

Invited commentary

Megaloblastic anaemia in vitamin B₁₂ deficiency

Deficiency of either folic acid or vitamin B₁₂ results in megaloblastic anaemia: the release into the circulation of immature erythrocytes due to a failure of the normal process of erythrocyte maturation in the bone marrow (Wickramasinghe, 1995, 1999). Pernicious anaemia is the megaloblastic anaemia due specifically to vitamin B₁₂ deficiency, in which there is also spinal cord degeneration, leading to peripheral neuropathy. It is a disease of later life; only about 10 % of patients are aged <40 years; by the age of 60 years about 1 % of the population are affected, rising to 2–5 % of people aged >65 years, as a result of atrophic gastritis (commonly due to autoimmune disease) and hence impaired secretion of intrinsic factor, which is required for the absorption of vitamin B₁₂ (Baik & Russell, 1999). Up to one-third of patients develop neurological signs without megaloblastosis, and high intakes of folate may prevent megaloblastosis in vitamin B₁₂ deficiency (Dickinson, 1995; Savage & Lindenbaum, 1995). As discussed later, it has long been believed that only man, and not other animals, develops megaloblastic anaemia as a result of vitamin B₁₂ deficiency, and indeed it is not obvious why vitamin B₁₂ deficiency should affect haematopoiesis.

Folate deficiency is relatively common; by contrast, dietary deficiency of vitamin B₁₂ is rare, and pernicious anaemia is usually due to impaired absorption. Dietary deficiency does occur in strict vegetarians, since there are no plant foods that are sources of the vitamin. The small amounts of biologically available vitamin B₁₂ that have been reported in algae (Watanabe *et al.* 2000; Takenaka *et al.* 2001; Kittaka-Katsura *et al.* 2002) are almost certainly due to bacterial contamination. A number of non-cobalamin corrinoids in algae are active in microbiological assays and thus appear to be vitamin B₁₂, although they have no vitamin activity, and may indeed be antimetabolites (Yamada *et al.* 1999).

The cause of megaloblastic anaemia is impaired DNA synthesis. Rapidly dividing cells, as in bone marrow, can either use preformed thymidine monophosphate (TMP) for DNA synthesis, or can synthesize it *de novo* from deoxyuridine monophosphate (dUMP). This reaction is catalysed by thymidylate synthetase, which uses methylenetetrahydrofolate as the methyl donor, so it is obvious that folate deficiency will result in impaired *de novo* synthesis of thymidylate. It is less obvious how vitamin B₁₂ deficiency affects thymidylate synthesis; the vitamin is required by only three mammalian enzymes: methionine synthetase, methylmalonyl CoA mutase and leucine

aminomutase, none of which is involved in nucleotide metabolism (Glusker, 1995; Marsh, 1999).

The reduction of methylenetetrahydrofolate to methyltetrahydrofolate, catalysed by methylenetetrahydrofolate reductase is irreversible, and the major source of folate for tissues is methyltetrahydrofolate. The only metabolic function of methyltetrahydrofolate is in the methylation of homocysteine to methionine, and this is the only way in which methyltetrahydrofolate can be demethylated to yield free tetrahydrofolate in tissues. Methionine synthetase thus provides the link between the physiological functions of folate and vitamin B₁₂.

Impairment of methionine synthetase activity in vitamin B₁₂ deficiency results in the accumulation of methyltetrahydrofolate, which can neither be utilized for other reactions nor demethylated to provide free tetrahydrofolate. Vitamin B₁₂ deficiency thus leads to functional folate deficiency, with much folate trapped as (unusable) methyltetrahydrofolate (Krebs *et al.* 1976; Horne *et al.* 1989). This 'methyl folate trap' hypothesis appears to explain many of the similarities between the symptoms and metabolic effects of folate and vitamin B₁₂ deficiency (Shane, 1985). However, it does not provide a completely satisfactory explanation of the effects of vitamin B₁₂ deficiency (Chanarin *et al.* 1985). Since most dietary folate is methylated during intestinal absorption, it is difficult to see how it is that a high intake of folate can mask the megaloblastic anaemia due to vitamin B₁₂ deficiency (Scott & Weir, 1994; Weir & Scott, 1998; Scott, 1999).

Isolated bone marrow cells and stimulated lymphocytes incubated with [³H]TMP will incorporate label into DNA. In the presence of adequate amounts of methylenetetrahydrofolate, the addition of dUMP as a substrate for thymidylate synthetase reduces the incorporation of [³H]TMP because of dilution of the pool of labelled material by newly synthesized TMP. The extent to which dUMP suppresses the incorporation of [³H]TMP into DNA thus reflects folate status. In normal cells, the incorporation of [³H]thymidine into DNA after pre-incubation with dUMP is 1.4–1.8 % of that without pre-incubation. By contrast, cells that are deficient in folate form little or no thymidine from dUMP, and hence incorporate nearly as much of the [³H]thymidine after incubation with dUMP as they do without pre-incubation. Either a primary deficiency of folic acid or functional deficiency secondary to vitamin B₁₂ deficiency has the same effect. In folate deficiency, addition of any biologically active form of

folate, but not vitamin B₁₂, will normalize the dUMP suppression of [³H]thymidine incorporation. In vitamin B₁₂ deficiency, addition of vitamin B₁₂ or methylenetetrahydrofolate, but not methyltetrahydrofolate, will normalize dUMP suppression (Killman, 1964; Pelliniemi & Beck, 1980).

Hitherto, it has been believed that the megaloblastic response to vitamin B₁₂ deficiency is unique to man. Deficient rats (Toyoshima *et al.* 1996), monkeys (Kark *et al.* 1974) and fruit bats (*Rousettus aegyptiacus*; Green *et al.* 1975) develop neuropathy, but have unimpaired haematopoiesis, suggesting that man is more reliant on the *de novo* synthesis of TMP, and less able to salvage it from DNA breakdown, than other species. The normal suppression of the incorporation of [³H]thymidine into DNA by added dUMP in the fruit bat is about 5%, and in the rat about 30%, of that seen in human subjects (Carmel, 2000). Ebara *et al.* (2003) have now shown that when vitamin B₁₂-deficient rats are subjected to the additional stress of hypoxia to induce haematopoiesis, they do indeed develop megaloblastosis, although vitamin B₁₂ deficiency alone is not sufficient.

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References

- Baik HW & Russell RM (1999) Vitamin B₁₂ deficiency in the elderly. *Annual Reviews of Nutrition* **19**, 357–377.
- Carmel R (2000) Current concepts in cobalamin deficiency. *Annual Reviews of Medicine* **51**, 357–375.
- Chanarin I, Deacon R, Lumb M, Muir M & Perry J (1985) Cobalamin–folate interrelations: a critical review. *Blood* **66**, 179–189.
- Dickinson CJ (1995) Does folic acid harm people with vitamin B₁₂ deficiency? *Quarterly Journal of Medicine* **88**, 357–364.
- Ebara S, Adachi S, Takenaka S, Enomoto T, Watanabe F, Yamaji R, Inui H & Nakano Y (2003) Hypoxia-induced megaloblastosis in vitamin B₁₂-deficient rats. *British Journal of Nutrition* **89**, 441–444.
- Glusker JP (1995) Vitamin B₁₂ and the B₁₂ coenzymes. *Vitamins and Hormones* **50**, 1–76.
- Green R, van Tonder S, Oettle G, Cole G & Metz J (1975) Neurological changes in fruit bats deficient in vitamin B₁₂. *Nature* **254**, 148–150.
- Horne DW, Patterson D & Cook RJ (1989) Effect of nitrous oxide inactivation of vitamin B₁₂-dependent methionine synthetase on the subcellular distribution of folate coenzymes in rat liver. *Archives of Biochemistry and Biophysics* **270**, 729–733.
- Kark J, Victor M, Hines J & Harris J (1974) Nutritional vitamin B₁₂ deficiency in rhesus monkeys. *American Journal of Clinical Nutrition* **27**, 470–478.
- Killman S-A (1964) Effect of deoxyuridine on incorporation of tritiated thymidine: difference between normoblasts and megaloblasts. *Acta Medica Scandinavica* **175**, 483–488.
- Kittaka-Katsura H, Fujita T, Watanabe F & Nakano Y (2002) Purification and characterization of a corrinoid compound from Chlorella tablets as an algal health food. *Journal of Agricultural and Food Chemistry* **50**, 4994–4997.
- Krebs HA, Hems R & Tyler B (1976) The regulation of folate and methionine metabolism. *Biochemical Journal* **158**, 341–353.
- Marsh EN (1999) Coenzyme B₁₂ (cobalamin)-dependent enzymes. *Essays in Biochemistry* **34**, 139–154.
- Pelliniemi TT & Beck WS (1980) Biochemical mechanisms in the Killmann experiment: critique of the deoxyuridine suppression test. *Journal of Clinical Investigation* **65**, 449–460.
- Savage DG & Lindenbaum J (1995) Neurological complications of acquired cobalamin deficiency: clinical aspects. *Baillieres Clinics in Haematology* **8**, 657–678.
- Scott J & Weir D (1994) Folate/vitamin B₁₂ inter-relationships. *Essays in Biochemistry* **28**, 63–72.
- Scott JM (1999) Folate and vitamin B₁₂. *Proceedings of the Nutrition Society* **58**, 441–448.
- Shane B (1985) Vitamin B₁₂–folate interrelationships. *Annual Reviews of Nutrition* **5**, 115–141.
- Takenaka S, Sugiyama S, Ebara S, Miyamoto E, Abe K, Tamura Y, Watanabe F, Tsuyama S & Nakano Y (2001) Feeding dried purple laver (nori) to vitamin B₁₂-deficient rats significantly improves vitamin B₁₂ status. *British Journal of Nutrition* **85**, 699–703.
- Toyoshima S, Watanabe F, Saido H, Pezacka E, Jacobsen D, Miyatake K & Nakano Y (1996) Accumulation of methylmalonic acid caused by vitamin B₁₂-deficiency disrupts normal cellular metabolism in rat liver. *British Journal of Nutrition* **75**, 929–938.
- Watanabe F, Takenaka S, Katsura H, Miyamoto E, Abe K, Tamura Y, Nakatsuka T & Nakano Y (2000) Characterization of a vitamin B₁₂ compound in the edible purple laver, *Porphyra yezoensis*. *Bioscience, Biotechnology and Biochemistry* **64**, 2712–2715.
- Weir D & Scott J (1998) Homocysteine as a risk factor for cardiovascular and related disease: nutritional implications. *Nutrition Research Reviews* **11**, 311–338.
- Wickramasinghe SN (1995) Morphology, biology and biochemistry of cobalamin- and folate-deficient bone marrow cells. *Baillieres Clinics in Haematology* **8**, 441–459.
- Wickramasinghe SN (1999) The wide spectrum and unresolved issues of megaloblastic anemia. *Seminars in Hematology* **36**, 3–18.
- Yamada K, Yamada Y, Fukuda M & Yamada S (1999) Bioavailability of dried asakusanori (*Porphyra tenera*) as a source of cobalamin (vitamin B₁₂). *International Journal of Vitamin and Nutrition Research* **69**, 412–418.