Megaloblastic anaemia in vitamin B_{12} deficiency

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Invited commentary

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Deficiency of either folic acid or vitamin B_{12} 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 B_{12} 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 B_{12} (Baik & Russell, 1999). Up to one-third of patients develop neurological signs without megaloblastosis, and high intakes of folate may prevent megaloblastosis in vitamin B_{12} 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 B_{12} deficiency, and indeed it is not obvious why vitamin B_{12} deficiency should affect haematopoiesis.

Folate deficiency is relatively common; by contrast, dietary deficiency of vitamin B_{12} 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 B_{12} 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 B_{12}, 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 B_{12} 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 B_{12}.

Impairment of methionine synthetase activity in vitamin B_{12} deficiency results in the accumulation of methyltetrahydrofolate, which can neither be utilized for other reactions nor demethylated to provide free tetrahydrofolate. Vitamin B_{12} 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 B_{12} deficiency (Shane, 1985). However, it does not provide a completely satisfactory explanation of the effects of vitamin B_{12} 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 B_{12} deficiency (Scott & Weir, 1994; Weir & Scott, 1998; Scott, 1999).

Isolated bone marrow cells and stimulated lymphocytes incubated with [^{3}H]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 [^{3}H]TMP because of dilution of the pool of labelled material by newly synthesized TMP. The extent to which dUMP suppresses the incorporation of [^{3}H]TMP into DNA thus reflects folate status. In normal cells, the incorporation of [^{3}H]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 [^{3}H]thymidine after incubation with dUMP as they do without pre-incubation. Either a primary deficiency of folic acid or functional deficiency secondary to vitamin B_{12} 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 [^{3}H]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 [^{3}H]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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