Diagnostic and Prognostic Values of Measurement of Serum Vitamin B₁₂-Binding Proteins

By Victor Herbert

That vitamin B₁₂ in serum is bound to protein¹ has been known since shortly after the isolation of the vitamin. Endogenous vitamin B₁₂ (which may be mainly methyl-B₁₂)² is bound primarily to an α₁-globulin ²,⁶,¹⁰ but vitamin B₁₂ added to serum in vitro binds primarily to β-globulin (presumably because B₁₂-binding α-globulin is normally almost saturated with endogenous vitamin B₁₂).⁶,¹⁰

Hall and Finkler¹¹ have reported that when vitamin B₁₂ is fed by mouth or injected, it attaches first to β-globulin, and, during the ensuing 24 hr., transfers to α-globulin. Our preliminary studies¹²,¹³ suggest an alternate explanation; namely, that ingested or injected vitamin B₁₂ attaches both to α- and β-globulin B₁₂-binding proteins, but that which is attached to β-globulin is delivered to tissues within twenty-four hours whereas that attached to α-globulin is retained by the serum. Our studies suggest that the two B₁₂-binding globulins act in relation to B₁₂ much as the two binding proteins for copper act in relation to that mineral. Ingested copper, after absorption, is picked up by albumin and delivered to the liver, where it is "packaged" in ceruloplasmin to be circulated therein as stored copper.¹¹ Thus, albumin is a transport protein for copper, and ceruloplasmin is a copper-storage protein.

While both B₁₂-binding globulins may deliver the vitamin to reticulocytes¹³,¹⁵ and liver¹³ (and presumably other tissues as well, including tumor cells¹⁶), our studies suggest that the B₁₂-binding β-globulin is primarily a transport protein,¹³ whereas the B₁₂-binding α-globulin functions mainly to conserve the vitamin. B₁₂-binding α-globulin has a greater affinity for B₁₂ and retains the vitamin more tenaciously than does the B₁₂-binding β-globulin,¹² doling it out sparingly when a short supply necessitates conservation of available vitamin. Since normally B₁₂-binding α-globulin is almost saturated with the vitamin, and B₁₂-binding β-globulin is totally unsaturated, in the normal situation absorbed vitamin B₁₂ attaches primarily to α-globulin and is delivered to tissues. In the B₁₂-deficient state, B₁₂-binding α-globulin is relatively

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unsaturated, and vitamin will attach to this globulin, which will husband it and allow its delivery to tissues at only a slow rate.

This operative assumption, which we have tested in several systems, provides a simple explanation for the fact that vitamin B₁₂ injected intravenously is cleared from the blood stream more slowly in vitamin B₁₂ deficiency states and chronic myelogenous leukemia than in the normal subject. In these situations, a large quantity of B₁₂-binding α-globulin is unsaturated and will take up the injected vitamin. In addition, it is possible that the large quantity of B₁₂-binding α-globulin present in chronic myelogenous leukemia is chemically abnormal. Vitamin B₁₂ attached to this α-globulin, for operative purposes, may be metabolically less available, since it is not delivered well to tissues.

A patient in point was recently reported: he had chronic myelocytic leukemia with coincidental pernicious anemia but a normal serum B₁₂ level; we would interpret this normal serum B₁₂ level to consist of metabolically inert vitamin B₁₂, since it was bound to the abnormal B₁₂-binding α-globulin. Supporting this was the fact that the patient had correction of his megaloblastosis when he was treated with injections of vitamin B₁₂ in sufficient quantity to more than saturate his B₁₂-binding α-globulin. We suspect that a similar phenomenon may be present in chronic liver disease; i.e., an abnormal B₁₂-binding globulin holds B₁₂ in serum in a metabolically inert state. Thus, patients with liver disease may have megaloblastosis due to B₁₂ deficiency despite a normal serum vitamin B₁₂ level. In such patients, erythrocyte and liver (and other tissue) stores of B₁₂ would be sharply reduced; this can be demonstrated by determination of erythrocyte or liver B₁₂ levels, as well as by response to injections of vitamin B₁₂ (or administration of a good diet).

It has gradually become clear that there are many conditions in which serum vitamin B₁₂ metabolism is deranged, as manifested by derangement in serum vitamin B₁₂ and total vitamin B₁₂-binding capacity (TBBC), unsaturated vitamin B₁₂-binding capacity (UBBC), and percentage of TBBC and UBBC due to physiologically normal α versus β globulin as well as physiologically abnormal α and β B₁₂-binding globulins. Measurement of these parameters is proving of interest not only in vitamin B₁₂-deficiency states but also in pregnancy, liver disease, the myeloproliferative disorders (polycythemia vera, myeloid metaplasia, myelogenous leukemia), chronic leucopenia, chronic leucocytosis, DiGuglielmo syndrome, and uremia. Figure 1, taken from data of Gottlieb et al., graphically summarizes the mean levels of serum vitamin B₁₂ and vitamin B₁₂-binding capacity in these various disturbances of vitamin B₁₂ metabolism. Ranges of B₁₂-binding protein in these disturbances are indicated in Table 2 of Retief et al. It is beginning to become clear that past studies of many investigators suggesting the existence of an “extragastric intrinsic factor” or “circulating intrinsic factor” actually reflect effects due to serum vitamin B₁₂-binding proteins.

As indicated in Figure 1, normally the TBBC is approximately one-third saturated, just as normally the total iron-binding capacity of serum is approximately one-third saturated. Just as the percentage saturation of trans-
Fig. 1.—Serum vitamin $B_{12}$ level and total vitamin $B_{12}$-binding capacity in various disturbances of vitamin $B_{12}$ metabolism.

The lower horizontal dash line represents the mean level of vitamin $B_{12}$ in 15 normal human sera; this vitamin $B_{12}$ is essentially all bound to $\alpha$-globulin. The upper horizontal dash line represents the normal level of total vitamin $B_{12}$-binding capacity of the same 15 normal human sera.

The bottom (black) bars represent sera vitamin $B_{12}$ levels. The middle (white) bars represent unsaturated $B_{12}$-binding $\alpha$-globulin; the height of the middle bar plus that of the lower bar represents total $B_{12}$-binding $\alpha$-globulin (except in liver disease, when some $B_{12}$ is on the $\beta$-globulin). The upper stippled bars represent $B_{12}$-binding $\beta$-globulin, which, except in liver disease, is generally all but devoid of vitamin $B_{12}$.

Averages of: (1) 15 normal healthy adults; (2) 20 patients with untreated pernicious anemia; (3) 15 patients with treated pernicious anemia; (4) 9 untreated patients with $B_{12}$ deficiency not due to pernicious anemia; (5) 11 women in the third trimester of pregnancy; (6) 24 patients with hepatic cirrhosis; (7) 31 patients with polycythemia vera; (8) 5 patients with "spent" polycythemia vera; (9) 8 patients with myeloid metaplasia; (10) 20 patients with chronic myeloid leukemia; (11) 8 patients with acute myelogenous leukemia; (12) 7 patients with chronic leucopenia for periods in excess of three months; (13) 4 patients with chronic leukocytosis; (14) 3 patients with Di Guglielmo syndrome, all of whom had leucopenia; (15) 6 patients with uremia.
ferrin falls as an index of developing iron deficiency, so does the percentage saturation of B₁₂-binding globulin fall as an index of developing vitamin B₁₂ deficiency. Note that, as reported from India²⁸ and the United States,¹⁹,²⁹ TBBC tends to be reduced in pernicious anemia. This may relate in part to a reduced bone marrow granulocyte reserve.³⁰ However, our experience has been that during the early stages of development of vitamin B₁₂ deficiency, TBBC tends to be elevated (see bar #4 in Fig. 2). It is only as the deficiency progresses that TBBC is reduced, perhaps concomitant with reduction in other globulins.¹¹

As pregnancy progresses, just as serum iron tends to fall and serum iron-binding capacity to rise,²⁶ so does serum vitamin B₁₂ level tend to fall and serum B₁₂-binding capacity to rise (Fig. 1),²⁵,³²,³³ and thus is higher in the mother than in the newborn.³²

In chronic liver disease, both serum vitamin B₁₂ level and UBBC tend to be moderately elevated (Fig. 1).²⁵,³²,³⁴,³⁵ The evidence is that the damaged liver releases into the bloodstream an abnormal B₁₂-binding protein which raises the serum vitamin B₁₂ level; this B₁₂ may be metabolically useless,¹⁸ and the liver stores of B₁₂ are generally well below normal.⁵⁶,⁵⁷

It is probable that a significant portion of vitamin B₁₂-binding α-globulin is derived from leukocytes.³⁸ This provides a convenient explanation for the elevated B₁₂-binding α-globulin in myeloproliferative disorders¹,⁶,⁷,²⁵ and leukocytosis²⁵ and also for the low unsaturated B₁₂-binding α-globulin in chronic leukopenia²⁵ and aplastic anemia.²⁸ In chronic leukopenia, the serum vitamin B₁₂ level may be elevated or normal, but the unsaturated B₁₂-binding α-globulin is almost invariably reduced.²⁵ This also was true in three cases of DiGuglielmo’s syndrome,²³ all of whom happened to be leukopenic (Fig. 1).

In the myeloproliferative disorders as a group, serum vitamin B₁₂ level and vitamin B₁₂-binding capacity are elevated above normal in rough proportion to the degree of white cell proliferation (Fig. 1).²⁵ Thus, serum vitamin B₁₂ level tends to be modestly above normal in polycythemia vera, still more above normal in myeloid metaplasia, and greatly above normal in chronic myelogenous leukemia (with, however, somewhat less elevation above normal in acute myelogenous leukemia). The same relative elevations occur, although generally more strikingly, in the UBBC in the various myeloproliferative disorders.

Table 2 graphically depicts the mean percentage of in vitro added radioactive vitamin B₁₂ which attaches to α- versus β-globulin B₁₂-binding protein in various disturbances of B₁₂ metabolism.²⁵ It should be noted that the terms “B₁₂-binding α-globulin” and “B₁₂-binding β-globulin” as used by us are usually loosely used terms. When we speak of “B₁₂-binding β-globulin” we usually mean all the B₁₂-binding protein which is eluted from “baby columns” of DEAE cellulose¹⁰ with 0.06 M phosphate buffer, pH 6.3, and this includes not only β, but also some globulin with mobility between α₂ and β, as well as some globulin with α₂ mobility; “B₁₂-binding α-globulin” is that protein fraction eluted by 1 M NaCl.¹⁰ To be more accurate, we should state the amount of B₁₂ eluted from the columns in each of the eleven fractions we col-
Fig. 2.—Per cent of unsaturated B₁₂-binding globulin which is α (black bars; left-hand ordinate) and β (white bars; right-hand ordinate) in various disturbances of vitamin B₁₂ metabolism. (See legend for Fig. 1.)

lect rather than lumping the counts in the first six fractions as "β" and the counts in the remaining five as "α." In fact, the counts in each of our first six fractions support the reports of Hall's group of an "abnormal" B₁₂-binding β globulin in polycythemia vera and in cord serum of the newborn, which elutes late with buffer. Our "B₁₂-binding β-globulin" includes Hall's "TC II," and our "B₁₂-binding α-globulin" includes his "TC I."

As shown in Figure 2, normally approximately 80 per cent of added B₁₂ binds to β (elutes in 0.6 M phosphate buffer); i.e., normally the UBBC is 20 per cent α and 80 per cent β. In vitamin B₁₂ deficiency states, endogenous vitamin B₁₂ level is low, and the percentage of unsaturated α is therefore elevated.

Elevation of UBBC also aids in the differential diagnosis of polycythemia vera from relative polycythemia. UBBC was above 2,000 pg/ml in 24 of 27 cases of active polycythemia vera, but in only 4 of 28 controls, in only 3 of 17 patients with relative polycythemia, and in only 7 of 23 patients with polycythemia vera treated with myelosuppressive agents. In 14 cases of polycythemia vera studied during active and treated phases, UBBC fell with response to therapy, and rose during relapse. The UBBC remained above normal in half, in whom erythroid suppression sufficient to maintain a normal hematocrit was produced during therapy. In polycythemia vera, serum B₁₂ levels paralleled the UBBC but did not fall in 6 of 14 treated patients, whereas UBBC fell toward or to normal in all 14. Positive correlation was found between the UBBC and the white cell count, but elevated UBBC occurred despite a normal white cell count in 9 of 50 patients with polycythemia vera. Thus, levels of UBBC may be affected by changes in white cell turnover not reflected in the peripheral white count. In summary, UBBC may be a useful parameter in (1) differentiating polycythemia vera from relative polycythe-
(2) assessing degree of total granulocytic proliferation as in polycythemia vera. (3) indicating effectiveness of therapy in suppressing hematopoiesis.

Similarly, measurement of serum B₁₂ level and vitamin B₁₂-binding protein may be used in differential diagnosis of chronic myelogenous leukemia from myeloid metaplasia, since both these parameters (as well as greater percentage elevation in α-globulin) are much more sharply elevated in the former situation.²⁵ Elevated UBBC appears to be a more reliable index of untreated chronic myelogenous leukemia than does the presence of the Philadelphia chromosome, since we found UBBC and B₁₂-binding α-globulin always to be elevated in such patients while the Philadelphia chromosome was absent in three of them.²⁵ The one partial exception was a patient with a double Philadelphia chromosome; his B₁₂ level (865 pg/ml.) was normal, his UBBC (2070 pg/ml.) was below the upper limit of normal (2200 pg/ml.), but his unsaturated α was 40.1 per cent (twice the normal mean).

Our knowledge of disturbances of vitamin B₁₂ metabolism in various illnesses stands in 1968, where our knowledge of disturbances of iron metabolism stood fifteen years ago. In fact, the Figure 1 we have here presented is in the same format as a figure for iron disturbances in Laurell's review of iron metabolism in 1952.²⁶

All workers with vitamin B₁₂-binding proteins must be constantly on guard against the introduction of artifacts into their results. For example, heparin increases the apparent B₁₂-binding capacity of serum.⁴³,⁴⁴

**ADDENDUM**

It has recently been reported that patients treated with long acting vitamin B₁₂ preparations may develop antibody to B₁₂-binding β globulin.⁴³ Such patients have a slower plasma clearance of B₁₂ than do control subjects. The significance of this finding awaits further study.

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