Determination of vitamin B_{12} (cobalamin) in serum and erythrocytes by radioassay, and of holo-transcobalamin II (holo-TC II) and holo-haptocorrin (holo-TC I and III) in serum by adsorbing holo-TC II on microfine silica

Kshitish C. Das, Cathy Manusselis, and Victor Herbert

Departments of Medicine and Pathology, Mount Sinai School of Medicine, New York, NY, USA; Hematology and Nutrition Laboratory, Veterans Administration Medical Center, Bronx, NY, USA

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Introduction

Deficiency of vitamin B_{12} produces hematological and neuropsychiatric damage reversible by appropriate therapy with this vitamin. These manifestations occur in classical pernicious anemia, a vitamin B_{12} deficiency disorder due to inadequate to absent production of gastric intrinsic factor.[1,2] Although neurological abnormalities have been considered to be late manifestations of vitamin B_{12} deficiency, occurring usually after blood damage, they have been known to precede or manifest in the absence of any hematological abnormalities.[1-5] This has been re-emphasized recently by Lindenbaum's group[6] and others.[7] The frequency of vitamin B_{12} deficiency in dementia and other neuropsychiatric disorders in geriatric subjects, Alzheimer's disease, and acquired immunodeficiency syndrome (AIDS) calls for evaluating vitamin B_{12} status in such subjects.[8,9]

Value of radioassays over microbiological assays

The determination of serum vitamin B_{12} level has been generally considered to be a highly sensitive test in the detection of clinical disorders caused by its deficiency. Competitive inhibition radioassays of vitamin B_{12} have virtually replaced microbiological assays[10,11] in almost all laboratories, since they are simple, rapid, and unaffected by presence of antibiot-

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Address reprint requests to Kshitish C. Das, c/o Dr. Victor Herbert, 130 West Kingsbridge Road, Bronx, New York 10468, USA.
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ics, antimetabolites, and other drugs.[12-14] The latter agents inhibit the growth of microorganisms, causing the microbiological assays to yield false low results. Additionally, differential radioassays can separate cobalamins (true vitamin B_{12}) from other corrinoids,[15,16] which cannot be done adequately by microbiological assays.[10,11,17]

Radioassays. The most widely employed radioassay[13] uses coated charcoal to separate free from bound vitamin B_{12} and is based on saturation analysis techniques, which assess the dilution of a standard amount of radiolabeled vitamin B_{12} (cobalamin) by the native (endogenous) cobalamin of serum, plasma or other tissues, using purified gastric intrinsic factor (IF) as the ligand-binding agent.

Human serum and tissues contain not only biologically active "true B_{12}" (i.e., cobalamin), but also analogues of vitamin B_{12} (i.e., noncobalamin corrinoids of varying to no activity).[15] Radioassays that do not use pure intrinsic factor (IF) or impure IF with its non-intrinsic factor (R-binder) blocked by an analogue of vitamin B_{12} measure total corrinoids (i.e., true B_{12} plus B_{12} analogues). Thus, assays using impure IF may fail to detect early vitamin B_{12} deficiency, since in early deficiency only true vitamin B_{12} levels may fall, and vitamin B_{12} analogues may still be in the normal range or even rise.[15,18] On the other hand, the differential radioassays in which both pure IF as well as R-binder are used in parallel (i.e., replicate assays) measure total corrinoids, and true vitamin B_{12} (cobalamin), from which cobalamin analogues can be derived.[15-19]

However, it is recognized that despite the use of technically advanced reliable assay systems (Kolhouse et al.[15] modification of the Lau et al. radioassay[13]), normal serum cobalamin level is found in a significant minority of patients with typical or atypical clinical features of vitamin B_{12} deficiency.[20-22] On the other hand, many patients with low serum cobalamin levels, vegetarians and vegans in particular, do not show significant clinical evidence of deficiency for a variable length of time.[20-23] It has been further observed that even when there is tissue depletion of vitamin B_{12}, serum vitamin B_{12} level may be normal or elevated as in liver disease,[19] transcobalamin II deficiency,[24] myeloproliferative disorders,[25,26] and after nitrous oxide exposure.[27,28]

Holo-transcobalamin II and vitamin B_{12} deficiency

Vitamin B_{12} (cobalamin) is bound to two major types of vitamin B_{12}-binding proteins: transcobalamin II (TC II) and haptocorrin (R-binders: TC I and III).[25,29] TC II binds 6–20% of endogenous vitamin B_{12} and delivers the vitamin to all metabolically active tissues.[25] Several investigators have shown that TC II carries only vitamin B_{12} in normal humans, but R-binders (TC I and III) also carry vitamin B_{12} analogues.[30,31] Herbert[5,19,22,32,33] has proposed an intriguing model of developing vitamin B_{12} deficiency, which envisages an overlapping continuum (occurring in a telescopic manner) of five discrete, slowly progressing stages of negative balance:

1. normality
2. early negative vitamin B_{12} balance
3. vitamin B_{12} depletion (i.e., loss of stores of B_{12})
4. biochemical deficiency, and
5. clinical deficiency.

Each of these stages can be defined by appropriate laboratory and clinical tests.[3,5,8,22,32,33] A negative balance of vitamin B_{12} begins when vitamin B_{12} absorption falls, and may continue to be low enough to reduce vitamin B_{12} content on its primary delivery protein, transcobalamin II (TC II) resulting in a low holo-TC II level, and reduced saturation of TC II, even though total vitamin B_{12} level in serum may remain within laboratory normal range. For any laboratory test, because of individual variability, deviation from the value normal for the individual often precedes deviation from the

laboratory range of normal[34] (e.g., an individual's low holo-TC II may be low for him and may indicate that he is in negative vitamin B_{12} balance before his holo-TC II level falls below the laboratory range of normal). Recent studies in patients with untreated pernicious anemia and with AIDS have indicated that in early vitamin B_{12} deficiency, this vitamin is preferentially depleted from TC II in comparison to TC I and TC III, and that a decrease in holo-TC II is the earliest detectable sign of developing vitamin B_{12} deficiency.[9,33]

In this paper, we describe the method used in our laboratory for competitive inhibition radioassay of vitamin B_{12} in serum (Kolhouse et al.[15] modification of Lau et al. method),[13] and in erythrocytes,[19] including the method of differential radioassays[13,15,19] to measure total corrinoids, true vitamin B_{12} (cobalamin), and vitamin B_{12} analogues. We also describe a method to determine vitamin B_{12} on TC II (i.e., holo-TC II) and on haptocorrin (i.e., holo-haptocorrin) by separation of TC II from haptocorrin (TC I and III) by treating the serum sample with microfine precipitate of silica (Quso).[30]

Principles

Competitive inhibition radioassay

Vitamin B_{12} is extracted from serum or plasma, red cells, or other tissues, and this unknown quantity of non-radioactive endogenous vitamin B_{12} is added to dilute the specific activity of a known quantity of radioactive vitamin B_{12} [^{57}Co]CN-B_{12}. A solution of pure IF or R-binder with a binding capacity less than the quantity of added [^{57}Co]CN-B_{12} (approximately 80%) is used to bind a portion of the mixture of radioactive and non-radioactive (extracted from the sample to be assayed) vitamin B_{12} (i.e., "to biopsy the pool of B_{12}." [13]) The vitamin B_{12} bound to the binder (IF or R-binder) then is separated by addition of hemoglobin-coated charcoal which adsorbs free or unbound vitamin B_{12}. Standards of vitamin B_{12} (cyanocobalamin) ranging from 0–1000 pg in the same volume as of the serum or other samples used are extracted and then treated simultaneously in the same manner as the samples. The radioactivity of the vitamin B_{12} bound to the binder (IF or R-binder), which remains in the supernatant after coated charcoal adsorption, is compared with those of the standards. Results are calculated from the standard curve. The steps are depicted in *Figure 1*.

R-binders bind "true" vitamin B_{12} (i.e., cobalamin) as well as non-cobalamin corrinoids (analogues), whereas pure IF binds only true vitamin B_{12}. Differential radioassays using both R-binders and IF measure total corrinoids (i.e., vitamin B_{12} analogues bound to R-binders) as well as true

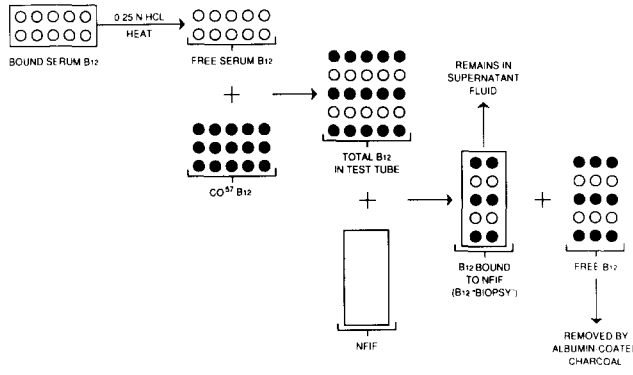

Figure 1 Schematic representation of serum vitamin B_{12} assay using radioisotope dilution and albumin-coated charcoal. Other large molecules, such as fibrinogen, globulins, dextrans, Ficoll (Pharmacia), and hemoglobin can be used in place of albumin to coat charcoal in the separation of free from bound B_{12}. For serum B_{12} assay we have found that hemoglobin solution used in the proportion of 1 part hemoglobin solution to 20 parts charcoal by weight (or 1 part hemoglobin powder to 10 parts charcoal by weight) will substitute for albumin-coated charcoal and has the advantage of giving consistently lower supernatant controls.

vitamin B_{12} (bound to IF). The total corrinoids minus the true vitamin B_{12} give the amount of analogues of vitamin B_{12}.[15,33]

Holo-transcobalamin II (holo-TC II) and holo-haptocorrin

When serum is treated with microfine precipitate of silica such as Quso G32 or Quso 761, TC II (including bound vitamin B_{12}) is adsorbed leaving haptocorrin (i.e., TC I and III) bound vitamin B_{12} in the supernatant solution. Radioassays performed on a sample of serum before and after adsorption with microfine silica precipitate estimate vitamin B_{12} bound to TC (I, II, and III) and that bound to TC (I and III), respectively. Holo-TC II (i.e., vitamin B_{12} bound to TC II) is obtained by subtracting the vitamin B_{12} value of a Quso-treated serum from that of the untreated or whole serum.[30]

Materials

Separation of holo-TC II from whole serum

Microfine precipitate of silica. Quso G32 or G761 (PQ Corporation, Valley Forge, PA). A slurry of 6% Quso is made by adding 6 g Quso to 100 ml of distilled water. This is mixed well and stored at 4° C. To each 0.1 ml of serum, 3 mg of Quso is added to adsorb TC II.

Extraction

Sodium acetate, 0.5 M (pH 4.5) (F.W. 82.03). Weigh out 4.1015 g Na Acetate on a scale; add this to about 80 ml sterile distilled water in a volumetric flask; adjust pH to 4.5 with 1 N HCl, and make up the volume to 100 ml with sterile distilled water.

NaCl, 0.9% with 50 μg/ml KCN. Weigh 12.5 mg (0.0125 g) KCN; put into 250 ml volumetric flask; add sterile 0.9% NaCl until the total volume is 250 ml.

Assay

Tris-HCl, 1.0 M (pH 10.0). Weigh out 12.114 g of Tris (Trizma Base) and put this into a 100 ml volumetric flask. Add about 80 ml of sterile distilled water; adjust pH to 10.0 with 1 N HCl; add sterile, distilled water until the volume is 100 ml. Store at 4° C.

Bovine serum album. *2mg/ml (BSA).* Weigh out 200 mg BSA and put this into a 100 ml volumetric flask. Add sterile distilled water until total volume is 100 ml. Mix well and store at 4° C.

Phosphate-buffered saline. *(PBS), 0.01 M (pH 7.5).* Make up 500 ml of 0.01 M potassium phosphate dibasic (0.8709 g) in 0.9% saline. Make up 250 ml of 0.01 M potassium phosphate monobasic (0.3402 g) in 0.9% saline. Adjust pH to 7.5 the dibasic phosphate (500 ml) by adding the monobasic component to it. Mix and store at 4° C.

Binder-buffer 0.01 M Tris HCl (pH 8.2) with 0.9% NaCl and 50 mg BSA. (This is used with the R-binder). Combine 0.6057 g Tris (Trizma Base) 0.0250 g BSA in about 450 ml 0.9% NaCl (preferably sterile, bottled). Adjust pH to 8.2 with 1 N HCl. Add sterile distilled water until the volume is 500 ml. Store at 4° C.

Preparation of hemoglobin for use in coated charcoal solution. Discarded human red cells are washed thrice with 0.9% NaCl. Saline is removed after the last wash; a volume of distilled water equal to the volume of the washed red cells, and one-half volume of toluene are added; the mixture is shaken vigorously for 5 minutes. This is then centrifuged for 15 minutes at 3000 rpm. The top two layers (toluene and cell debris) are discarded. The hemoglobin solution is filtered through Whatman #1 filter paper. Hemoglobin concentration is measured and adjusted to 12.5% if it is higher. Aliquot and freeze at −20° C.

Hemoglobin-coated charcoal (HbCC) 1:10. Weigh out 2.5 g activated charcoal of Norit "A" pharmaceutical grade (Amend Drug and Chemical Co., Inc., New York, NY). Take 0.25 g Hgb (i.e., 2 ml of 12.5 g% hemoglobin solution) and add to 100 ml sterile distilled water, mix well, and store overnight at 4° C before use.

Radioactive vitamin B_{12} [^{57}Co]B_{12}. [^{57}Co]B_{12} (Amersham Corporation, Arlington Heights, IL) was diluted with 0.9% saline so as to obtain a concentration of 1000 pg/ml (containing approximately 200 μCi/μg). Use 50 μl of this solution (containing 50 pg of radioactive vitamin B_{12} as tracer) in each assay tube.

IF—Pure Intrinsic Factor (Sigma Chemicals, St. Louis, MO). This is diluted so that 200 μl of diluted IF binds approximately 40 pg of vitamin B_{12}. This is done as follows:
Original. Sigma IF contains 1000 units/bottle (1 unit IF binds 1 ng vitamin B_{12} at pH 7.7 and 25° C).
Stock solution. Original Sigma (1000 units/bottle) plus 10 ml phosphate buffered saline (pH 7.5) with 5% bovine serum albumin (BSA). Pipette 1 ml of stock solution into 10 tubes and label "IF-100 units" and store at −20° C.
Working stock solution. Dilute "stock" solution 1:10 (with saline). This provides 10 units/ml.
Assay dilution. Dilute working stock solution 1:35 (with saline). This will provide approximately 0.15–0.30 units/ml. This must be made fresh before use. In the assay protocol, 200 μl of IF binder solution (assay dilution) is added to all tubes containing vitamin B_{12} standards or sample extracts (i.e., tubes 5 to 22 in *Table 1*), and it is expected that approximately 30 to 60 pg of vitamin B_{12} will bind to the IF binder. This is necessary to obtain a Bo value ≥40%. The binding ability of each new batch of IF should be tested using radioactive vitamin B_{12} ([^{57}CO]B_{12}) as previously described by Gottlieb et al.[35]

R-binder (Sigma Chemicals). The assay dilution of R-binder will provide a solution so that 200 μl will bind approximately 40 pg of vitamin B_{12}. Prepare different solutions as follows:
Original. Sigma R-binder contains 1000 units/bottle.
Stock solution. Original Sigma (1000 units/bottle), plus 10 ml PBS with 5% BSA provides 100 units/ml solution. Label and store this in 1 ml aliquots labeled "R-100 units" at −20° C.
Working solution. Dilute "stock" solution 1:10 with binder buffer.
Assay dilution. Dilute working stock solution 1:35 with binder buffer. The working stock solution must be made fresh before use. In the actual assay, 200 μl of R-binder solution (assay dilution) is added to all tubes containing vitamin B_{12} standards or sample extract (i.e., tubes 23–40 in *Table 1*), and approximately 30–60 pg of vitamin B_{12} is expected to bind to the R-binder. The addition of this quantity of R-binder yields a Bo value of ≥40%. The binding ability should be tested for each new batch of R-binder using radioactive vitamin B_{12} ([^{57}Co]B_{12}) as stated above for IF.

Preparation of vitamin B_{12}. Cold or non-radioactive standards are done as follows:

Vitamin B_{12} standards. A stock solution of 50 ng/ml CN-B_{12} may be stored at 4° C.
A. 1000 pg/ml. To 9.8 ml PBS, add 0.2 ml of 50 ng/ml CN-Cbl.
B. 500 pg/ml. To 3.0 ml PBS, add 3.0 ml of solution A.
C. 250 pg/ml. To 2.0 ml PBS, add 2.0 ml of solution B.
D. 100 pg/ml. To 9.0 ml PBS, add 1.0 ml of solution A.
E. 0 pg/ml. Use 2.0 ml PBS.

Table 1 Intrinsic factor

Tube contents	Tube No.	Standard (extracts) [Std. ext.]	Sample (extracts) [S. ext.]	1.0 M Tris-HCl buffer (pH 10)	BSA 2 mg/ml	Saline 0.9%	$[^{57}Co]B_{12}$ 1000 pg/ml		Binder IF	Binder R	Hbcc 1:10
A	1,2	—	—	225 µl	50 µl	1.325 ml	50 µl	V	—	—	0
B	3,4	—	—	↓	↓	1.325 ml	↓	O	—	—	0.5 ml
C1	5,6	Stud. ext.	—	↓	↓	325 µl	↓	R	200 µl	—	↓
C2	7,8	↓	—	↓	↓	↓	↓	T	↓	—	↓
C3	9,10	↓	—	↓	↓	↓	↓	E	↓	—	↓
C4	11,12	↓	—	↓	↓	↓	↓	X	↓	—	↓
C5	13,14	↓	—	↓	↓	↓	↓	↓	↓	—	↓
D	15,16	—	S. ext.	↓	↓	↓	↓	T	↓	—	↓
E	17,18	—	↓	↓	↓	↓	↓	U	↓	—	↓
F	19,20	—	↓	↓	↓	↓	↓	B	↓	—	↓
G	21,22	—	↓	↓	↓	↓	↓	E	↓	—	↓
					R-Binder			S	↓	—	↓
					↓						
C_1	23,24	Std. ext.	—	225 µl	50 µl	325 µl	50 µl	V	—	200 µl	0.5 ml
C2	25,26	↓	—	↓	↓	↓	↓	O	—	↓	↓
C3	27,28	↓	—	↓	↓	↓	↓	R	—	↓	↓
C4	29,30	↓	—	↓	↓	↓	↓	T	—	↓	↓
C5	31,32	↓	—	↓	↓	↓	↓	E	—	↓	↓
D	33,34	—	S. ext.	↓	↓	↓	↓	X	—	↓	↓
E	35,36	—	↓	↓	↓	↓	↓	↓	—	↓	↓
F	37,38	—	↓	↓	↓	↓	↓	↓	—	↓	↓
G	39,40	—	↓	↓	↓	↓	↓	↓	—	↓	↓

Tube A: Contents: $[^{57}Co]B_{12}$ standard; tube B: Contents: Hbcc control; tube C_1: B_{12} Standards: 0 pg/ml; tube C_2: B_{12} Standards: 100 pg/ml; tube C_3: B_{12} Standards: 250 pg/ml; tube C_4: B_{12} Standards: 500 pg/ml; tube C_5: B_{12} Standards: 1000 pg/ml; tube D: Known control; tube E: Serum sample; tube F: Serum Quso; tube G: Red cells.

* Vortex and incubate at 37° C for 45 min.

† Vortex, centrifuge at 3000 rpm for 15 min. Decant supernatant into tubes and count supernatants (and tubes A [#1 and #2]) in a gamma counter.

Red cell vitamin B_{12} assay

Reagents for extraction. Prepare acetate buffer as follows:

Acetic Acid, 0.4 M, is made from 17.47 M glacial acetic acid. 2.3 ml of 17.47 M acetic acid is made up to 100 ml with distilled water in a volumetric flask.

Sodium acetate, 0.4 M, is made up from powder (F.W. 82.04)

Sodium acetate, 0.4 M, (pH 4.9). Take 20 ml of 0.4 M acetic acid and 0.4 M Na acetate, titrating to pH 4.9; approximately 30 ml of 0.4 M Na acetate solution is required.

Procedure

1. Turn water bath on.
2. Thaw samples.
3. Label tubes (polypropylene test tubes 5 ml, 75 mm for extraction and assay and 1.5 ml microfuge tubes for Quso treatment).
4. Stir Quso. It takes about one half-hour at room temperature to liquefy.
5. Make standards for standard curve.
6. For the Quso treatment, place 0.1 ml Quso slurry in a microfuge tube (1.5 ml) and add 0.2 ml of serum sample. Vortex and let sit for 10 min. Centrifuge the sample in a microfuge for 10 min. Pour off the supernatant into polypropylene tubes.
7. Extraction of red cells.
   a Determine the hematocrit on an aliquot from the anticoagulated blood (EDTA).
   b. Use EDTA blood in quantity (ml) equal to 200% Hct. For example,

if Hct is 50, use (200/50 = 4) 4 ml blood; if Hct is 20, use (200/20 = 10) 10 ml blood. Spin, make level mark on tube, and discard supernatant solution; wash sediment once with saline; spin, discard supernatant, leaving a 2 ml red cell pellet. If the amount of blood or red cell is less than 2 ml, calculate amount of red cell as: (quantity of blood × Hct/100)

c. Prepare a 10% red cell solution as follows: To 2 ml red cell pellet, add 2 ml 0.4 M acetate buffer (pH 4.9), 0.4 ml 0.1% NaCN, and 15.6 ml distilled water. Vortex; autoclave for 15 min; cool; spin; and remove pink supernatant as vitamin B_{12} extract. Freeze at −20° C if not used immediately.

8. Extract serum and standard. To all tubes except 1–4, (i.e., isotope and Hb-coated charcoal control) containing 0.2 ml serum or standard (vitamin B_{12}) curve points, add 0.2 ml sodium acetate (0.5 M), pH 4.5 and 0.4 ml NaCl with 50 μg/ml KCN. Vortex all tubes and cover rack with aluminum foil. Place in 100° C water bath for 45 min. Cool to room temperature. Either assay immediately or store at −20° C until assayed. Each assay is carried out in duplicate; therefore, for each serum sample, 8 tubes of 200 μl of serum should be used for extraction (i.e., 4 tubes for IF (2 tubes for Quso treated and 2 tubes for untreated serum), 4 tubes for R-binder (2 tubes for Quso treated and 2 tubes for untreated serum).
9. Follow the assay protocol by adding extracts of standards and samples (i.e., serum, red cells, etc.), and other reagents as shown in *Table 1.*
10. Decant the supernatant after Hbcc (hemoglobin-coated charcoal) adsorption into a test tube (16 × 100 mm), and count the radioactivity in a gamma counter usually 5 min for each sample. Average counts per minute is used for calculation.

Calculations

1. Average of the counts in tubes 3 and 4 represent the "blank" and should be subtracted from counts in all other tubes to get the corrected counts.
2. Average of the counts in tubes 1 and 2 gives the "Total Count" of the radioactive B_{12} (1000 pg/ml) used per assay.
3. Divide the average of the corrected counts for tubes 5 and 6 by the corrected Total Count to get the Trace Binding Bo. This value should be greater than 40%. If less than 40%, the binder is inadequate or deteriorated. A fresh binder should be prepared.

$$\text{Bo} = \text{Trace Binding} = \frac{\text{Average Corrected Counts for tubes 5 and 6}}{\text{Total Count} \times 100} \quad (1)$$

4. Divide the Corrected Counts for each tube by the Average Corrected Counts for tubes 5 and 6 to give the % Trace Binding for each concentration of standard.

$$\%\ \text{of Trace Binding} = \frac{(\text{Corrected Count} \times 100)}{\text{Average Corrected Counts for tubes 5 and 6}} \quad (2)$$

5. A standard curve may be plotted for IF as follows:
Using logit-log paper plot % Trace Binding as the ordinate versus pg/ml vitamin B_{12} standard for IF. Similarly, a standard curve can be plotted for R-binder. The concentrations of true vitamin B_{12} or total corrinoids are determined by interpolation from the standard curves of % Trace Binding versus pg/ml of vitamin B_{12} for IF or R-binder, respectively (*Figure 2*).

Discussion

We describe a competitive inhibition radioassay for vitamin B_{12} in which the endogenous vitamin B_{12} extracted from the sample competes with the radioactive vitamin B_{12} ([^{57}Co]cobalamin) for binding sites on the IF or

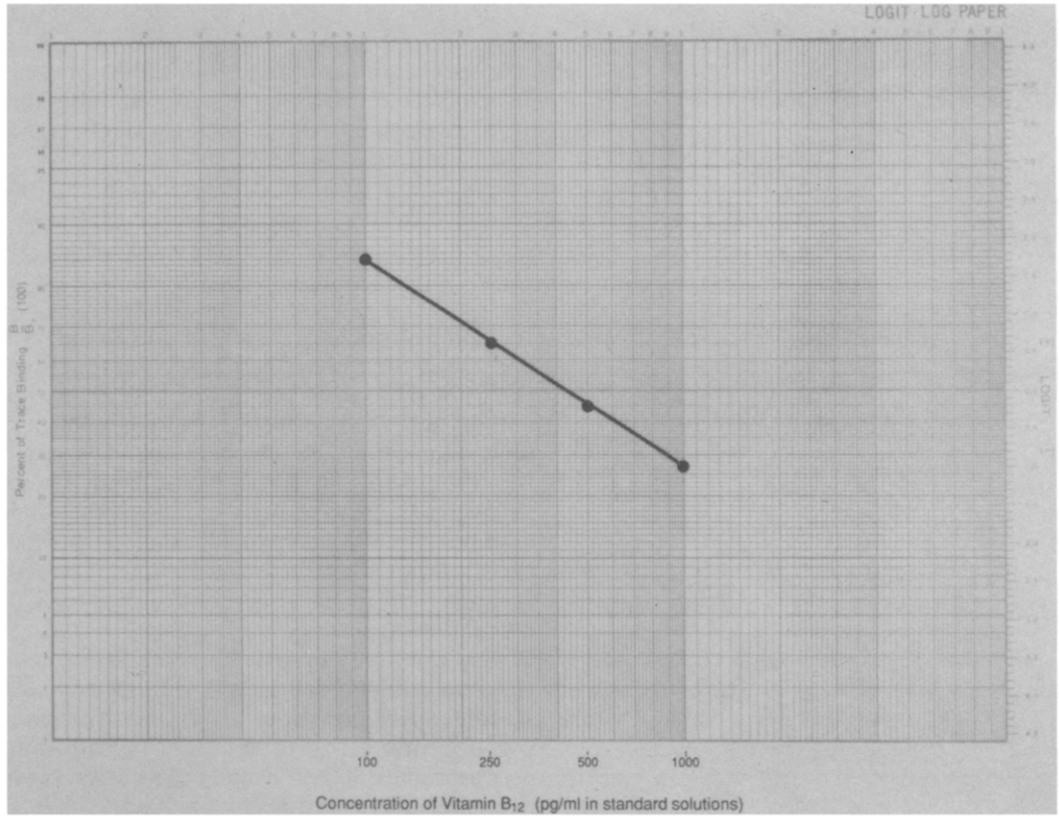

Figure 2 A standard curve for IF.

R-binder. The percent inhibition of [^{57}Co]cobalamin binding to the binder increases as the concentration of endogenous vitamin B_{12} (i.e., vitamin B_{12} content of the unknown sample) or of the standards increases.[13,15] This assay has been applied successfully to measure vitamin B_{12} content in serum,[9,15,19,29,36] erythrocytes,[19,37] and tissues.[19,37] IF binds only vitamin B_{12}, but R-binders bind both vitamin B_{12} as well as non-cobalamin vitamin B_{12} analogues. Competitive inhibition radioassays with IF measure true vitamin B_{12}, whereas assays with R-binders measure both vitamin B_{12} and analogues of vitamin B_{12}[15-17] and yield higher values for this vitamin. Kolhouse et al.[15] reported that a large number of commercial radioassay kits contained a variable quantity of R-binders (51–85%) mixed with "IF" binders. Radioassays of serum vitamin B_{12} levels with these kits may yield higher "vitamin B_{12}" values due to the presence of analogues in serum and may even mask the diagnosis of vitamin B_{12} deficiency.[4,15-19] The radioassay described in this paper obviates this problem by using both pure IF and R-binders in the assay, which measures the true vitamin B_{12} (cobalamin) and the total corrinoids content simultaneously.

In spite of these improvements, it has been observed that a small but significant number of patients with clinically significant vitamin B_{12} deficiency show serum vitamin B_{12} levels within normal laboratory range.[6,20,21] Herbert et al. also observed that within a month after stopping vitamin B_{12} therapy, pernicious anemia patients become depleted of serum holo-TC II, even though total vitamin B_{12} levels remained within laboratory range of normal.[9,38] Further, a proportion of patients with AIDS who had normal total serum vitamin B_{12} levels, but low serum holo-TC II showed significant hematologic and neuropsychiatric improvement within 3 weeks after intramuscular injection of 1 mg vitamin B_{12}.[9] These observations indicated that the earliest detectable serum marker of negative vitamin B_{12} balance and

developing vitamin B_{12} deficiency is low serum holo-TC II[9,32,33,34] despite normal total serum vitamin B_{12} level. The determination of holo-TC II described in this paper involves an additional step in the radioassay for vitamin B_{12},[33] (i.e., separation of TC II from haptocorrin [TC I and TC III] by adsorption with Quso [microfine precipitate of silica]). Estimation of the vitamin B_{12} content of the adsorbed serum, which yields vitamin B_{12} content on haptocorrin, and subtraction of this value from the vitamin B_{12} content of the untreated serum provides vitamin B_{12} content on TC II (holo-TC II). This relatively simple additional step in this procedure provides valuable information on the vitamin B_{12} status of patients at a very early stage of developing deficiency, even when the total serum vitamin B_{12} level may remain within the laboratory range of normal.[9,33] This procedure is likely to have a wide application in the study of clinical disorders due to vitamin B_{12} deficiency.

Summary

We describe here a competitive inhibition radioassay of vitamin B_{12} in serum and erythrocytes, including differential radioassays to measure total corrinoids, "true" vitamin B_{12} (cobalamin), and vitamin B_{12} analogues in the assay of Lau et al.[13] as modified by Kolhouse et al.[15], in which the ligands are pure IF and R-binders. We also describe a method to determine holo-TC II (i.e., vitamin B_{12} on TC II) and holo-haptocorrin (i.e., vitamin B_{12} on TC I and TC III) by adsorbing TC II from haptocorrin (TC I and III) with microfine precipitate of silica (Quso). This procedure adds only one step to the vitamin B_{12} assay, and delineates negative vitamin B_{12} balance of patients at a very early stage of developing deficiency, well before the total serum vitamin B_{12} level falls below the laboratory range of normal.

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