A New Case of Deficiency of the R Binder for Cobalamin, With Observations on Minor Cobalamin-Binding Proteins in Serum and Saliva

By Ralph Carmel

A patient presented at the age of 77 yr with a low serum cobalamin level. Subsequent study showed that he had persistently very low R binder (TC I) cobalamin-binding capacity in serum (< 5 ng/liter versus 213 ± 171 ng/liter in normal controls), and that almost all of his endogenous serum cobalamin was carried by TC II instead of TC I. His saliva also demonstrated virtually undetectable R binder (binding capacity of 31–38 ng/liter versus 41,690 ± 23,820 ng/liter for control subjects). Unlike previous cases of R binder deficiency, he seemed to maintain normal serum cobalamin levels while receiving monthly cyanocobalamin injections. This and his normal serum unsaturated binding capacity were due to elevated TC II levels. TC II carried 72%-98% of his endogenous cobalamin, the rest being attached to minor binders. As incidental findings, the patient had a serum component of molecular weight of approximately 70,000 that carried 7%-8% of his endogenous cobalamin and also had small quantities of TC II demonstrable in his saliva. Both these heretofore unappreciated minor peaks were identifiable because of the lack of R binder. The patient’s clinical presentation supports the conclusion that R binder deficiency is a benign disorder. Whether his mild hypersegmentation of neutrophils and neuropathy were related to the R binder deficiency or, more likely, arose from coexisting folate deficiency and alcohol abuse, the overall picture contrasts dramatically with the severe clinical sequelae of TC II deficiency.

R BINDER is an immunologically distinct cobalamin-binding protein that is ubiquitous in human serum [where its chief component is transcobalamin (TCII)], secretions, and many blood cells. Though various roles have been suggested, its function remains unknown. Deficiency of R binder seemed to carry no obvious metabolic penalty in the two brothers in whom it was first described, other than a falsely decreased serum cobalamin level. This apparently rare disorder has not been encountered since. The new subject with R binder deficiency reported here provides a further bonus, the opportunity to assess the presence of unsuspected minor cobalamin-binding proteins in serum and saliva. These are difficult to identify in normal subjects whose R binders obscure their detection.

CASE REPORT

N.C., a 77-yr-old man, was noted in 1973 to be mildly anemic. Review of his record revealed hemoglobin (Hb) values of 16.6 and 14.0 g/dl in 1962, and persistent anemia since 1965 (Hb 11.2 g/dl, MCV 90 fl, WBC 3500/μl). Iron studies and serum bilirubin were normal in 1970. Retrospective review of a 1970 blood smear revealed a borderline-elevated neutrophil lobe count (3.30 nuclear lobes/neutrophil); 7% of the neutrophils had 5-lobe nuclei, which is an increased proportion. The only other abnormal laboratory data were AS hemoglobin by electrophoresis, mildly elevated serum uric acid and creatinine, and a weakly positive rheumatoid factor. He had been treated with oral folic acid since 1970.

Past history included multinodular goiter, gout treated with colchicine, positive VDRL test for syphilis, shingles, and increased alcohol consumption. There was no family history suggestive of any cobalamin-related disorders, but he had no children or living siblings.

Abnormal physical findings in 1973 were those of mild congestive failure, goiter, residua of cataract surgery, and abnormal vibratory sense in his feet. His tongue was described as normal. Hb was 10.3 g/dl, MCV 92 fl, and WBC 3200/μl with normal differential count (no mention was made of neutrophil morphology). Serum iron and iron-binding capacity were 40 and 284 μg/dl, respectively. Serum folate was 3.0 μg/liter (normal 5–21) and cobalamin was <50 ng/liter (normal 170–760). Folic acid was discontinued and he was given vitamin B complex and iron tablets at discharge.

Several months later, monthly injections of cyanocobalamin were begun. He has continued these without interruption because he feels better after the injections. The following year he apparently felt better, though his blood counts remained unchanged over the next 6 yr. His alcohol intake diminished during this time.

In 1980 he was readmitted with tiredness and weight loss. His Hb was 8.7 g/dl, MCV 93 fl, WBC 4300/μl, platelet count 134,000/μl, and corrected reticulocyte count 1.4%. Neutrophil lobe average was normal (2.70) and only 3% of neutrophils had 5-lobe nuclei. Serum cobalamin was 636 ng/liter (a few days after injection), folate 1.3 μg/liter (normal 2.5–20.0), and ferritin 230 μg/liter. Anti-intrinsic factor antibody (blocking type) was not present in serum. Bone marrow aspirate was hypoplastic but was not megaloblastic; the only abnormal features were slight plasmacytosis and the iron distribution of “anemia of chronic disease.” Multiple blood studies were normal except for slightly elevated serum creatinine and acid phosphatase, and low T₄ with otherwise normal thyroid function studies. Upper gastrointestinal x-ray studies showed mild duodenal bulb deformity; endoscopy revealed erythematous, edematous folds at the pylorus and atrophic gastritis; intravenous pyelography showed slightly small kidneys; prostatic biopsy showed benign hypertrophy; bone x-rays showed diffuse osteoporosis and bone scan was interpreted as compatible with Forrestier’s disease. The patient slowly improved during hospitalization.

The following year he had some progression in congestive heart
failure. His vibratory sense perception remained abnormal in his feet. His Hb was 10.7 g/dl and MCV was 93 fl. Neutrophil lobe average (3.00) was normal; only 1% of neutrophils had 5-lobed nuclei. He refused further studies related to his cobalamin problem, but a few months later consented to a gastric analysis. The basal collection (60 ml in 1 hr) had a pH of 1.6 and the betazole-stimulated collection (91 ml) had a pH of 1.5. Unfortunately, the specimen was discarded, so binder assays could not be done. He underwent a Schilling test 6 mo later, which revealed 7.8% excretion (normal >8.0%) in a urine volume of 785 ml. At that time his serum creatinine was 2.0 mg/dl.

MATERIALS AND METHODS

Venous blood was immediately centrifuged after clotting. Since EDTA-anticoagulated plasma gave identical results in this study, only serum results are presented. Whole-mouth saliva was obtained in the fasting state by direct spitting into a plastic cup. The saliva was centrifuged to remove debris. All specimens were assayed fresh or after storage at −20°C.

Serum cobalamin radioassay was done by a modification of the method of Kolhouse et al., using gastric juice from a previously reported R binder-deficient subject which contains only intrinsic factor. In some experiments, radiodilution assay was also done using saliva R binder.

Serum or saliva were fractionated by Sephadex G-200 gel filtration as previously described, using 0.1 M Tris−1 M NaCl buffer, pH 8.6, containing 0.02% NaN3. Unsaturated cobalamin-binding capacity was quantitated from the elution pattern. Radioactive cyanocobalamin (57CoB12) of 15 µCi/µg specific activity was obtained from Amersham/Searle Corp., Arlington Heights, Ill. For some experiments where low binding capacities were noted or fractions had to be used, higher specific activity 57CoB12 (220 µCi/µg) was used. Anti-R-binder antiserum was raised in rabbits injected with human saliva. Rabbit anti-TC-II antiserum was a gift from Dr. Charles A. Hall, VA Medical Center, Albany, N.Y. Both antisera were shown to be specific in their reactions.

Endogenous cobalamin distribution was determined on Sephadex G-200 gel eluate fractions whose pH was adjusted to the appropriate assay pH. The fractions were shown not to vary in their undenatured residual binding of 57CoB12, as determined by supernatant controls run without adding binding protein. The serum specimens were applied to the column in 0.2−0.5 ml aliquots, depending on their cobalamin concentrations.

Serum and saliva, obtained in 1979 from W.B., one of the initially reported R-binder deficient brothers, was also studied.

RESULTS

Serum

In 1980, three serum specimens were collected spanning a 5-wk period from 1 wk after cyanocobalamin injection until the next such injection. Cobalamin concentrations ranged from 355 to 460 ng/liter by radio assay using pure intrinsic factor. A serum obtained in 1981 had a level of 385 ng/liter. Concentrations in all sera were slightly lower when measured by R binder radioassay.

The endogenous cobalamin, as fractionated on Sephadex G-200 gel, was largely carried by TC II in the three specimens tested. Figure 1 shows two such fractionations compared to a normal serum. The latter carries virtually all its cobalamin on R binder or TC I, with a smaller fraction in the void volume ("TC O"); only a very small amount is attached to TC II. The patient's sera from 1980 and 1981 had, respectively, only 0% and 3% of their cobalamin attached to TC I, while 72% and 79% was carried by TC II and 20% and 11% by "TC O." Approximately 8% and 7% in the two respective patient sera were eluted in the region of fractions 32−34, which corresponds to a molecular weight of about 70,000. Normal serum could not be accurately assessed for the latter "binder" because of the large overlap from the TC I peak. Another serum from the patient in 1980 (not shown) contained 98% of its endogenous cobalamin in the TC II region and 2% in the "TC O"; none was carried by TC I.

In 1980, the patient's serum unsaturated cobalamin-binding capacity ranged from 1280 to 1320 ng/liter (normal mean ± 1 SD, 1041 ± 194 ng/liter). Less
than 5 ng/liter was TC I (<0.5% of the binding capacity). Normal TC I unsaturated binding capacity is $213 \pm 171$ ng/liter, or about 20% of serum unsaturated binding capacity. In 1981, the patient’s binding capacity had risen to $2314$ ng/liter. Once again, less than 5 ng/liter of this was TC I. At all times, 4%-5% of the binding capacity was “TC O,” as is seen in normal sera.7,8

The 1981 serum, labeled with $^{57}$CoB$_{12}$, was incubated separately with anti-TC-II and anti-R-binder antisera. All the binding protein reacted with anti-TC-II and was shifted to the void volume on gel chromatography (Fig. 2). No reaction occurred with anti-R-binder antiserum. The pattern with the latter in Fig. 2 is identical to that of the patient’s serum filtered by itself; the small void volume peak represents the patient’s “TC O.” The reaction with anti-TC-II antiserum may incidentally have unmasked a small 70,000-molecular weight cobalamin-binding component.

The patient’s serum contained no inhibitors of cobalamin binding. Its incubation with a normal serum or with serum from W.B. produced strictly additive binding patterns on gel chromatography. No R binder appeared in the mixture with WB serum.

**Saliva**

Saliva specimens were collected from the patient in 1980 and 1981. Neither contained measurable cobalamin. The first specimen had an unsaturated cobalamin-binding capacity of 220 ng/liter, virtually all of it “TC O” and TC II. The second specimen’s binding capacity was 645 ng/liter, with similar fractionation (Fig. 3). Saliva from 24 normal and ill subjects had a mean binding capacity of $41,690 \pm 23,820$ ng/liter, with no value below 12,000 ng/liter.

As suggested in Fig. 3, a minute R binder fraction seems present in the patient’s saliva. This was estimated to be only 31 ng/liter in binding capacity in the 1980 specimen and 38 ng/liter in the 1981 specimen. Its presence was confirmed by reaction of the 1981 sample with anti-R-binder antiserum.

That the patient’s saliva contained TC II was confirmed by its positive reaction with anti-TC-II antiserum. Chromatographic quantitation gave values of 130 and 378 ng/liter for TC II in the two specimens. A saliva specimen from WB was shown to contain TC II in similarly small concentrations. That his salivary TC II did not represent simply some occult
blood in whole-mouth saliva (none of the tested saliva specimens were visibly blood-tinged) was supported by demonstration of TC II even in parotid saliva collected with a cap over the parotid duct opening.9

Normal saliva reacted only with anti-R-binder antiserum. No reaction was detectable with anti-TC II antiserum, presumably because of the huge preponderance of R binder obscuring any possible small TC II component.

DISCUSSION

This is the second report of R binder deficiency. Many of the details resemble and therefore support those in the two brothers described previously.1 The patient clearly lacked R binder in his serum and saliva. Other secretions and cells were not similarly examined because of his unwillingness to undergo further study, but the two fluids studied provide adequate support for the diagnosis. That serum (as well as plasma) contained virtually no R binder suggests that his leukocytes were deficient too, since these cells otherwise usually leak the protein into serum in vitro.10-12

Nevertheless, decreased R binder in serum is not in itself diagnostic of the entity of R binder deficiency and can occur in various conditions.13,14 However, the latter patients have normal salivary R binder concentrations;14 only their serum seems deficient in the binder, and usually the TC I decrease is moderate. I have found this to be the case particularly in patients with multiple myeloma (unpublished data). A further example is a patient with immune agranulocytosis who had marked deficiency of serum TC I (12 ng/liter) but had 133,050 ng/liter binding capacity in her saliva, all of it R binder (unpublished data). It is the concurrent and persistent lack of R binder in saliva that establishes the diagnosis of R binder deficiency in the present patient. Virtually no healthy or ill subjects have salivary R binder concentrations substantially lower than 10,000 ng/liter, and most have had levels of 30,000-40,000 ng/liter.15-18 My data from 24 control subjects are quite typical in this respect.

It is worth noting, however, that R binder capable of binding cobalamin was not totally absent in my patient. Serum contained a very small TC I unsaturated cobalamin-binding capacity and an equally small TC I component carrying endogenous cobalamin. Together these constituted much less than 5% of normal serum TC I concentrations. Similarly, a small R binder component was identifiable in saliva, the 31-38 ng/liter estimated concentration representing about 0.1% of the normal amount.

Like the first patient reported,1 the present one was initially misdiagnosed as having pernicious anemia because of a falsely low serum cobalamin level. (His Schilling test result was borderline, but this seemed to be due to his mild renal failure, which depresses urinary excretion of the isotope,19 and he had normal gastric acid secretion.) Unlike the initial patient,1 he maintained normal serum cobalamin levels after cyanocobalamin injections. However, all his cobalamin was carried by TC II instead of TC I, the reverse of the normal pattern.20-22 His serum unsaturated cobalamin-binding capacity was similarly normal because of his elevated TC II levels.

A hereditary disorder was suggested in the initial brothers.1,22 If the disorder is indeed hereditary, its first detection at the advanced age of the present patient is noteworthy. This supports the previous conclusion that lack of R binder is a benign condition. Whether it is entirely without metabolic consequence is somewhat less clear. My patient never seemed to have a megaloblastic anemia and his present unexplained anemia is unrelated to cobalamin deficiency, but he had hypersegmentation of neutrophils initially. Although the hypersegmentation was probably due to coexisting folate deficiency (serum folate level was low and he was initially treated with folic acid), patient W.B. also had mild hypersegmentation (but had a normal marrow deoxyuridine suppression test) attributed to folate deficiency.1 Furthermore, the present patient had impaired vibratory sense perception in his feet, a mild defect perhaps attributable to alcoholic neuropathy. Patient W.B. had a progressive debilitating neurologic disorder featuring spastic paraparesis and dementia,23 which is not typical for cobalamin-deficiency-related neurologic disease and was initially diagnosed as multiple sclerosis.1 While all these findings in both cases seem unrelated to cobalamin deficiency, the accumulated coincidences must give some pause.

Finally, the absence of R binder in my patient allowed a more ready identification of some minor cobalamin-binding protein components that are probably otherwise obscured in the normal individual. Thus, 7%-8% of his endogenous cobalamin was carried by a binder of approximately 70,000 molecular weight. This resembles a minor binder peak in Fig. 4 of a study by Hall et al.24 of a TC-II-deficient child receiving frequent cobalamin injections. The nature of this binder remains to be established. Clearly, it is easier to recognize when one or the other normal cobalamin-binding protein of blood is missing. I have also observed such a peak carrying a large amount of cobalamin in patients who had normal binders but who had recently been injected with cobalamin (unpublished data).

Similarly, the lack of R binder in saliva allowed the demonstration of a small amount of TC II in saliva of
both this patient and W.B. Hall has also noted this in W.B. saliva (Hall CA, personal communication). The presence of TC II even in parotid saliva indicated that it could not have been a plasma contaminant arising from undetected bleeding from the gums. Presumably, normal salivas contains a similarly small TC II component that is hidden by the huge R binder component, though I have been unable to demonstrate this directly. Heretofore, all studies but one (which from undetected bleeding from the gums. Presumably, it could not have been a plasma contaminant arising presence of TC II even in parotid saliva indicated that

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