

Haemoglobin A₂ levels in vitamin B₁₂ and folate deficiency

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SUMMARY Haemoglobin A₂ levels were measured in 50 patients with vitamin B₁₂ deficiency, 50 patients with folate deficiency, and six patients with combined deficiencies of these vitamins. All were normal except for three patients with vitamin B₁₂ deficiency, who had a slightly elevated Hb A₂ level; this fell to normal after vitamin B₁₂ therapy. It is concluded that haemoglobin A₂ levels are usually normal in vitamin B₁₂ or folate deficiency. However, raised levels of haemoglobin A₂ may be found, but these are not as high as is found in β thalassaemia trait and should not cause difficulty in diagnosis.

In normal adult peripheral blood several minor haemoglobin components may be found, including haemoglobin A₂. Relatively little is known concerning the functional significance of this haemoglobin. Its level is held constant in health, and consistent alterations have been observed only in thalassaemia and some unstable haemoglobin variants (Bradley and Ranney, 1973). However, acquired changes in Hb A₂ level have been reported in iron deficiency (Josephson *et al.*, 1958; Wasi *et al.*, 1968; Alperin *et al.*, 1977) and megaloblastic anaemia (Josephson *et al.*, 1958; Alperin *et al.*, 1977). It is the purpose of this report to assess how frequently the Hb A₂ level is altered by vitamin B₁₂ and folate deficiencies.

Methods

In this study vitamin B₁₂ deficiency was considered to be present when a serum level below the normal range was observed. An isotope dilution technique was used (Lau *et al.*, 1965), the normal range in our laboratory being 200-1000 ng/l. Folate deficiency was diagnosed when both serum and red cell folate levels were reduced and where there was no reason to suppose that any inhibitory substance was present in the serum assayed. A microbiological assay using *Lactobacillus casei* was employed (Waters and Mollin, 1961; Hoffbrand *et al.*, 1966). The normal ranges in our laboratory are 6-21 μ g/l for serum folate and 160-640 μ g/l for red cell folate.

Patients were assigned to the combined vitamin B₁₂/folate deficiency group when all three parameters

were reduced. It is recognised that this may not represent a combined B₁₂/folate deficiency state in the strict sense. In each patient the blood sample collected into EDTA for red cell folate was also analysed for its haemoglobin A₂ content. The method employed for A₂ estimation is based on that of Marengo-Rowe (1965). Cellulose acetate strips, 4.5 × 12 cm, are soaked in tris-EDTA-borate buffer, pH 9.1, and are placed in an electrophoretic tank. Ten microlitres of a 10 g/dl haemolysate is applied in duplicate 4 cm from the cathodal end of each strip. Electrophoresis is carried out for 100 minutes at 250 volts. At the end of the separation the strips are cut into the various haemoglobin fractions, and the duplicates are combined and eluted into tris-EDTA-borate buffer. The OD of each fraction is read at 413 nm and the percentage of Hb A₂ present is calculated from this. The normal range in our laboratory is from 1.8 to 3.3 with a mean of 2.63%. Repeated assays on normal samples did not vary by more than \pm 0.15%.

Results

These are summarised in Tables 1 and 2. No significant difference in mean Hb A₂ levels was found when each group of vitamin B₁₂ and/or folate deficiency states was compared to the established normal range. In three patients with vitamin B₁₂ deficiency, increased Hb A₂ levels were found. In each patient the level fell into the normal range after B₁₂ therapy, the fall being greater than could be explained by technical variation in haemoglobin A₂ assays. The levels of A₂ at 3.7, 3.7, and 3.8% were only slightly increased and below that found in

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Table 1 Mean haemoglobin A₂ levels in patients with vitamin B₁₂ and folate deficiencies

Vitamin deficiency present	No. of patients or normal subjects	Hb A ₂ levels %	
		Mean	Observed range
B ₁₂	50	2.38	1.1—3.8
Folate	50	2.47	1.5—3.1
B ₁₂ and folate	6	2.45	1.6—3.0
Normal range	130	2.63	1.8—3.3

β thalassaemia trait. No correlation was observed between the A₂ level and the degree of anaemia (Figure).

Comment

In the normal adult about 90% of the circulating

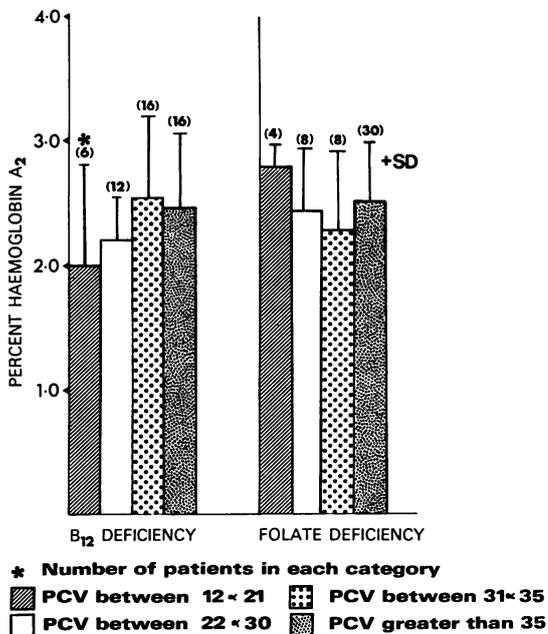


Figure Haemoglobin A₂ levels correlated with packed cell volume in patients with vitamin B₁₂ and folate deficiencies.

Table 2 Findings in three patients with reduced serum vitamin B₁₂ and raised haemoglobin A₂ levels before and after therapy

Patient	Hb (g/dl)	Serum vitamin B ₁₂ (ng/l)	Serum folate (μg/l)	RBC folate (μg/l)	Hb A ₂ %	
					Before B ₁₂ therapy	After B ₁₂ therapy
AL	8.1	75	21.0	340	3.7	3.0
IB	10.8	150	4.0	170	3.8	3.1
WJ	12.5	160	6.5	250	3.7	3.2

haemoglobin is haemoglobin A. Various minor components make up the remaining 10%, and these include haemoglobins F (less than 1%) and A₂ (2-3%), and their production is genetically determined (Horton and Huisman, 1965). In addition, haemoglobin A may be modified following its synthesis leading to haemoglobins A_{1a} and A_{1b} (together about 2%) and A_{1c} (5%) (Trivelli *et al.*, 1971). The evolutionary and functional significance of haemoglobin A₂ is unknown, but genetic factors may alter the observed level of haemoglobin A₂ in the peripheral blood. In nearly all patients with β thalassaemia trait the A₂ level is increased, from the mean normal of 2.55% to mean levels of between 5.15% (Alperin *et al.*, 1977) and 6.49% (Josephson *et al.*, 1958). Raised levels of A₂ have also been observed in patients with unstable abnormal haemoglobins (Rieder *et al.*, 1965).

Acquired changes in Hb A₂ levels have been documented less often. A decrease in Hb A₂ has been found in iron deficiency, mean levels returning to normal after iron therapy (Wasi *et al.*, 1968; Alperin *et al.*, 1977). The original report of A₂ levels in megaloblastic anaemia described raised levels in five of eight patients with pernicious anaemia (Josephson *et al.*, 1958). In four the increase was slight, and the fifth probably carried β thalassaemia trait. The effect of B₁₂ therapy on the A₂ level was not assessed. The comprehensive report by Alperin *et al.* (1977) described increased levels of A₂ in both B₁₂ and folate deficiencies. In both these groups the mean A₂ levels, though raised, were lower than is found in β thalassaemia trait. An inverse relationship between the Hb A₂% and the total haemoglobin level was observed. The results in the present study cannot be directly compared to that of Alperin *et al.* Our criteria for the diagnosis of vitamin B₁₂ and folate deficiencies did not include routine bone marrow examination. Only three of our 106 patients with vitamin B₁₂ or folate deficiencies had raised A₂ levels, and mean A₂ levels for the various groups did not differ from normal. We found no relationship between the Hb A₂ level and the degree of anaemia (Figure). It seems unlikely that the differences in the results between our study and that of Alperin *et al.* could

be explained solely by the different methods used. In neither study was associated iron deficiency rigidly excluded, but there is no reason to suppose that it was more frequent in the present study.

The present study documents that in vitamin B₁₂ deficiency minor increases in Hb A₂ may be found but that this is uncommon. Furthermore, these increases are too slight to cause any diagnostic confusion. The precise explanation for the increase in Hb A₂ noted in our three patients remains obscure, although the level fell into the normal range after vitamin B₁₂ therapy.

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