ELEVATED VITAMIN B12 BLOOD LEVELS

Anecdotic but not rare clinical cases lead health care professionals to question the reason for finding high or very high blood levels for cobalamin (vitamin B12). Most cases are considered as having been supplemented, possibly through self-medication or from another practitioner, and very often no practical measure is foreseen.

But very frequently among those patients, careful inquiries demonstrate that no external human intervention may explain the finding. So we have to find some explanation to the increase within the metabolism. The presence of vitamin B12 in human faeces doesn't only correspond to what is left from the absorption in the ileum, but it also reflects the production of significant quantities of cobalamin by the colonic microflora [1].

The fact that intestinal micro-organisms produce significant amounts of vitamins B is fully accepted and has been published by peer-reviewed international medical journals [2, 3]. Intestinal bacterial vitamins B biosynthesis concerns at least vitamin B1 (thiamine) [4], vitamin B2 (riboflavin) [5], vitamin B5 (pantothenic acid) [6], vitamin B8 (biotin) [6-8], vitamin B9 (folic acid) [9, 10],and vitamin B12 (cobalamin) [1]. As a matter of fact, bacteria obtained from dairy and belonging to the genus *Propionibacterium* (also abundant in the human intestinal microflora) are extensively used for the biological production of **cobalamin** [11].

Concerning vitamin B8, also called **biotin**, "it has long been recognized that the normal microflora of the large intestine synthesize considerable amounts of biotin" [6]. In fact, several studies have shown that the colon is capable of absorbing free biotin and *HM Said* has shown, for the first time in 1998, the functional existence of a specialized carrier-mediated system for biotin uptake in colonic epithelial cells [7]. "In addition, the uptake process is shared by another water-soluble vitamin, **pantothenic acid**, (…) which is also synthesized by the normal microflora of the large intestine", as biotin inhibited the uptake of vitamin B5 and *vice versa* [6].

This transporter has been cloned in the rabbit intestine by another team in 1999 [12] and named the *sodium-dependent multivitamin transporter* (*SMVT*). This transporter is also highly expressed in human enterocytes [12, 13], where it uptakes not only pantothenate and biotin, but also lipoate (the ion from lipoic acid) [12].

Half a century ago, vitamin B2 (also called **riboflavin**) was known to be synthesized by intestinal bacteria and the amount provided by this source appears to become significantly higher following the consumption of a vegetable-based diet [14]. Interestingly, as he did for other water-soluble vitamins B, the same author demonstrated in 2000 "for the first time, the existence of a specialized carrier-mediated mechanism for riboflavin uptake in an in vitro cellular model of human colonocytes" [5]. Once again in 2001, *HM Said* has shown that a model of human-derived colonic epithelial cells possesses a specific carrier-mediated system for **thiamine** (vitamin B1) uptake [4]. "It is suggested that bacterially synthesized thiamine in the large intestine may contribute to thiamine nutrition of the host, especially towards (…) the local colonocytes" [4].

Certain bacterial species present in the rat colon are also capable of *de novo* synthesis of vitamin B9, better known as **folic acid** [9]. As clearly evidenced by the use of tritiated (marked with radioactive hydrogen) para-aminobenzoic acid (3H PABA), the experimental "data provide direct evidence that some of the folate synthesized by the microflora in the rat large intestine is incorporated into the tissue folate of the host" [9].

More recently, the same methodology has been utilized with humans in order to determine whether folate synthesized by the small intestinal flora - opposed to what we have covered until now, which concerned exclusively the colonic microflora - was assimilated by the human host [10]. Indeed, the perfusion of tritiated PABA, a classic precursor substrate for the bacterial folate synthesis, led to the identification of bacterially synthesized (as marked) folates aspirated from in the small intestine. Then, the presence of tritiated 5-mlethyltetrahydrofolate, a major metabolite of folate, was isolated from the human host urine, demonstrating that the human host did absorb and consequently metabolized these bacterially synthesized folates [10].

Coming back to **cobalamin**, it has been shown, already in 1980, that "at least two groups of organisms in the small bowel, *Pseudomonas* and *Klebsiella sp.*, may synthesize significant amounts of the vitamin [B12]" [1]. Obviously, the two main dogma of vitamin B metabolism in the digestive tract don't seem to correspond to the reality of the field: several compounds (vitamins B1, B2, B5, B8 and B9) supposedly absorbed by the small intestine may be assimilated by the colonocytes, while several compounds (vitamins B9 and B12) supposedly synthesized by colonic bacteria may be generated in the small intestine! Unfortunately, if we wanted to explain the high vitamin B12 blood levels by some colonic absorption, we must underline that absolutely nothing has been published about it and what seems true for other vitamins B would not be for cobalamin.

Consequently, we should rather focus on the possibility for bacterial vitamin B12 to be absorbed in the small intestine, where most of the assimilation process of vitamins B takes place. Two different specific proteins ensure the uptake of **thiamine** (vitamin B1) in the enterocytes of proximal small intestine and are structurally close to a specific **folic acid** carrier [15]. Indeed, the intestinal folate (vitamin B9) absorption process occurs via a specialized mechanism that involves the *reduced folate carrier* (*RFC*) in the jejunum [16, 17]. We have already mentioned earlier the existence, in the proximal small intestinal enterocytes, of a *sodium-dependent multivitamin transporter* (*SMVT*) taking care of **biotin** (vitamin B8) and of **pantothenic acid** (vitamin B5). The involvement of a specialized carrier-mediated mechanism for **pyridoxine** (vitamin B6) by the intestinal epithelial cells has been demonstrated for the first time in 2003 [18]. Finally, a specialized carrier for **niacin** (vitamin B3) has been uncovered very recently, the article only being published in July 2005 [19].

Oppositely to all the other B vitamins, **cobalamin** is not absorbed in the jejunum or in the proximal ileum as they are, but only in the terminal ileum from a quite complex absorption process. This makes it very sensitive to diseases affecting specifically, or more frequently, this portion of the digestive tract such as Crohn's disease. We should rather speak about "cobalamins", due to the presence of four different metabolically important forms in the diet: **methylcobalamin**, **hydroxocobalamin**, **cyanocobalamin**, and **deoxyadenosylcobalamin**, mostly bound to proteins in our foods. In first, the cobalamins must be released from their protein complexes through the action of *acid* or *pepsin* in the stomach. In second, they have to bind *R proteins* (secreted in saliva and in gastric juice) which consist in cobalamin-binding glycoproteins. In third, these cobalamin-protein complexes must be degraded by *pancreatic proteases* and the whole process of absorption may be ruined in case of pancreatic insufficiency [20]. In fourth, the free cobalamin combines in the duodenum with another glycoprotein called *intrinsic factor* and secreted by the stomach parietal (oxyntic) cells; this glycoprotein dimerises and each part of the dimer binds one molecule of cobalamin, the complex resisting to digestion [21].

The formation of the cobalamin-intrinsic factor complex appears indispensable for the vitamin to be absorbed in the terminal ileum via an active transport system [20]. In fifth, the brush border membrane of those enterocytes contains a *specific receptor* for the dimeric complex and its importance in the process is shown by a congenital vitamin B12 malabsorption syndrome due to a defect in the receptor. The absorption is hampered by an abnormally low ileum pH, which may occur in some diseases such as the Zollinger-Ellison syndrome.

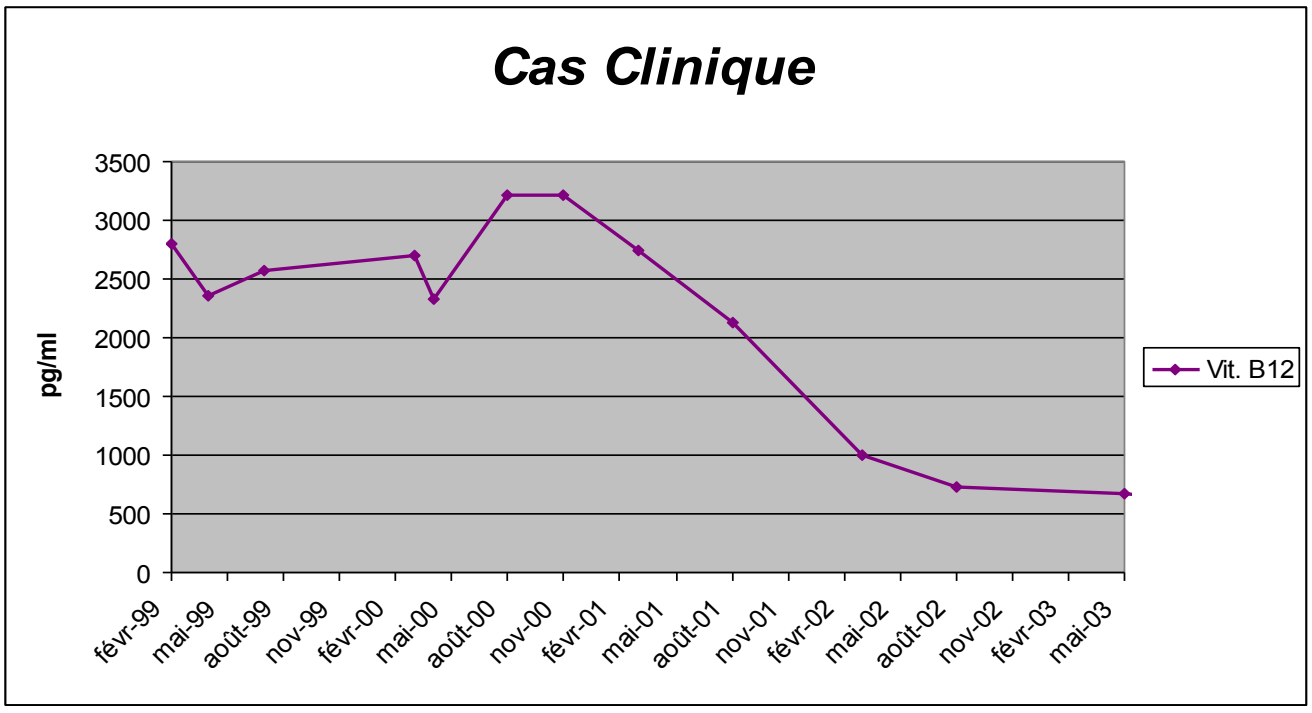
The problem lies in the small security margin between the dietary requirements for vitamin B12 and the maximal absorptive capacity. Cobalamins can also be absorbed passively, but the passive pathway concerns only 1 or 2 % of the ingested vitamin, explaining the development of anemia when one of the five steps is not fulfilled [21]. The most frequent cause for **vitamin B12** malabsorption is of course represented by the lack of intrinsic factor [20], which may be explained by a genetic defect, an auto-immune condition (auto-antibodies targeting either the parietal cells or the intrinsic factor itself), or a surgical gastrectomy. But further problems can occur at the level or blood carriers, transcobalamin I and transcobalamin II, which may be impaired [22].

Now, supposing that all these steps leading to an effective absorption of vitamin B12 function adequately, the presence in significant amounts of bacteria producing cobalamin in the terminal ileum would explain - at least theoretically - a sharp increase in absorption and lead to higher blood levels of this vitamin. If we consider some specific circumstances in the above mentioned study about folate absorption [10], we might find out which mechanisms could lead to an excessive absorption of cobalamine and to an elevation of blood levels.

In this exemplary study for functional medicine, two groups of patients were formed - healthy volunteers and subjects suffering from atrophic gastric - and were studied before and after omeprazole (a proton pump inhibitor, which turns off the gastric acid production) administration [10]. Logically, both patients with atrophic gastritis and receiving omeprazole showed an increased duodenal pH (which stands for less acidity), but also an overgrowth of the small intestinal microflora [10]. In physiological conditions, bacterial growth in the small intestine is limited by the acidic environment due to the presence of hydrochloric acid. The small intestinal environment, normally hostile to the local microflora, becomes friendlier and enables what is called a "**small intestinal bacterial overgrowth**" (**SIBO**) either in case of atrophic gastritis [23] or in case of drug-induced hypochlorhydria [24], especially among "subjects taking a hydrogen pump blocking agent [such as] omeprazole" [25]. Interestingly, SIBO seems to provide "a unifying framework for understanding **irritable bowel syndrome** (**IBS**) and other functional disorders" [26], among which **fibromyalgia** [26, 27].

We come back once again to the experimentation with labelled folate to give its conclusions as presented in the corresponding abstract: "(1) Mild bacterial overgrowth caused by atrophic gastritis and administration of omeprazole are associated with *de novo* folate synthesis in the lumen of the small intestine; (2) the human host absorbs and uses some of these folates" [10]. Indeed, the unexplained increase of blood levels that we are describing about vitamin B12 may also occur with folic acid. We present a first **case study** concerning a four-year old boy who suffered from diarrhoea and abdominal bloating. Cœliac disease had been dismissed but he showed an increase of specific urinary organic metabolites corresponding to a bacterial overgrowth, typically from *Clostridium*. This child had never been treated with vitamins at the time of his first blood check, though his erythrocytic folate level (**folic acid** in red blood cells) was measured at **913**µg/l whereas 257µg/l - 582µg/l represent the lab's normal range for the parameter. Besides, the plasmatic level for **cobalamins** was raised at **1324**ng/l, contrasting with the laboratory's normal range going from 450ng/l up to 1200ng/l. He was treated for intestinal dysbiosis and put on a casein-free diet, improved dramatically… and was not blood tested again!

We present a second **case study** concerning a thirty-year old woman (in 1999) whose blood parameters were monitored for unrelated matters but strikingly presented repetitive high **vitamin B12** levels without any related supplementation neither from the vitamin itself, nor through vitamin B complexes / multivitamin formulas.



We present the original data from our records, in French unfortunately. All the results for vitamin B12 are expressed in pg/ml and the normal range provided by the Belgian laboratory starts from 200pg/ml up to 900pg/ml, even if the lower limit could be considered as too low to be compatible with optimal health. We see that the five first measurements, from February 1999 to April 2000, were quite consistently fluctuating around 2500pg/ml (respectively **2796**pg/ml on 6/2/99, **2355**pg/ml on 19/4/99, **2572**pg/ml on 30/7/99, **2697**pg/ml on 7/3/2000 and **2325**pg/ml on 17/4/00), which is much too much! At that time, the patient's blood had to be monitored in relation with a drug-based anti-epileptic treatment. But the young woman was not complaining about her digestive system, even if she occasionally mentioned some severe but transitory abdominal cramps.

Her digestive problems started during the summer season in 2000, with IBS like symptoms, bloating, diarrhoea and excruciating pain in the belly. She was explored thoroughly and the gastroenterologist suspected initial Crohn's disease due to the presence of mucosal ulcerations in the proximal small intestine. During that period of major clinical deterioration, blood vitamin B12 level increased even further as seen from two measurements performed on 25/08/00 (**3220**pg/ml) and on 28/11/00 (**3221**pg/ml). Then, she refused to take the corticoids prescribed by the specialist and went on a natural treatment based on diet modifications (exclusion of high IgG foods, in her case: dairy products, beef, bananas and black pepper), supplements (according her biological results in blood and in 24-hour urine), antimicrobial herbs (such as grapefruit seed extracts) and probiotics.

She didn't improve dramatically, but slowly started to complain less within a few weeks, then was feeling slightly better in March 2001 and significantly better when she came back five months later, in August 2001. Very interestingly, vitamin B12 blood levels started to withdraw to **2740**pg/ml on 24/3/01 and then down to **2132**pg/ml on 22/08/01. In fact, the last result provided her lower blood value from the beginning of the study. In September 2001, we then asked the gastroenterologist to perform a new endoscopy, in order to dismiss the diagnosis of Crohn's disease and make sure that we were not harming her by not giving the prescribed drugs. The digestive exploration was then considered as normal, besides some "non specific mucosal inflammation".

So the case was much less worrying and it took about seven months before she consulted again, in March 2003. She was symptom-free, finally expressing a much better digestive capacity since she was on this diet, even though she hadn't renewed her supplements for a while. The cramps had disappeared and her blood reading for the vitamin B12 was **1001**pg/ml on 26/3/02, almost back to the normal range. She definitely reached and stayed within the normal range on further checks with 726pg/ml on 31/08/02, 677npg/ml on 21/5/03 and finally 516pg/ml on 15/5/04. The last time, she was still symptom-free, but also dairy-free. She might have to consider taking vitamin B12 supplements one day in the future, but that's another story…

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1. Albert, M.J., V.I. Mathan, and S.J. Baker, *Vitamin B12 synthesis by human small intestinal bacteria.* Nature, 1980. **283**(5749): p. 781-2.
2. Hill, M.J., *Intestinal flora and endogenous vitamin synthesis.* Eur J Cancer Prev, 1997. **6 Suppl 1**: p. S43-5.
3. Cummings, J.H. and G.T. Macfarlane, *Role of intestinal bacteria in nutrient metabolism.* JPEN J Parenter Enteral Nutr, 1997. **21**(6): p. 357-65.
4. Said, H.M., et al., *Mechanism of thiamine uptake by human colonocytes: studies with cultured colonic epithelial cell line NCM460.* Am J Physiol Gastrointest Liver Physiol, 2001. **281**(1): p. G144-50.
5. Said, H.M., et al., *Riboflavin uptake by human-derived colonic epithelial NCM460 cells.* Am J Physiol Cell Physiol, 2000. **278**(2): p. C270-6.
6. Said, H.M., *Cellular uptake of biotin: mechanisms and regulation.* J Nutr, 1999. **129**(2S Suppl): p. 490S-493S.
7. Said, H.M., et al., *Biotin uptake by human colonic epithelial NCM460 cells: a carrier-mediated process shared with pantothenic acid.* Am J Physiol, 1998. **275**(5 Pt 1): p. C1365-71.
8. Noda, H., N. Akasaka, and M. Ohsugi, *Biotin production by bifidobacteria.* J Nutr Sci Vitaminol (Tokyo), 1994. **40**(2): p. 181-8.
9. Rong, N., et al., *Bacterially synthesized folate in rat large intestine is incorporated into host tissue folyl polyglutamates.* J Nutr, 1991. **121**(12): p. 1955-9.
10. Camilo, E., et al., *Folate synthesized by bacteria in the human upper small intestine is assimilated by the host.* Gastroenterology, 1996. **110**(4): p. 991-8.
11. Zarate, G., S. Gonzalez, and A.P. Chaia, *Assessing survival of dairy propionibacteria in gastrointestinal conditions and adherence to intestinal epithelia.* Methods Mol Biol, 2004. **268**: p. 423-32.
12. Prasad, P.D., et al., *Molecular and functional characterization of the intestinal Na+-dependent multivitamin transporter.* Arch Biochem Biophys, 1999. **366**(1): p. 95-106.
13. Balamurugan, K., A. Ortiz, and H.M. Said, *Biotin uptake by human intestinal and liver epithelial cells: role of the SMVT system.* Am J Physiol Gastrointest Liver Physiol, 2003. **285**(1): p. G73-7.
14. Iinuma, S., *Synthesis of riboflavin by intestinal bacteria.* J Vitaminol (Kyoto), 1955. **1**(2): p. 6-13.
15. Subramanian, V.S., J.S. Marchant, and H.M. Said, *Targeting and trafficking of the human thiamine transporter-2 (hTHTR2) in epithelial cells.* J Biol Chem, 2005.
16. Matherly, L.H., *Molecular and cellular biology of the human reduced folate carrier.* Prog Nucleic Acid Res Mol Biol, 2001. **67**: p. 131-62.
17. Subramanian, V.S., N. Chatterjee, and H.M. Said, *Folate uptake in the human intestine: promoter activity and effect of folate deficiency.* J Cell Physiol, 2003. **196**(2): p. 403-8.
18. Said, H.M., A. Ortiz, and T.Y. Ma, *A carrier-mediated mechanism for pyridoxine uptake by human intestinal epithelial Caco-2 cells: regulation by a PKA-mediated pathway.* Am J Physiol Cell Physiol, 2003. **285**(5): p. C1219-25.
19. Nabokina, S.M., M.L. Kashyap, and H.M. Said, *Mechanism and regulation of human intestinal niacin uptake.* Am J Physiol Cell Physiol, 2005. **289**(1): p. C97-103.
20. Festen, H.P., *Intrinsic factor secretion and cobalamin absorption. Physiology and pathophysiology in the gastrointestinal tract.* Scand J Gastroenterol Suppl, 1991. **188**: p. 1-7.
21. Oh, R. and D.L. Brown, *Vitamin B12 deficiency.* Am Fam Physician, 2003. **67**(5): p. 979-86.
22. Carmel, R., et al., *Update on cobalamin, folate, and homocysteine.* Hematology (Am Soc Hematol Educ Program), 2003: p. 62-81.
23. Saltzman, J.R. and R.M. Russell, *The aging gut. Nutritional issues.* Gastroenterol Clin North Am, 1998. **27**(2): p. 309-24.
24. Pereira, S.P., N. Gainsborough, and R.H. Dowling, *Drug-induced hypochlorhydria causes high duodenal bacterial counts in the elderly.* Aliment Pharmacol Ther, 1998. **12**(1): p. 99-104.
25. Paiva, S.A., et al., *Interaction between vitamin K nutriture and bacterial overgrowth in hypochlorhydria induced by omeprazole.* Am J Clin Nutr, 1998. **68**(3): p. 699-704.
26. Lin, H.C., *Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome.* Jama, 2004. **292**(7): p. 852-8.
27. Pimentel, M., et al., *A link between irritable bowel syndrome and fibromyalgia may be related to findings on lactulose breath testing.* Ann Rheum Dis, 2004. **63**(4): p. 450-2.