Diagnostic and Prognostic Values of Measurement of Serum Vitamin $B_{12}$-Binding Proteins

By Victor Herbert

That vitamin $B_{12}$ in serum is bound to protein has been known since shortly after the isolation of the vitamin. Endogenous vitamin $B_{12}$ (which may be mainly methyl-$B_{12}$) is bound primarily to an $\alpha$-globulin but vitamin $B_{12}$ added to serum in vitro binds primarily to $\beta$-globulin (presumably because $B_{12}$-binding $\alpha$-globulin is normally almost saturated with endogenous vitamin $B_{12}$).

Hall and Finkler have reported that when vitamin $B_{12}$ is fed by mouth or injected, it attaches first to $\beta$-globulin, and, during the ensuing 24 hr., transfers to $\alpha$-globulin. Our preliminary studies suggest an alternate explanation; namely, that ingested or injected vitamin $B_{12}$ attaches both to $\alpha$- and $\beta$-globulin $B_{12}$-binding proteins, but that which is attached to $\beta$-globulin is delivered to tissues within twenty-four hours whereas that attached to $\alpha$-globulin is retained by the serum. Our studies suggest that the two $B_{12}$-binding globulins act in relation to $B_{12}$ 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. Thus, albumin is a transport protein for copper, and ceruloplasmin is a copper-storage protein.

While both $B_{12}$-binding globulins may deliver the vitamin to reticulocytes and liver (and presumably other tissues as well, including tumor cells), our studies suggest that the $B_{12}$-binding $\beta$-globulin is primarily a transport protein whereas the $B_{12}$-binding $\alpha$-globulin functions mainly to conserve the vitamin. $B_{12}$-binding $\alpha$-globulin has a greater affinity for $B_{12}$ and retains the vitamin more tenaciously than does the $B_{12}$-binding $\beta$-globulin, doling it out sparingly when a short supply necessitates conservation of available vitamin. Since normally $B_{12}$-binding $\alpha$-globulin is almost saturated with the vitamin, and $B_{12}$-binding $\beta$-globulin is totally unsaturated, in the normal situation absorbed vitamin $B_{12}$ attaches primarily to $\beta$-globulin and is delivered to tissues. In the $B_{12}$-deficient state, $B_{12}$-binding $\alpha$-globulin is relatively saturated with the vitamin, and $B_{12}$-binding $\beta$-globulin is totally unsaturated, in the normal situation absorbed vitamin $B_{12}$ attaches primarily to $\beta$-globulin and is delivered to tissues.
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 B₁₂ level and total vitamin B₁₂-binding capacity in various disturbances of vitamin B₁₂ metabolism.

The lower horizontal dash line represents the mean level of vitamin B₁₂ in 15 normal human sera; this vitamin B₁₂ is essentially all bound to α-globulin. The upper horizontal dash line represents the normal level of total vitamin B₁₂-binding capacity of the same 15 normal human sera.

The bottom (black) bars represent sera vitamin B₁₂ levels. The middle (white) bars represent unsaturated B₁₂-binding α-globulin; the height of the middle bar plus that of the lower bar represents total B₁₂-binding α-globulin (except in liver disease, when some B₁₂ is on the β-globulin). The upper stippled bars represent B₁₂-binding β-globulin, which, except in liver disease, is generally all but devoid of vitamin B₁₂.

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 B₁₂ 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 leukopenia for periods in excess of three months; (13) 4 patients with chronic leukocytosis; (14) 3 patients with Di Guglielmo syndrome, all of whom had leukopenia; (15) 6 patients with uremia.
ferrin falls as an index of developing iron deficiency, so does the percentage saturation of $\text{B}_{12}$-binding globulin fall as an index of developing vitamin $\text{B}_{12}$ deficiency. Note that, as reported from India$^{28}$ 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 $\text{B}_{12}$ 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.$^{31}$

As pregnancy progresses, just as serum iron tends to fall and serum iron-binding capacity to rise,$^{26}$ so does serum vitamin $\text{B}_{12}$ level tend to fall and serum $\text{B}_{12}$-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 $\text{B}_{12}$ 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 $\text{B}_{12}$-binding protein which raises the serum vitamin $\text{B}_{12}$ level; this $\text{B}_{12}$ may be metabolically useless,$^{18}$ and the liver stores of $\text{B}_{12}$ are generally well below normal.$^{36-37}$

It is probable that a significant portion of vitamin $\text{B}_{12}$-binding $\alpha$-globulin is derived from leukocytes.$^{38}$ This provides a convenient explanation for the elevated $\text{B}_{12}$-binding $\alpha$-globulin in myeloproliferative disorders$^{1,6,7,25}$ and leukocytosis$^{25}$ and also for the low unsaturated $\text{B}_{12}$-binding $\alpha$-globulin in chronic leukopenia$^{25}$ and aplastic anemia.$^{28}$ In chronic leukopenia, the serum vitamin $\text{B}_{12}$ level may be elevated or normal, but the unsaturated $\text{B}_{12}$-binding $\alpha$-globulin is almost invariably reduced.$^{25}$ This also was true in three cases of DiGuglielmo’s syndrome,$^{25}$ all of whom happened to be leukopenic (Fig. 1).

In the myeloproliferative disorders as a group, serum vitamin $\text{B}_{12}$ level and vitamin $\text{B}_{12}$-binding capacity are elevated above normal in rough proportion to the degree of white cell proliferation (Fig. 1).$^{25}$ Thus, serum vitamin $\text{B}_{12}$ 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 $\text{B}_{12}$ which attaches to $\alpha$- versus $\beta$-globulin $\text{B}_{12}$-binding protein in various disturbances of $\text{B}_{12}$ metabolism.$^{25}$ It should be noted that the terms “$\text{B}_{12}$-binding $\alpha$-globulin” and “$\text{B}_{12}$-binding $\beta$-globulin” as used by us are usually loosely used terms. When we speak of “$\text{B}_{12}$-binding $\beta$-globulin” we usually mean all the $\text{B}_{12}$-binding protein which is eluted from “baby columns” of DEAE cellulose$^{10}$ with 0.06 M phosphate buffer, pH 6.3, and this includes not only $\beta$, but also some globulin with mobility between $\alpha_2$ and $\beta$, as well as some globulin with $\alpha_2$ mobility; “$\text{B}_{12}$-binding $\alpha$-globulin” is that protein fraction eluted by 1 M NaCl.$^{10}$ To be more accurate, we should state the amount of $\text{B}_{12}$ eluted from the columns in each of the eleven fractions we col-
I)AGNOSTIC ANt) PROGNOSTIC VALUES

Fig. 2.—Per cent of unsaturated $B_{12}$-binding globulin which is $\alpha$ (black bars; left-hand ordinate) and $\beta$ (white bars; right-hand ordinate) in various disturbances of vitamin $B_{12}$ metabolism. (See legend for Fig. 1.)

lect rather than lumping the counts in the first six fractions as "$\beta$" and the counts in the remaining five as "$\alpha$." In fact, the counts in each of our first six fractions support the reports of Hall’s group of an “abnormal” $B_{12}$-binding $\beta$ globulin in polycythemia vera and in cord serum of the newborn, which elutes late with buffer. Our “$B_{12}$-binding $\beta$-globulin” includes Hall’s “TC II,” and our “$B_{12}$-binding $\alpha$-globulin” includes his “TC I.”

As shown in Figure 2, normally approximately 80 per cent of added $B_{12}$ binds to $\beta$ (elutes in 0.6 M phosphate buffer); i.e., normally the UBBC is 20 per cent $\alpha$ and 80 per cent $\beta$. In vitamin $B_{12}$ deficiency states, endogenous vitamin $B_{12}$ level is low, and the percentage of unsaturated $\alpha$ 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 $B_{12}$ 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 polycythemia-
mia, (2) assessing degree of total granulocytic proliferation as in polycythemia vera, (3) indicating effectiveness of therapy in suppressing hematopoiesis.\(^4\)

Similarly, measurement of serum B\(_{12}\) level and vitamin B\(_{12}\)-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 \(\alpha\)-globulin) are much more sharply elevated in the former situation.\(^{25,30,42}\) 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 B\(_{12}\)-binding \(\alpha\)-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 B\(_{12}\) level (865 pg/ml.) was normal, his UBBC (2070 pg/ml.) was below the upper limit of normal (2200 pg/ml.), but his unsaturated \(\alpha\) was 40.1 per cent (twice the normal mean).

Our knowledge of disturbances of vitamin B\(_{12}\) 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 B\(_{12}\)-binding proteins must be constantly on guard against the introduction of artifacts into their results. For example, heparin increases the apparent B\(_{12}\)-binding capacity of serum.\(^{43,44}\)

**ADDENDUM**

It has recently been reported that patients treated with long acting vitamin B\(_{12}\) preparations may develop antibody to B\(_{12}\)-binding \(\beta\) globulin.\(^{45}\) Such patients have a slower plasma clearance of B\(_{12}\) than do control subjects. The significance of this finding awaits further study.

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