZAK, A. HOCHMAN, J. ARONOVITCH and N. GROSSOWICZ