Megaloblastic anaemia in vitamin B_{12} deficiency

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Invited commentary

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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 vitamin B₁₂ absorption (Baik & Russell, 1999). The small amounts of biologically available vitamin B₁₂ that have been reported in algae (Watanabe 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 methylene-tetrahydrofolate 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 methylethyltetrahydrofolate, catalysed by methylenetetrahydrofolate reductase is irreversible, and the major source of folate for tissues is methyltetrahydrofolate. The only metabolic function of methylethyltetrahydrofolate 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) methylethyltetrahydrofolate (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 methylene-tetrahydrofolate, the addition of dUMP as a substrate for thymidylate synthetase reduces the incorporation of [³H]TMP into DNA thus reflects folate status. In normal cells, the incorporation of [³H]thymidine into DNA during 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 B$_{12}$, will normalize the dUMP suppression of $[^3H]$thymidine incorporation. In vitamin B$_{12}$ deficiency, addition of vitamin B$_{12}$ 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 B$_{12}$ 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 B$_{12}$-deficient rats are subjected to the additional stress of hypoxia to induce haematopoiesis, they do indeed develop megaloblastosis, although vitamin B$_{12}$ deficiency alone is not sufficient.

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