Deficiency of Vitamin B₁₂-Binding Alpha Globulin in Two Brothers

By Ralph Carmel and Victor Herbert

Endogenous vitamin B₁₂ circulates bound to an alpha-1 glycoprotein.²³ Added vitamin binds primarily to a globulin with beta mobility electrophoretically, which may serve mainly a temporary transport function.³⁴ This beta globulin readily gives up its vitamin B₁₂ to cells, while the alpha globulin seems to serve as a circulating storage depot.³⁵⁶

With the exception of some conditions involving longstanding neutropenia,⁷ˣ⁻⁸ deficiency of B₁₂-binding proteins has been reported in only two instances. In one,⁹ various features suggested poor B₁₂ binding by serum, but subsequently, the serum B₁₂-binding capacity was found to be normal.¹⁰ The other report¹¹ was of transient deficiency of B₁₂-binding beta globulin in a subject with pernicious anemia.

The following is a study of persistent B₁₂-binding alpha globulin deficiency in two brothers, which, to our knowledge, is the first report of such a defect.

Case Reports

F.B., a 47 year old Puerto Rican-Corsican male, had a subtotal gastrectomy for recurrent gastric ulcer in July 1963. In May 1966, his serum B₁₂ level was 0 by coated charcoal assay,¹² with a hemoglobin of 11.9 Gm. per cent, white cell count of 11,300/mm³ with normal differential count, and platelet count of 260,000/mm³. Lobe average was normal (3.12 lobes/neutrophil); there were no megaloblastic changes. He had no antibody to either parietal cells or intrinsic factor. Gastric analysis revealed intrinsic factor; his Schilling test was normal (9.2 per cent excretion of a 2 µg. dose in 48 hours). Upper GI x-ray studies showed a normal gastric stump; small bowel follow-through revealed rapid transit time and slight dilation of the small intestine but no other abnormalities. Serum
iron was 67 μg. per cent, total iron-binding capacity 335 μg. per cent. Radioactive iodine uptake was normal.

In 1958 he had undergone mitral commissurotomy for rheumatic mitral stenosis. During that year hemoglobin ranged between 11.7 and 15.5 Gm per cent, and white cells between 7,900 and 9,500/mm.³ with normal differential counts. Blood morphology was unremarkable except for some red cell hypochromia. In 1966, 0.8–1.2 Gm. quinidine orally daily was begun for atrial fibrillation, and maintained to the present. It has been the only medication other than B₁₂ taken regularly.

Family history was not known except that his mother died of carcinoma of the colon, and his father of "stroke."

Physical examination revealed only the cardiac findings of a diastolic blowing murmur along the left sternal border with an opening snap, and a low-pitched apical rumble, and patchy dark pigmentation about the left ankle.

He was discharged on monthly vitamin B₁₂ (100 μg.) injections. In 1968, the patient was reevaluated because of low B₁₂-binding capacity by coated charcoal assay¹³ in his serum during a routine screening study. (The B₁₂ and B₁₂-binding protein values are listed in Table 1.) Serum B₁₂ levels were low by microbiologic assay¹⁴ as well as by coated charcoal assay. Hemoglobin was 13.5 Gm. per cent, white cell count 9,500/mm.³ with a normal differential count, platelet count 190,000/mm.³ and reticulocyte count 1.5 per cent. Red cell and white cell morphology was normal. Leukocyte alkaline phosphatase was normal. Hemoglobin electrophoresis revealed 30.5 per cent S, 63.4 per cent A, 4.1 per cent A₂, and 2.0 per cent F hemoglobin. Urine analysis, serum folate, bilirubin, urea nitrogen, cholesterol, SCOT, LDH, sugar, uric acid and lysozyme* levels were normal. A Schilling test was normal once again (22.9 per cent excretion of a 2 μg. dose in 48 hours). Urinary excretion of methylmalonic acid § was within normal limits. Small bowel x-ray study was now entirely normal, as were 72 hour fecal fat excretion, serum Vitamin A and carotene levels, and 5-hour d-xylene excretion. Glucose tolerance test showed a mildly diabetic pattern. X-ray of the skull was normal, as were serum corticosteroid, electrolyte, protein-bound iodine, and T₃ uptake levels.

Serum protein electrophoresis was unremarkable (4.38 Gm. per cent albumin, 0.31 Gm per cent α₁ globulin, 0.75 Gm. per cent α₂ globulin, 0.88 Gm. per cent β globulin, and 1.19 Gm. per cent γ globulin), as were immunoglobulin determinations. Haptoglobin was 158 mg. per cent, serum iron 59 μg. per cent, total iron-binding capacity 432 μg. per cent, and ceruloplasmin 155.8 mg. per cent (normal: 20–40). Thyroid-binding globulin was 22.3 μg. per cent and pre-albumin 161 μg. per cent (normal: 17–25 and 180–300 respectively). § Urinary amino acid excretion was quantitatively normal.

Vitamin B₁₂ therapy has been discontinued at present.

Summary

P. B., a 47 year old male, was found to have a low serum B₁₂ with low levels of B₁₂-binding capacity, primarily of the alpha globulin component. No other protein abnormalities could be found. Other conditions present in the patient were rheumatic heart disease, postgastrectomy status, sickle cell trait, iron deficiency, and mild diabetes mellitus; the only drug taken regularly was quinidine. No evidence of any of the changes of B₁₂ deficiency could be found.

W.B., 46 year old brother of P.B., was discovered to have low serum B₁₂ by coated charcoal and microbiologic assays during survey of the family. The only positive historical and physical findings are those pertaining to his 10 year history of multiple sclerosis. He has not been taking any medications.

*Assayed by Dr. E. Osserman, Columbia University College of Physicians & Surgeons, N. Y.
† Assayed by Dr. A. Giorgio, New York Medical College, N. Y.
‡ Assayed by Drs. I. Sternlieb and I. H. Scheinberg, Albert Einstein College of Medicine, N. Y.
§ Assayed by Dr. J. H. Oppenheimer, Montefiore Hospital, N. Y.
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Laboratory data include 40.5 per cent hematocrit, white cell count of 10,500/mm.3 with normal differential count, and platelet count of 240,000/mm.3 No macro-ovalocytosis was seen. Although few 5-lobed and no 6-lobed neutrophil nuclei were seen, there was a mild increase in 4-lobed nuclei; lobe average was increased slightly at 3.45 lobes/neutrophil (normal: 3.17 ± 0.25). Serum folate was in the borderline-low range (4.5 ng/ml). Bone marrow examination showed no evidence of megaloblastic changes. In vitro study of deoxyuridine suppression of H3-thymidine incorporation into DNA by marrow cells was normal.15 Schilling test was normal (6.2 per cent excretion of a 2 µg. dose in an incomplete 24 hour urine collection). There was no circulating antibody to intrinsic factor. His B12 and B12-binding protein values are listed in Table 1. Hemoglobin electrophoresis was normal. Serum protein electrophoresis was normal. Serum haptoglobin, iron, iron-binding capacity, and quantitative urinary amino acid excretion were all within normal limits. Chromosomal analysis was unremarkable.*

Summary

The 46 year old brother of P. B. also had low serum B12 with low B12-binding alpha globulin. The only other condition present was multiple sclerosis, and he had been taking no medications. Although he had minimal white cell changes, no other evidence of B12 deficiency, including the sensitive test of deoxyuridine suppression of H3-thymidine incorporation into DNA by bone marrow in vitro, could be found.

Family

Survey of all but one of the known blood relatives revealed normal B12-binding capacity and serum B12 levels in all members (Table 2). Other findings were unremarkable except for 3 members with sickle cell trait, one of whom had mild folate deficiency and anemia.

METHODS

Serum was either tested immediately or stored at -20 C. until used. All studies were performed in duplicate, and there was no significant difference between results of frozen and fresh samples. Saliva was obtained by expectoration, occasionally with the chewing of Parafilm®, and stored at -20 C. Gastric juice was aspirated, depepsinized, neutralized, and stored at -20 C. Leukocyte extracts were prepared by sedimentation in 3 per cent dextran, pH 6.8, with 0.2 per cent EDTA, pH 6.8, several washings with cold 0.1M phosphate buffer, pH 6.8, hypotonic shock lysis to remove red cells, sonication, and solution in acetate buffer (pH 5.5).16 After centrifugation at 20,000 g. for 10 minutes, the supernate was passed through a G-25 sephadex column. The extracts were stored at -20 C.

Serum B12 was assayed by the coated charcoal method,12 as was the B12-binding capacity of the various specimens.18 Fractionation of B12-binding proteins was done by rapid DEAE-cellulose chromatography on a “baby” column.17 The beta globulin fraction was eluted with 0.06M phosphate buffer, pH 6.3, and the alpha with 1M NaCl. Horizontal starch gel electrophoresis of serum overnight at 2 volts/cm. was done in a discontinuous system described by Poulik.18 (The serum had previously been saturated with excess Co57B12 of specific activity of 19.29 µc./µg., the unbound vitamin being removed with coated charcoal.) The gel was cut in 1 cm. strips which were counted in a well-type scintillation counter for 10,000 counts.

Intravenous B12 disappearance study was performed by injecting 0.45 µg. sterile Co57B12 (19.29 µc./µg.) into a fore-arm vein, and obtaining venous blood samples from the other arm at various intervals. In addition, all stool and urine, and periodic saliva specimens, were collected for the first 72 hours.

Inhibitory effect of the subject’s serum on serum binding of vitamin B12 was studied, using normal sera as simultaneous controls. Various volumes, up to 0.5 ml., of P.B. serum

*Performed by Dr. L. Hsu, The Mount Sinai Hospital, N. Y. Laboratory of Dr. K. Hirschhorn.
Table 1.—B₁₂ and B₁₂-Binding Capacity of P.B. and W.B.

<table>
<thead>
<tr>
<th>Date</th>
<th>Bu</th>
<th>UBₐ BC *</th>
<th>% UBₐBCa</th>
<th>Total α +</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.B.</td>
<td>5/23/66</td>
<td>0</td>
<td>904</td>
<td>21.8</td>
</tr>
<tr>
<td></td>
<td>10/23/67</td>
<td>62</td>
<td>874</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td>3/18/68</td>
<td>34</td>
<td>750</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/22/68</td>
<td>31</td>
<td>968</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/28/68</td>
<td>74</td>
<td>1328</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td>4/5/68</td>
<td>172 †</td>
<td>654</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/27/68</td>
<td>87</td>
<td>638</td>
<td>15.4</td>
</tr>
<tr>
<td>W.B.</td>
<td>4/27/68</td>
<td>93</td>
<td>964</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>5/16/68</td>
<td>34</td>
<td>1014</td>
<td>11.6</td>
</tr>
<tr>
<td>Normal</td>
<td>&gt;150 pg./ml.</td>
<td>980-1756 pg./ml.</td>
<td>15-25%</td>
<td>347-1282 pg./ml.</td>
</tr>
</tbody>
</table>

* UB₁₂BC = Unbound B₁₂ binding capacity.
† Calculated by adding serum B₁₂ value to unbound α B₁₂BC (obtained by rapid chromatography). ¹
‡ 3 days after receiving 1000 µg B₁₂ IM.
§ Other random UB₁₂BC values: 768, 825, 805, 728.
" Total α: (Mean ± S.D.) = 235 ± 70; 19 normal subjects = 619 ± 215; the difference is highly significant (p < 0.005).

Table 2.—Family Survey

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>B₁₂ B₂</th>
<th>UB₁₂ BC *</th>
<th>% UB₁₂BCa</th>
<th>Total α +</th>
<th>Saliva UB₁₂ BC (pg./0.1 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B₁₂</td>
<td>UB₁₂ BC *</td>
<td>% UB₁₂BCa</td>
<td>Total α +</td>
<td>Saliva UB₁₂ BC (pg./0.1 ml.)</td>
</tr>
<tr>
<td>L.B.</td>
<td>23 F</td>
<td>451</td>
<td>1716</td>
<td>12.9%</td>
<td>672</td>
<td>3389</td>
</tr>
<tr>
<td>P.B. Jr.</td>
<td>20 M</td>
<td>274</td>
<td>1333</td>
<td>19.1%</td>
<td>526</td>
<td>3360</td>
</tr>
<tr>
<td>S.B.</td>
<td>9 F</td>
<td>443</td>
<td>1068</td>
<td>19.1%</td>
<td>647</td>
<td>1295</td>
</tr>
<tr>
<td>T.B.</td>
<td>4 F</td>
<td>787</td>
<td>1844</td>
<td>18.6%</td>
<td>1130</td>
<td></td>
</tr>
<tr>
<td>D.B.</td>
<td>3 M</td>
<td>664</td>
<td>1296</td>
<td>21.9%</td>
<td>948</td>
<td></td>
</tr>
<tr>
<td>H.B.</td>
<td>42 M</td>
<td>186</td>
<td>1360</td>
<td>19.7%</td>
<td>454</td>
<td>2599</td>
</tr>
<tr>
<td>D.B.</td>
<td>14 F</td>
<td>352</td>
<td>1484</td>
<td>18.0%</td>
<td>619</td>
<td>1769</td>
</tr>
<tr>
<td>N.B.</td>
<td>11 M</td>
<td>569</td>
<td>1844</td>
<td>27.4%</td>
<td>1074</td>
<td>3948</td>
</tr>
</tbody>
</table>

* UB₁₂BC = Unbound B₁₂ binding capacity.
† Calculated by adding serum B₁₂ value to α UB₁₂BC.

were presaturated with non-radioactive B₁₂, the excess free B₁₂ then being removed with hemoglobin-coated charcoal. These were then incubated with 0.1 ml of various normal sera, or with high-alpha-binder content sera from patients with chronic myelogenous leukemia, for 30 minutes at room temperature. Two ng Co⁵⁷B₁₂ were added and, after 30 minutes' incubation, unbound Co⁵⁷B₁₂ was removed by coated charcoal. The amount bound was then compared to that bound by the sera without preincubation with test serum. The effect of leukocyte extract on serum binding was similarly tested.

Results

Serum B₁₂-binding Protein

Serum B₁₂-binding capacity was low in 10 of 11 determinations of subject P.B. and in both determinations of W.B. (Table 1). This decrease was most prominent in the alpha globulin component, as determined by rapid DEAE-
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Fig. 1.—Serum B12-binding capacity of P.B. and W.B., and of various control groups (number in parentheses indicates number of subjects). Bars represent mean and range. The lowest value in the untreated pernicious anemia group is a patient with transient B12-binding beta globulin deficiency, discussed below.

cellulose chromatography (Fig. 1) and starch gel electrophoresis (Fig. 2). There was also decrease in beta globulin binding, P.B. demonstrating a mean of 757 pg./ml. beta globulin B12-binding capacity for 4 separate determinations and W.B. 845 pg./ml. for 2 determinations, compared to 1058 pg./ml. for the 19 normal subjects tested. (The difference between the values of P.B. and normals is significant, p being < 0.01).

Leukocyte B12-binding Protein

Leukocyte extracts of both subjects manifested virtually absent B12 binding (P.B. 24 pg./10^7 leukocytes and W.B. 43 pg./10^7 leukocytes). This is in contrast to various control subjects in our laboratory (Fig. 3) and others' published data.19,20 The only condition in which nearly as low levels were seen was chronic lymphocytic leukemia with marked preponderance of lymphocytes, which are poor in B12-binding proteins.19,21,22 Both P.B. and W.B., however, had normal numbers of neutrophils and lymphocytes.

Furthermore, the minute amounts of binding protein present eluted with the beta globulin fraction on DEAE-cellulose chromatography, unlike the usual elution with alpha globulin of leukocyte B12-binding proteins.

Saliva and Gastric Juice B12-binding Protein

Saliva of the 2 subjects also demonstrated virtual absence of B12-binding protein as compared to various controls, including the rest of the family (Fig. 4). Specimens from P.B., collected at various times between 1966 and 1968, had levels between 9 and 36 pg./0.1 ml.; W.B. saliva showed a binding capacity of 25 pg./0.1 ml.

The total B12-binding capacity of P.B. gastric juice was low as well (102 pg./0.1 ml. in the residual, 156 pg./0.1 ml. in the basal, and 202 pg./0.1 ml. in the histamine-stimulated specimens); approximately half of this binding was shown to be due to intrinsic factor, which elutes with the beta globulins on DEAE-cellulose chromatography.17 Such low B12-binding values are rarely seen in subjects with intrinsic factor, but occasionally in patients with pernicious anemia. Thus, of 100 consecutive gastric juice specimens containing intrinsic factor, only 5 bound less than 1000 pg./0.1 ml., whereas of 100 con-
secutive specimens lacking intrinsic factor, 28 had such levels (unpublished data from our laboratory).

Intravenous Co\textsuperscript{57}B\textsubscript{12} Disappearance Study

P.B. was given 0.45 \(\mu\)g Co\textsuperscript{57}B\textsubscript{12}, a less than saturating dose, intravenously (Fig. 5). The early, rapid phase of disappearance probably reflects the transient binding by beta globulin, whereas the later, slow phase reflects the tight binding by alpha globulin.\textsuperscript{3} In P.B. the early phase was comparable to that seen in normal subjects. The later phase may have been at a slightly lower plateau than is seen usually in normal subjects, and was much lower than is seen in B\textsubscript{12} deficient states.\textsuperscript{23-26} (His serum B\textsubscript{12} level on the day of the test, 4/27/68, was 87 pg./ml.) Over the first 3 days of the study, only 2.1 per cent of the injected dose appeared in the urine and 1.2 per cent in stool; none appeared in saliva.

Effect of P.B. Serum on B\textsubscript{12} Binding by Other Sera

Incubation of as much as 0.5 ml. P.B. serum with 0.1 ml. of various normal or high-alpha-binder content sera resulted in no significant decrease of B\textsubscript{12}-binding capacity. Similarly, P.B. leukocyte extracts had no effect on B\textsubscript{12} binding by the various sera.
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Fig. 3—B₁₂-binding capacity of leukocyte extracts of P.B. and W.B., and of various control groups (number in parentheses indicates number of subjects). Bars represent mean and range. A = normals; B = chronic myelogenous leukemia; C = leukemoid reaction; D = polycythemia vera; E = secondary polycythemia; F = myeloid metaplasia; G = chronic lymphocytic leukemia.

Fig. 4—B₁₂-binding capacity of saliva of P.B. and W.B., and of various control subjects. 1 = normals, 2 = pernicious anemia; 3 = chronic myelogenous leukemia; 4 = subjects' family; 5 = chronic leukopenia with low serum B₁₂-binding capacity.

Urinary B₁₂-binding Protein Excretion

Urine at no time contained measurable B₁₂-binding capacity, indicating no loss of the proteins by that route.

DISCUSSION

The alpha-1 globulin, a molecule with an approximate molecular weight of 120,000,²⁷,²⁸ carries endogenous vitamin B₁₂. It binds the vitamin tightly, and transfers B₁₂ poorly to cells in vitro.³,⁵,⁶ It appears to be closely similar to the B₁₂-binding protein found in saliva and neutrophils.²⁰,²⁸

When excess vitamin B₁₂ is added, the majority binds to a beta globulin,³,⁴,¹⁷ only 15-25 per cent binds to alpha globulin.¹⁷ This beta globulin, approximately 1/3 the size of the alpha,⁵,²⁷ appears to be primarily a transport protein. It gives up B₁₂ fairly readily to cells in vitro.³,⁶,⁸ A postulated
schema is that, as vitamin B₁₂ enters the bloodstream, it is bound primarily by beta globulin and delivered rapidly to cellular sites, and thence transferred to alpha globulin, some of which is intracellular or cell-bound and some freely circulating. The alpha globulin may be an altered form of the beta or they may be two distinct proteins.

Elevated levels of these B₁₂-binding proteins occur in various conditions. Most of these are associated with a proliferative increase of neutrophils, the most striking example being chronic myelogenous leukemia. Low levels are rare, having been observed in long-standing neutropenia. The exact relationship between B₁₂-binding protein levels and these diseases is not established.

Lawrence reported a patient with pernicious anemia who exhibited deficiency of the beta globulin binder, which normalized after parenteral vitamin B₁₂ therapy. We have observed this phenomenon in a 66 year old man with pernicious anemia, who presented with a serum unsaturated B₁₂-binding capacity of 746 pg./ml., of which only 61 per cent was due to beta globulin (normal: 75-85 per cent). After monthly injections of 100 µg. B₁₂ for 18 months, unsaturated B₁₂-binding capacity was 1876 pg./ml., of which 79 per cent was now due to beta globulin. Our patient with pernicious anemia initially did not have an abnormal Schilling test when given exogenous intrinsic factor, suggesting that the transient B₁₂ malabsorption in Lawrence's patient was the intestinal defect of B₁₂ absorption seen not infrequently in untreated pernicious anemia and unrelated to the serum binding abnormality.

Horrigan and Heinle reported a patient with possible defective in vivo binding of B₁₂, who required frequent injections of the vitamin or intravenous administration of normal plasma to maintain remission of her megaloblastosis. Intramuscular administration of 0.5 µg. Co²⁰B₁₂ resulted in excessive urinary excretion (60 per cent of the dose in 24 hours). Subsequently, she became increasingly refractory to therapy. The nature of the B₁₂-binding problem was never established, since the B₁₂-binding capacity of her serum proved to be normal.

The two brothers reported here have persistent decrease in serum B₁₂-binding alpha globulin in the absence of chronic neutropenia. This was unresponsive
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to vitamin B12 therapy. The beta globulin binder for B12 was slightly decreased as well, but transferrin, ceruloplasmin, haptoglobin, and thyroxine-binding protein were not reduced. The primary manifestation in both cases was a low serum B12 level by coated charcoal assay. This low B12 value was not a technical artifact, being also demonstrable on microbiologic assay. The serum unsaturated and total B12-binding alpha globulin levels in the 2 subjects were lower than in normals or in subjects with pernicious anemia or B12 deficiency due to other causes. Low values for total B12-binding alpha globulin were sometimes noted in untreated pernicious anemia, but this appears to be primarily associated with the low B12 levels, since the unsaturated alpha globulin binder values were not low. Thus, in patients with pernicious anemia in therapeutic remission, total alpha globulin levels were normal (Fig. 1). In contrast to this, there was no change in the levels of subject P.B. over 2 years, despite monthly injections of 100 μg. B12. Both B12-binding alpha globulin and serum B12 levels remained low. (One value was in the low normal range, but 10 others, earlier and later, were low.)

P.B. manifested no evidence of metabolic deficiency of vitamin B12. Determination of methylmalonic acid excretion in the urine was, unfortunately, not done until after B12 therapy was begun. It was normal despite the persistently low serum B12 levels. W.B. also showed no abnormality save minimal white cell changes, which are the earliest morphologic changes of B12 deficiency; his bone marrow was morphologically normal. Deoxyuridine suppression of H3-thymidine incorporation by his marrow in vitro, a sensitive measure of B12 or folate deficiency, was normal and was unaffected by adding B12 into the system, even when normal serum was added. It may be that in the case of W.B. the binding defect was just enough to produce only the very mildest manifestation of vitamin deficiency, although unrelated factors may have accounted for even this change.

The rapid disappearance from the blood stream of P.B. of Co57B12 given intravenously suggests that tissues were still able to store the vitamin adequately, as no significant amount was lost by excretion; this may account for the absence of metabolic evidence of B12 deficiency. However, it is possible that maldistribution of the vitamin may have occurred.

In addition to deficiency of the protein in the serum, virtual absence of it in leukocyte extracts and saliva was noted. Since in the normal individual most, if not all, B12-binding protein in these substances is alpha globulin, its absence is easily demonstrable. This is not always the case with serum, where a large B12-binding beta globulin fraction may obscure absence of the alpha globulin component due to technical problems. The absence in saliva, a fluid which normally has very high B12-binding capacity, has not been observed in any condition by us. Subject P.B. also had low B12-binding capacity in his gastric juice, although this could be a reflection of changes due to gastrectomy.

The absence of B12 binding by the subjects’ leukocyte extracts is unique. The only condition in which values approaching this are found is chronic lymphocytic leukemia, where the cause appears related to deficiency of neutrophils, which bind much B12 normally (lymphocytes bind little B12). Both subjects had normal white cell counts with normal numbers of neutro-
phils. With the exception of the minimal hypersegmentation of neutrophil nuclei of W.B., the cells appeared normal microscopically.

The source of the B₁₂-binding alpha globulin in serum is unknown. Due to its high concentration in neutrophils, the high serum values seen in chronic myelogenous leukemia and various myeloproliferative disorders, and the low values seen in some neutropenias of long duration, it has been postulated that neutrophils are a major source.²⁷,²⁰,²⁸ However, the electrophoretic mobility of leukocyte alpha globulin B₁₂-binder differs slightly from the alpha-1 globulin in serum. A distinct possibility exists that sialic acid content accounts for this difference,²⁸ and that the two are otherwise identical. Indeed, the possibility also exists that the beta globulin binder is a monomer, or other variant, of the alpha globulin binder, and that cells, possibly including neutrophils, act primarily in converting one form to the other.⁵ Thus, it is worth noting that although the change in B₁₂-binding alpha globulin is the striking one in the disorders listed above and in our 2 subjects, beta globulin changes in the same direction, although of smaller magnitude, occur.³³

The cause of the binder deficiency, probably inherited, in the 2 subjects is hard to define. It may be that only circulating protein was absent, tissue binding by cells other than leukocytes being intact. Protein production may have been decreased, or a defective protein produced. Alternatively, release of the protein into leukocytes and the circulation may have been defective. There did not appear to be destruction or inhibition of B₁₂-binding capacity, as these could not be demonstrated in vitro, and no urinary excretory loss was found.³⁴

**Summary**

Persistent deficiency of serum B₁₂-binding alpha-1 globulin was demonstrated in two brothers, manifesting primarily as low serum B₁₂ levels. Despite the inability of one subject to maintain normal levels of B₁₂ in the blood, as shown by persistently low serum values despite monthly injections of B₁₂, no evidence of metabolic B₁₂ deficiency could be found. Tissue B₁₂-storing ability appeared to be intact. His brother exhibited only minimal hypersegmentation of neutrophil nuclei; otherwise, he too presented a completely normal picture.

The normally present alpha globulin B₁₂-binder was virtually absent from saliva and peripheral leukocyte extracts of both subjects.

Current indirect evidence favors neutrophils as at least a partial source of the serum globulin.³³ The cause of the possibly hereditary defect in the 2 subjects is unknown. Neither excessive B₁₂-binding protein excretion³⁴ nor a destructive factor in their serum or leukocytes was found.

**SUMMARIO IN INTERLINGUA**

Un persistente carentia de seral globulina a-1 a ligation de vitamina B₁₂ esseva demonstrate in duo fratres in qui le manifestation primari de ille abbrevation esseva basse nivellos seral de vitamina B₁₂. In despecto del incapacitate de un del subjectos de mantener normal nivellos de vitamina B₁₂ in le sanguine — un facto rendite evidente per le persistentemente basse valores seral malgrado injectiones mensual de vitamina B₁₂ — nulle prova de carentia metabolic de vitamina B₁₂ poteva esser establite. Le capacitate tissular pro thesaurisar vitamina B₁₂ pareva esser intacte. Le fratre de iste subjecto exhibiva solo minime grades de
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hypersegmentation in su nucleo de neutrophilo. Alteremente etiam ill presentava un totallyente normal tableau.

Le normalmente presente globulina a ligator de vitamina B₁₂ eseva virtualmente absente ab le saliva e ab extractos de leucocytos peripheric in ambele subjectos.

A base del currentemente disponibile evidentia indirecte ii pote esser stipulate que le neutrophilos es le font del globulina seral. Le causa del defecto, possibilemente hereditari, in le duo subjectos hic presentate non es cognoscite. Esseva trovate nulle excessive excretion de proteina a ligation de vitamin B₁₂ e nufle factor destructive in le sero o in le leucocytos del probandos.

ACKNOWLEDGMENTS

We are grateful to Dr. Samuel Waxman for the marrow study of DNA synthesis, Dr. Winnifred Seegers for the starch gel electrophoresis, and Dr. Stephen Krauss and Miss Gertrude Lurinsky for preparing the leukocyte extracts. We also thank Le Teng Go, Lois Brenner and Leona Bandel for invaluable technical assistance.

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