Haemoglobin A\textsubscript{2} levels in vitamin B\textsubscript{12} and folate deficiency

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SUMMARY Haemoglobin A\textsubscript{2} levels were measured in 50 patients with vitamin B\textsubscript{12} deficiency, 50 patients with folate deficiency, and six patients with combined deficiencies of these vitamins. All were normal except for three patients with vitamin B\textsubscript{12} deficiency, who had a slightly elevated Hb A\textsubscript{2} level; this fell to normal after vitamin B\textsubscript{12} therapy. It is concluded that haemoglobin A\textsubscript{2} levels are usually normal in vitamin B\textsubscript{12} or folate deficiency. However, raised levels of haemoglobin A\textsubscript{2} may be found, but these are not as high as is found in \( \beta \) thalassaemia trait and should not cause difficulty in diagnosis.

In normal adult peripheral blood several minor haemoglobin components may be found, including haemoglobin A\textsubscript{2}. 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 A\textsubscript{2} 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 A\textsubscript{2} level is altered by vitamin B\textsubscript{12} and folate deficiencies.

Methods

In this study vitamin B\textsubscript{12} 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 \textit{Lactobacillus casei} was employed (Waters and Mollin, 1961; Hoffbrand et al., 1966). The normal ranges in our laboratory are 6-21 \( \mu \)g/l for serum folate and 160-640 \( \mu \)g/l for red cell folate.

Patients were assigned to the combined vitamin B\textsubscript{12}/folate deficiency group when all three parameters were reduced. It is recognised that this may not represent a combined B\textsubscript{12}/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 A\textsubscript{2} content. The method employed for A\textsubscript{2} estimation is based on that of Marengo-Rowe (1965). Cellulose acetate strips, 4.5 x 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 A\textsubscript{2} 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 A\textsubscript{2} levels was found when each group of vitamin B\textsubscript{12} and/or folate deficiency states was compared to the established normal range. In three patients with vitamin B\textsubscript{12} deficiency, increased Hb A\textsubscript{2} levels were found. In each patient the level fell into the normal range after B\textsubscript{12} therapy, the fall being greater than could be explained by technical variation in haemoglobin A\textsubscript{2} assays. The levels of A\textsubscript{2} at 3.7, 3.7, and 3.8 % were only slightly increased and below that found in
Haemoglobin A$_2$ levels in vitamin B$_{12}$ and folate deficiency

<table>
<thead>
<tr>
<th>Vitamin deficiency present</th>
<th>No. of patients or normal subjects</th>
<th>Hb A$_2$ levels %</th>
<th>Mean</th>
<th>Observed range</th>
</tr>
</thead>
<tbody>
<tr>
<td>B$_{12}$</td>
<td>50</td>
<td>2.38</td>
<td>1.1-3.8</td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>50</td>
<td>2.47</td>
<td>1.5-3.1</td>
<td></td>
</tr>
<tr>
<td>B$_{12}$ and folate</td>
<td>6</td>
<td>2.45</td>
<td>1.6-3.0</td>
<td></td>
</tr>
<tr>
<td>Normal range</td>
<td>130</td>
<td>2.63</td>
<td>1.8-3.3</td>
<td></td>
</tr>
</tbody>
</table>

β thalassaemia trait. No correlation was observed between the A$_2$ level and the degree of anaemia (Figure).

Comment

In the normal adult about 90% of the circulating haemoglobin is haemoglobin A. Various minor components make up the remaining 10%, and these include haemoglobins F (less than 1%) and A$_2$ (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 A$_2$ is unknown, but genetic factors may alter the observed level of haemoglobin A$_2$ in the peripheral blood. In nearly all patients with β thalassaemia trait the A$_2$ 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 A$_2$ have also been observed in patients with unstable abnormal haemoglobins (Rieder et al., 1965).

Acquired changes in Hb A$_2$ levels have been documented less often. A decrease in Hb A$_2$ 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 A$_2$ 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 B$_{12}$ therapy on the A$_2$ level was not assessed. The comprehensive report by Alperin et al. (1977) described increased levels of A$_2$ in both B$_{12}$ and folate deficiencies. In both these groups the mean A$_2$ levels, though raised, were lower than is found in β thalassaemia trait. An inverse relationship between the Hb A$_2$% 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 B$_{12}$ and folate deficiencies did not include routine bone marrow examination. Only three of our 106 patients with vitamin B$_{12}$ or folate deficiencies had raised A$_2$ levels, and mean A$_2$ levels for the various groups did not differ from normal. We found no relationship between the Hb A$_2$ 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

![Figure](http://jcp.bmj.com/)

**Figure** Haemoglobin A$_2$ levels correlated with packed cell volume in patients with vitamin B$_{12}$ and folate deficiencies.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hb (g/dl)</th>
<th>Serum vitamin B$_{12}$ (ng/l)</th>
<th>Serum folate (µg/l)</th>
<th>RBC folate (µg/l)</th>
<th>Hb A$_2$ %</th>
<th>Before B$_{12}$ therapy</th>
<th>After B$_{12}$ therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>8.1</td>
<td>75</td>
<td>21.0</td>
<td>340</td>
<td>3.7</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>10.8</td>
<td>150</td>
<td>4.0</td>
<td>170</td>
<td>3.8</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>WJ</td>
<td>12.5</td>
<td>160</td>
<td>6.5</td>
<td>250</td>
<td>3.7</td>
<td>3.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Findings in three patients with reduced serum vitamin B$_{12}$ and raised haemoglobin A$_2$ levels before and after therapy
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.

References


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doi: 10.1136/jcp.31.10.960