Mild Transcobalamin I (Haptocorrin) Deficiency and Low Serum Cobalamin Concentrations

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Background: Low cobalamin concentrations are common, but their causes are often unknown. Transcobalamin I/haptocorrin (TC I/HC) deficiency, viewed as a rare cause, has not been examined systematically in patients with unexplained low serum cobalamin.

Methods: Total TC I/HC was measured by RIA in three subgroups of 367, 160, and 38 patients with different categories of low cobalamin concentrations and three comparison subgroups of 112, 281, and 119 individuals with cobalamin concentrations within the reference interval. Additional studies, including family studies, were done in selected patients found to have low TC I/HC concentrations.

Results: Low TC I/HC concentrations suggestive of mild TC I/HC deficiency occurred in 54 of 367 (15%) patients with low cobalamin identified by clinical laboratories and 24 of 160 (15%) patients whose low cobalamin was unexplained after absorption and metabolic evaluation, but in only 2 of 38 patients with malabsorptive causes of low cobalamin concentrations (5%). The prevalence was only 3% (8 of 281 plasma samples) to 5% (6 of 112 sera) in patients with cobalamin concentrations within the reference interval and 3% (4 of 119) in healthy volunteers. Three patients with low cobalamin (0.6%) had severe TC I/HC deficiency with undetectable TC I/HC. Presumptive heterozygotes for severe TC I/HC deficiency in two families had the findings of mild TC I/HC deficiency; mild deficiency was also found in at least three of seven studied families of patients with mild TC I/HC deficiency.

Conclusions: Mild TC I/HC deficiency is frequently associated with low cobalamin, is often familial, and its biochemical phenotype appears identical to the heterozygous state of severe TC I/HC deficiency. Severe TC I/HC deficiency also appears to be more common than suspected. Both diagnoses should be considered in all patients with unexplained low serum cobalamin.

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Serum cobalamin concentrations are frequently below the reference interval in patients who have no obvious causes for such low concentrations, and it is often not clear what should be done about them (1, 2). Only one-half or fewer of low cobalamin concentrations are explained by malabsorption of either free or food-bound cobalamin, and dietary insufficiency is uncommon even among the elderly (2–6). Moreover, metabolic studies indicate that 15–40% of patients with low serum cobalamin do not have cobalamin deficiency (1, 2, 7–10), which suggests that as many as 15–40% of cobalamin values below the reference interval may be diagnostically misleading. This diagnostic dilemma is not resolved by changing reference intervals for cobalamin because low concentrations that represent true metabolic deficiency and malabsorption are not always lower than low concentrations that do not.

Among the few known nonmalabsorptive causes of low cobalamin concentrations is hereditary absence of transcobalamin I/haptocorrin (TC I/HC) (11–16), which causes low serum cobalamin concentrations because most cobalamin circulates in the blood attached to TC I/HC (17–19). Neither malabsorption nor cellular deficiency of cobalamin results from the absence of TC I/HC, which unlike transcobalamin II is not needed for cellular uptake of cobalamin.

Only a handful of patients with hereditary absence of TC I/HC have been reported (11, 13–16), which has fostered the impression that the condition is rare. The present report describes the results of surveys of several subpopulations with selected characteristics that were undertaken because of observations in two instructive

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1 Nonstandard abbreviations: HC, haptocorrin; TC I, transcobalamin I; and MMA, methylmalonic acid.
2 Because many names have been used for TC I, such as R binder, cobalophilin, α-globulin binder, and more recently HC, and because unanimity still does not exist on nomenclature, this report will use a hybrid of the two most frequently used names, TC I/HC, until a formal consensus is established.
families with low cobalamin concentrations associated with decreased rather than absent concentrations of TC I/HC. The findings in those two families, as well as informative family studies in patients uncovered in the surveys, are also presented, along with further studies in some of the individuals found to have low TC I/HC in the surveys. As discussed elsewhere (20), most assays of TC I/HC have measured its ability to bind cobalamin added in vitro, thus detecting only unsaturated apo-TC I/HC. However, cobalamin-saturated holo-TC I/HC usually exceeds apo-TC I/HC in plasma (21, 22), which limits the informativeness of measurements of apo-TC I/HC alone. The present study measures TC I/HC with a RIA that quantifies total TC I/HC directly. The reference interval for this RIA was comparable to but slightly lower (20) than the intervals for two reported additive methods of determining total TC I/HC (21, 22). As presaged in a preliminary abstract of part of the data (23), the findings reported here suggest that mild TC I/HC deficiency is relatively common and is responsible for many otherwise unexplained low cobalamin concentrations.

**Participants and Methods**

Individuals from several sources, reflecting different subpopulations, were tested (groups 1–6). Each group was selected to address slightly different but interrelated questions about TC I/HC deficiency.

**Patients with Low Serum Cobalamin**

Because low cobalamin concentrations were the abnormality of interest that prompted the study and are the chief "clinical" expression of TC I/HC absence (11–16), patients with cobalamin concentrations known to be low were targeted first. Study of this subset also was the most effective initial strategy to identify affected patients, especially because the frequency of TC I/HC deficiency in the general population was unknown and thought to be very small. Two sources of specimens were studied, one prospective and one retrospective.

**Group 1.** Group 1 consisted of serum samples with low cobalamin concentrations identified prospectively in three clinical laboratories. These laboratories, at a teaching community hospital, a municipal hospital, and a university hospital, each set aside all leftover, low-cobalamin serum specimens identified during routine, clinically requested assays. The specimens were stored at −20 °C. All consecutive specimens were obtained, excepting only exhausted samples and the occasional, inadvertently discarded sample. The three laboratories provided 367 sera with low cobalamin concentrations (of >4000 specimens that were assayed), but the use of these samples involved several limitations. One hospital required patient anonymity, which prevented follow-up or ascertainment of clinical and demographic information. Because cobalamin assays are always done on serum in clinical laboratories, the TC I/HC assay also had to be performed with serum samples instead of the more reliable (24, 25) EDTA-anticoagulated plasma. This shortcoming was compensated to some degree by establishing a separate reference interval for TC I/HC in serum. A final limitation was that each laboratory used a different cobalamin assay with a slightly different cutpoint (148 to 181 pmol/L) to define low concentrations. Because it formed the basis on which the laboratories set aside the samples for this study and because sample volume was usually inadequate for reassembly, each laboratory’s identification of low concentrations according to its assay criterion was accepted. Reassembly of 22 samples of adequate volume from the three laboratories with the radiodilution assay for cobalamin in my laboratory (26) uncovered only one case in which the characterization was not confirmed.

**Group 2.** A separate group of 160 consecutive patients with low cobalamin concentrations whom I had studied and who had no malabsorptive or other known causes of cobalamin deficiency were selected retrospectively to determine the prevalence of low TC I/HC concentrations among patients with low cobalamin concentrations (<140 pmol/L) for which no alternative explanation was found. Schilling tests and/or egg-yolk cobalamin absorption tests (27) gave results within the appropriate reference intervals in all cases; normal food-cobalamin absorption implies a normal absorption of free cobalamin by Schilling test as well (6). Serum anti-intrinsic factor antibody was undetectable in all cases, and dietary histories were adequate for intake of food sources of cobalamin (meat, poultry, and dairy products). No metabolic evidence of cobalamin deficiency was found by homocysteine concentrations, methylmalonic acid (MMA) concentrations, and/or deoxyuridine suppression test results in any of the 160 patients, and none had clinical signs of deficiency. The only study exclusion factor was unavailability of a properly processed plasma sample.

**Group 3.** For comparison with group 2, which featured unexplained low cobalamin concentrations, EDTA-anticoagulated plasma samples were tested in 38 patients with low cobalamin concentrations, in whom clinically and metabolically expressed cobalamin deficiency was documented and whose malabsorptive causes were identified by appropriate abnormalities of the Schilling test and/or history of gastric surgery (29 had pernicious anemia, 5 had ileal disease, and 4 had partial gastrectomy). The only selection factor was availability of pretreatment plasma samples with low cobalamin concentrations.

**Individuals with Cobalamin Concentrations within the Reference Interval**

Two groups of individuals were tested to examine the prevalence of TC I/HC deficiency in patients with cobalamin concentrations within the reference intervals. Group 4 consisted of 281 patients whose cobalamin concentrations were within the reference interval for my laboratory.
assay during the same time periods that patients in group 2 were studied and whose EDTA-anticoagulated plasma was available for direct TC I/HC comparison with group 2, who had low cobalamin concentrations. Group 5, consisting of 112 blindly selected sera found by the three above-mentioned clinical laboratories to have cobalamin concentrations within the respective reference intervals, provided a direct serum comparison with group 1.

PRESUMABLY HEALTHY VOLUNTEERS
The prevalence of low TC I/HC concentrations in the healthy population at large, rather than patients, was estimated prospectively in EDTA-anticoagulated plasma collected from 119 blood donors, medical personnel, and other volunteers (group 6); their cobalamin status was unknown before enrollment. Samples were taken anonymously, as specified by the Institutional Review Board.

The study and all sources of participants were approved by the Institutional Review Board. All participants who underwent venipuncture for the study gave informed, written consent; when anonymity was mandated, the consent forms were separated from the coded blood samples.

METHODS
All EDTA-anticoagulated blood specimens were centrifuged, and plasma was separated within 1–2 h of blood drawing to minimize in vitro release of TC I/HC into the plasma (24, 25). Serum specimens were processed by the clinical laboratories in their routine fashion.

The RIA for total TC I/HC was performed as described previously, with determinations done in triplicate (14, 20). TC I/HC concentrations were shown to be stable even after prolonged storage and freezing and thawing and to have interassay CVs of 7–13%, depending on the TC I/HC concentration (20). Saturating plasma samples by adding cobalamin did not alter TC I/HC results (data not shown), suggesting that the RIA recognizes holo-TC I/HC and apo-TC I/HC equally. A reference interval of 165–454 pmol/L was established with use of EDTA plasma from 434 healthy volunteers (20); for serum, the reference interval was 234–557 pmol/L, based on results for 102 healthy volunteers (23).

Cobalamin was measured in my laboratory by a previously reported radioisotope dilution assay using pure intrinsic factor (26). A reference interval of 140–750 pmol/L was established with use of results from 269 healthy volunteers. The cobalamin assay methods used in the three clinical laboratories were the Quantaphase II radiodilution assay (Bio-Rad), the Immuno-1 immunoassay (Bayer Diagnostics), and the IMx immunoassay (Abbott Diagnostics).

The Student t-test, Fisher exact test, and other analyses were performed with use of SAS software, release 8.0 (SAS Institute).

## Results

TC I/HC deficiency in serum from patients with low cobalamin. TC I/HC concentrations were low (<234 pmol/L) in 54 of the 367 sera (15%) that had low cobalamin concentrations identified by the clinical laboratories (group 1), compared with 6 of 112 sera (5%) with cobalamin concentrations within the reference interval from the same sources (group 5; P = 0.009). Among the 60 low TC I/HC concentrations identified in these two groups, three specimens had the undetectable concentrations characteristic of severe TC I/HC deficiency (see below). The low TC I/HC concentrations in the other 57 sera ranged from 47 to 233 pmol/L (median, 171 pmol/L).

TC I/HC deficiency in patients with unexplained low cobalamin. Low EDTA-plasma TC I/HC concentrations (<165 pmol/L) were significantly more prevalent in group 2 (24 of 160; 15%), the patients who had low cobalamin concentrations that were unexplained by malabsorption or dietary insufficiency, than in plasma samples from group 4 (8 of 281; 3%), the patients with cobalamin concentrations within the reference interval, or group 3 (2 of 38; 5%), the patients with low cobalamin concentrations caused by standard malabsorptive disorders (P <0.00001). The 34 low plasma TC I/HC values in the three groups ranged from 69 to 164 pmol/L (median, 138 pmol/L).

TC I/HC deficiency in presumably healthy volunteers. In EDTA plasma from healthy volunteers (group 6), only 4 of 119 TC I/HC values (3%) were <165 pmol/L, a rate comparable to that in patients with normal cobalamin status, such as group 4. Two of these four low values were in volunteers who also were found to have low cobalamin concentrations (77 and 131 pmol/L). Because the donors were anonymous, they could not be investigated further.

CHARACTERISTICS OF SEVERE AND MILD TC I/HC DEFICIENCY
Severe TC I/HC deficiency. In groups 1 and 2, TC I/HC concentrations were undetectable in 3 of the 537 (0.6%) samples with low cobalamin concentrations. Serum and plasma are equally reliable in identifying severe TC I/HC deficiency because the absence of granulocytic TC I/HC precludes artifactual release of the protein into serum (11). Two of the three patients could not be studied further. One patient, a 77-year-old black woman with liver disease, renal failure, alcohol abuse, and gastrointestinal bleeding, died before she could be contacted. Reassay of her undiluted serum (the RIA uses serum diluted 1:2) suggested that trace amounts of TC I/HC were present; her serum cobalamin concentration was 119 pmol/L. The second patient, whose anonymity prevented restudy, had undetectable TC I/HC even in undiluted serum and a cobalamin of 20 pmol/L. Lactoferrin concentrations were within the reference interval in both cases.
The third patient with undetectable TC I/HC was studied further. Complete absence of immunoreactive TC I/HC was confirmed in a fresh plasma sample. She also had undetectable TC I/HC in saliva, which is characteristic of severe TC I/HC deficiency. Her findings, along with those of her daughter, a presumptive heterozygote for TC I/HC deficiency, are shown in Table 1 (family A). A low cobalamin concentration had been noted several years earlier in the 57-year-old propositus, for which she received intermittent cobalamin injections. Her history included rheumatic heart disease, probable coronary artery disease, depression and anxiety, arthritis, and carpal tunnel syndrome. Her ancestry was Puerto Rican, Caribbean Indian, and African, and the family history included low cobalamin concentrations in a sister. There were no abnormal neurologic findings. Except for mildly increased hepatic enzymes and the presence of hemoglobin AS, her laboratory data were within reference values, including the blood count and plasma homocysteine. Her 34-year-old daughter was also receiving frequent cobalamin injections because of a low cobalamin concentration discovered the previous year. She had a past history of pseudotumor cerebri (attributed to obesity), keratoconus, and asthma. Her TC I/HC concentration was low-normal in plasma and normal in saliva. Her laboratory data, aside from a mild iron deficiency anemia, were within reference values, including a Schilling test, hemoglobin electrophoresis, and homocysteine and MMA concentrations (measured a few weeks after cobalamin injection). Lactoferrin concentrations were within reference values in both the mother and daughter.

Presumptive heterozygosity for TC I/HC deficiency. Unlike family A, families B and C were identified before the surveys and helped prompt the undertaking of those surveys, as mentioned earlier. Family B provided the first view of presumptive heterozygosity for TC I/HC deficiency, albeit the precise deficiency in the two original propositi (11) was severe combined TC I/HC and lactoferrin deficiency (28). The presumptively heterozygous sons of the two propositi were found to have low but measurable plasma TC I/HC and cobalamin concentrations (Table 1). The son of propositus 1 had a 20-year history of Crohn's disease with resection of 2 feet of terminal ileum. He took a multivitamin that contained folic acid and intermittently self-injected cobalamin. The son of propositus 2 had no medical problems other than hyperlipidemia. Both sons had blood counts and homocysteine concentrations within reference values. The data from these presumptive heterozygotes and those of their uncle, who had colon cancer, renal insufficiency, and normochromic anemia at the time of testing and did not take cobalamin, are shown in Table 1; the previous data from the original propositi are included for comparison only. As in the presumptively heterozygous daughter in family A, the noteworthy findings in the heterozygotes were the mildly decreased or borderline TC I/HC concentrations in plasma and the normal concentrations in saliva. This can be contrasted with the complete absence of TC I/HC in both plasma and saliva in all of the severely deficient propositi.

The findings in family C, who had pure TC I/HC deficiency without lactoferrin involvement and had European, American Indian, and Jewish ancestry, also helped stimulate the undertaking of the surveys because the family had no identifiable member with severe TC I/HC deficiency (Table 2). The propositus was found to have a cobalamin concentration of 94 pmol/L at the age of 6 months and was treated intermittently with cobalamin for several months. His history was positive only for a presumed hepatitis 2 months before. His MMA concentration was minimally increased (390 nmol/L), but the results of neurologic examination, growth and development assessment, blood count, homocysteine concentration, and the Schilling test were normal. The boy's father was discovered to have low cobalamin concentrations and mild TC I/HC deficiency also (Table 2). His blood count, MMA, homocysteine, Schilling test, and food-cobalamin absorption test results were within the appropriate reference values, and the results of his neurologic examination were normal, but he had slightly abnormal posterior tibial sensory evoked potentials and P300 event-related potentials. Both father and son had plasma and saliva TC I/HC findings similar to those of the presumptive heterozy-

| Table 1. Family studies of propositi with severe TC I/HC deficiency that identify family members who are obligate heterozygotes for the condition.a |
|------------------|---------------|---------------|---------------|
| Patient          | Plasma, pmol/L| Saliva, nmol/L| Cobalamin, pmol/L |
| Family A         |               |               |               |
| Propositus       | 0b            | 0b            | 65c           |
| Daughter         | 169           | 21.5          | –c            |
| Family B         |               |               |               |
| Propositus 1a    | Absent        | Absent        | Low           |
| Propositus 2a    | 0b            | 0b            | 39            |
| Brother of both  | 98            | 23.1          | 157           |
| propositi2f      |
| Son of propositus| 74            | 24.2          | 139           |
| Son of propositus| 111           | 19.4          | 127           |
| Reference interval | 165–454     | 6.5–98.1      | 140–750       |

a The propositus in family A was found during the survey to have classic severe TC I/HC deficiency in this study (lactoferrin concentrations were within the reference interval). Combined TC I/HC and lactoferrin deficiency was identified previously in family B (28).

b Below the limits of detection by RIA.

c Receiving cobalamin injections (the propositus intermittently and her daughter regularly); both had low cobalamin concentrations before cobalamin therapy was begun.

d Presumptive heterozygote for severe TC I/HC deficiency.

e The previously published data for the two severely affected propositi (11, 12) are shown for comparison only. The specific values for propositus 1 are not given because they were obtained by methods considerably different from those used here.

f Probable heterozygote for severe TC I/HC deficiency.
gotes shown in Table 1. The child’s mother had TC I/HC concentrations within the reference interval. Studies in patients with low TC I/HC in the surveys. Non-anonymous patients found in the surveys to have mildly low TC I/HC and cobalamin concentrations were studied further whenever possible, and their families were also tested (Table 2). One or more affected relatives were identified in families D and E. The father of the propositus in family F had a low cobalamin concentration and low-normal TC I/HC and may thus have had mild TC I/HC deficiency, but this remains unconfirmed, as does the somewhat similar picture in the brother; their findings, like the borderline findings in the mother in family E, closely resemble the low-normal TC I/HC concentrations in the obligate heterozygote in family A (Table 1). None of the patients and family members had megaloblastic anemia, hyperhomocysteinemia, evidence of cobalamin malabsorption, or other known causes of cobalamin deficiency, and none showed any clinical or laboratory improvement with cobalamin therapy. A few propositi had neurologic, cognitive, or electrophysiologic abnormalities (e.g., abnormal evoked potentials), which were often the reason that the cobalamin assay was requested in the first place. However, their symptoms did not improve with cobalamin therapy. Family studies in four additional TC I/HC-deficient patients who were discovered in the survey identified no other affected family members, but only one or two relatives were tested in each case (data not shown).

Twenty-four additional nonanonymus patients with low TC I/HC concentrations found in the survey were studied in further detail, but relatives were not available for study. The TC I/HC concentrations in the follow-up EDTA-anticoagulated plasma samples were again low in 22 cases and borderline low-normal in the other 2. These results confirmed their serum or plasma results in the survey and suggested that the low TC I/HC concentrations were not transient. None of the 24 patients had megaloblastic anemia, increased homocysteine or MMA concentrations, or evidence of malabsorption by either Schilling tests or egg-yolk cobalamin absorption tests. As in all cases of mild TC I/HC deficiency, the TC I/HC concentrations in saliva were within the reference interval in all 19 patients tested for it.

### Discussion

Patients heterozygous for severe TC I/HC deficiency (Table 1) have a mild biochemical phenotype, characterized only by usually mildly decreased TC I/HC concentrations in plasma, whereas severe deficiency is characterized by an absence of TC I/HC in plasma and in

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma, pmol/L</th>
<th>Saliva, nmol/L</th>
<th>Cobalamin, pmol/L</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family C (mother was unaffected)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propositus</td>
<td>165&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.7</td>
<td>94</td>
<td>Low cobalamin noted at 14 months; MMA borderline high</td>
</tr>
<tr>
<td>Father&lt;sup&gt;c&lt;/sup&gt;</td>
<td>117</td>
<td>11.9</td>
<td>139</td>
<td>Mild abnormality of evoked potentials</td>
</tr>
<tr>
<td><strong>Family D (mother was unaffected)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propositus</td>
<td>139</td>
<td>11.7</td>
<td>137&lt;sup&gt;d&lt;/sup&gt;</td>
<td>47 years of age; chronic laryngitis; vague neurologic symptoms unresponsive to cobalamin</td>
</tr>
<tr>
<td>Son&lt;sup&gt;c&lt;/sup&gt;</td>
<td>115</td>
<td>18.6</td>
<td>165&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>Family E (one brother was unaffected)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propositus</td>
<td>162</td>
<td>11.7</td>
<td>139</td>
<td>Asymptomatic; discovered in survey; 36 years of age</td>
</tr>
<tr>
<td>Mother</td>
<td>193&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.4</td>
<td>91</td>
<td>Dementia at age 71 years</td>
</tr>
<tr>
<td>Brother</td>
<td>128</td>
<td>13.3</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td><strong>Family F (mother and one brother were unaffected)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propositus</td>
<td>112</td>
<td>105&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>Memory and concentration deficits; neuropathy (legs) at age 23 years; no response to cobalamin</td>
</tr>
<tr>
<td>Father&lt;sup&gt;d&lt;/sup&gt;</td>
<td>185&lt;sup&gt;e&lt;/sup&gt;</td>
<td>52.5</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Brother&lt;sup&gt;d&lt;/sup&gt;</td>
<td>239&lt;sup&gt;e&lt;/sup&gt;</td>
<td>150</td>
<td>150&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The deficiency in the propositus in family C was identified before the survey, whereas all the other propositi were identified in the surveys reported here. Malabsorption and other known causes of cobalamin deficiency were excluded. Only data from family members with abnormal, borderline, or low-normal cobalamin and TC I/HC concentrations are shown. The propositi are shown in bold font.

<sup>b</sup> Serum value (reference interval, 234–557 pmol/L). All other values in the column are in plasma.
<sup>c</sup> The MMA and homocysteine concentrations were within the reference intervals.
<sup>d</sup> The serum cobalamin shown was the initial value at another institution, before the patient was started on cobalamin therapy.
<sup>e</sup> Low-normal value.
secretions. Table 3 summarizes the known biochemical characteristics of mild and severe TC I/HC deficiencies gleaned from the present study and published data. The unexplained discrepancy between the patterns found in blood and secretions suggests that TC I/HC is expressed, regulated, or elaborated differently in different cellular sources. The same kind of discrepancy between plasma and secretion concentrations in homozygotes and heterozygotes was evident for lactoferrin in family B, who had combined TC I/HC and lactoferrin deficiency (28).

As for serum cobalamin concentrations in TC I/HC deficiency, published data (11–16) and the three new cases found in the present survey indicate that severe TC I/HC deficiency is accompanied by very low (<100 pmol/L) or occasionally by mildly low cobalamin concentrations, which are hard to maintain within the reference interval despite cobalamin injections. The present data show that cobalamin concentrations are usually only mildly decreased in mild TC I/HC deficiency; the median cobalamin value was 125 pmol/L in those samples measured with my isotopic assay. However, the range of cobalamin concentrations was 12–370 pmol/L. That mildly low cobalamin concentrations predominate but are not inevitable in TC I/HC deficiency is not surprising, but the explanation for the occasionally normal concentrations is unknown.

The finding deserving the greatest emphasis is that mild TC I/HC deficiency may be one of the most common causes of low cobalamin concentrations. The two surveys of patients with low cobalamin concentrations, one prospective and based on serum samples and one retrospective and based on plasma samples, showed that a remarkably consistent 15% of the low cobalamin concentrations were associated with low TC I/HC concentrations suggestive of mild TC I/HC deficiency. The family data also suggest that many of these patients’ findings, which resembled those in obligate heterozygotes for TC I/HC deficiency, were hereditary in origin (Table 2). However, it seems probable that some patients have acquired TC I/HC deficiencies whose mechanisms remain to be defined, as do the comparative frequencies of hereditary and acquired cases.

The contrast between the 15% prevalence of low TC I/HC concentrations in patients with unexplained low cobalamin concentrations and the much lower prevalence of 3–5% in comparable groups of patients and volunteers with cobalamin concentrations within the reference interval strengthens the association between low cobalamin and TC I/HC concentrations. The observation that patients with low cobalamin concentrations attributable to known malabsorptive causes, such as pernicious anemia, had TC I/HC deficiency as infrequently (5%) as patients with cobalamin concentrations within the reference interval indicates that mild TC I/HC deficiency is the probable cause rather than a consequence of low cobalamin concentrations.

In accordance with the relatively high frequency of mild TC I/HC deficiency, the 0.6% prevalence of severe TC I/HC deficiency in the survey of low cobalamin concentrations suggests that it too is not the rare event that was assumed from the few individual case reports (11–16). However, determining its true prevalence in the population at large will require the testing of thousands of healthy individuals. No cases of severe TC I/HC deficiency were detected in the 393 volunteers and patients with cobalamin concentrations within the reference interval in the present study.

The results indicate that TC I/HC deficiency should be added to the list of common causes of low cobalamin concentrations. With a frequency approximating 15% for

### Table 3. Characteristics of mild TC I/HC deficiency, often the heterozygous state of severe TC I/HC deficiency, compared with severe TC I/HC deficiency.

<table>
<thead>
<tr>
<th>Findings in blood</th>
<th>Severe TC I/HC deficiency</th>
<th>Mild TC I/HC deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma TC I/HC</td>
<td>Absent</td>
<td>Decreased (or low-normal)</td>
</tr>
<tr>
<td>Serum TC I/HC</td>
<td>Absent</td>
<td>Decreased (or low-normal)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum cobalamin</td>
<td>Decreased</td>
<td>Decreased (or low-normal)</td>
</tr>
<tr>
<td>Response of cobalamin concentration to cobalamin therapy</td>
<td>Remains low or increases transiently</td>
<td>May move to within reference values</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Findings in secretions</th>
<th>Severe TC I/HC deficiency</th>
<th>Mild TC I/HC deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva TC I/HC</td>
<td>Absent</td>
<td>Within reference interval</td>
</tr>
<tr>
<td>Tear TC I/HC</td>
<td>Absent</td>
<td>Present&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Findings in cells</th>
<th>Severe TC I/HC deficiency</th>
<th>Mild TC I/HC deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocyte TC I/HC</td>
<td>Absent</td>
<td>Present&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tissue cobalamin</td>
<td>Within reference interval</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

<sup>a</sup> These are based in large part on the findings in families A and B in this report and other published (11–16) and unpublished data on severe TC I/HC deficiency, and confirmed in patients identified in the surveys.

<sup>b</sup> The TC I/HC concentration may occasionally be well within the reference interval. In addition, TC I/HC concentrations are higher in serum than in plasma in unaffected as well as mildly TC I/HC-deficient individuals (because of in vitro release of TC I/HC from granulocytes during the clotting process).

<sup>c</sup> Demonstrated in the propositus in family C (TC I/HC concentration in tears, 41.6 nmol/L).

<sup>d</sup> Demonstrated in the affected father in family C and the propositus in family E (data not shown).
mild TC I/HC deficiency and 0.6% for severe deficiency in patients with low cobalamin concentrations. TC I/HC deficiency may be exceeded in frequency as a cause of low cobalamin concentrations only by food-cobalamin malabsorption (6). It also appears likely that mild TC I/HC deficiency may exist in some patients whose plasma TC I/HC concentrations exceed the mathematical cutpoint of <165 pmol/L used in the present report. Several patients who were presumptively heterozygous for TC I/HC deficiency, such as the daughter in family A and several relatives in Table 3, had "low-normal" TC I/HC concentrations. It is not unusual for phenotypic expression, including protein concentrations, to overlap with normal in the heterozygous state. More precise and reliable identification should become possible once molecular diagnosis of TC I/HC deficiency becomes available.

Until then, TC I/HC quantification can be clinically useful by identifying many unexplained low cobalamin concentrations that do not require treatment. TC I/HC deficiency does not appear to cause clinically recognizable cobalamin deficiency despite the low cobalamin concentrations. Neurologic problems have been described occasionally (11, 28), but as illustrated in Table 2, they have atypical characteristics, do not respond to cobalamin therapy, and may be coincidental. Nevertheless, further study, including electrophysiologic testing, will be needed, especially in view of cases such as the father in family C, and the issue of neurologic associations remains open.

Metabolic studies have given negative results in TC I/HC deficiency (11–16), although most reported patients were identified and tested only after undergoing trials of cobalamin therapy. Most patients with TC I/HC deficiency in the present study had metabolite concentrations within the reference intervals when tested, but information about previous therapy was not always available. Rare cases showed borderline increases in MMA, such as the 6-month-old boy in family C. The increase in his MMA may be unrelated to TC I/HC deficiency, however, in view of the observation that infants often have mildly increased MMA concentrations (29).

Several technical concerns deserve comment. The study consisted of six surveys, some conducted under different conditions. This arose from study design choices made to allow different questions to be addressed but was accompanied by the disadvantage that two of the six surveys unavoidably depended on serum samples rather than plasma. The steps taken to compensate for this problem are described in the Participants and Methods section, such as separate reference intervals and the inclusion of comparison groups for both serum and plasma sampling. It should be noted that the use of serum, with its in vitro elevation of TC I/HC content compared with plasma, may underestimate rather than overestimate the prevalence of TC I/HC deficiency. The dependence of identification of low cobalamin concentrations on different cobalamin assays in two of the subgroups was a lesser technical concern. Different laboratories and cobalamin assay methods often differ in the reference intervals they produce, but classification discrepancies have been relatively few, at least among isotopic and microbiological assays (30–32). Indeed, reassay of 22 samples from the three clinical laboratories with my cobalamin assay showed a diagnostic discrepancy rate within the expected intraassay variation, and it is unlikely that the TC I/HC deficiency rates were affected substantially by the screening use of different cobalamin assays in two subgroups. Most importantly, the discussed concerns are mitigated by the fact that the survey of several distinct groups of individuals, each with its own targets, advantages, and disadvantages, formed a remarkably consistent and coherent pattern of findings in relation to TC I/HC results. Nevertheless, testing of patients should involve EDTA-anticoagulated plasma whenever possible, because some cases of mild TC I/HC deficiency may not be detected with serum.

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