SHORT COMMUNICATION

The forms of vitamin $B_{12}$ on the transcobalamins

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Summary

1. The transcobalamins from normal serum were obtained in two fractions. One contained transcobalamin I and transcobalamin III: the other contained transcobalamin II. The forms of vitamin $B_{12}$ in the two fractions were then examined.

2. Methylcobalamin and adenosylcobalamin were found in both fractions. Hydroxocobalamin was found in the fraction containing transcobalamin I and transcobalamin III. Cyanocobalamin was found in both fractions in two cases, in the transcobalamin III fraction only in one case and was absent in one case.

Key words: transcobalamins, vitamin $B_{12}$.

Abbreviations: TC, transcobalamin.

Introduction

Four forms of vitamin $B_{12}$ are normally present in human serum. Methylcobalamin is the predominant form. Adenosylcobalamin and hydroxocobalamin account jointly for an average of 33% and cyanocobalamin for an average of 2% (Linnell, Hoffbrand, Hussein, Wise & Matthews, 1974). Two of these forms have established biochemical functions: methylcobalamin in the synthesis of homocysteine from methionine and adenosylcobalamin in the isomerization of methylmalonyl coenzyme A to succinyl coenzyme A (Babior, 1975). The functions of hydroxocobalamin and cyanocobalamin have not been established.

Vitamin $B_{12}$ is transported in plasma on specific carrier proteins designated transcobalamins (TC). One of these proteins, transcobalamin II, is involved in intravascular transport of vitamin $B_{12}$ and in the entry of vitamin $B_{12}$ into the cells (Allen, 1975, 1976; Mahoney & Rosenberg, 1975), and absence of this protein is characterized by a life-threatening illness (Hakami, Neiman, Canellos & Lazeron, 1971; Scott, Hakami, Teng & Sagerson, 1972; Hitzig, Dohman, Pluss & Vischer, 1974). In contrast the absence of the other carrier proteins in serum shows only as a low serum vitamin $B_{12}$ concentration and does not imperil life (Carmel & Herbert, 1969).

Only a small proportion of the vitamin $B_{12}$ in plasma is bound to TCII (Hall, 1969; Benson, Rapazzo & Hall, 1972; Burger, Mehlman & Allen, 1975; England, Down, Wise & Linnell, 1976) but TCII mediates 33–99% of the total plasma vitamin $B_{12}$ clearance and 99% of the clearance to cells other than hepatocytes (Allen, 1976). The forms of vitamin $B_{12}$, which are normally transported on this vitally important protein have not been determined, however, and this point was therefore investigated.

Materials and methods

Samples of venous blood were obtained from four normal subjects by clean venepuncture with foil-wrapped syringes and transferred to foil-
wrapped glass tubes in a darkroom illuminated by two Ilford Safelight bulbs and F904 dark-brown filters. After clotting, the samples were centrifuged and the serum was removed in the darkroom and stored at -20°C in foil-wrapped glass tubes.

Preparation of the transcobalamin fractions and extraction and separation of cobalamins were all carried out under darkroom conditions. Serum was fractionated on a K26/70 column (Pharmacia Ltd) containing Sephadex G-200 (Pharmacia Ltd) with a bed volume of 300 ml and a bed height of 56 cm by upward elution with degassed Tris/HCl buffer, pH 8.0, at a flow rate of 15 ml/h maintained by a persitaltic pump (12000 Varioperpex LKB Ltd). Before separation of the test samples, the column was calibrated with 4 ml volumes of serum containing [57Co]cyanocobalamin and the eluent volumes of the two transcobalamin fractions were ascertained. The test samples were applied to the column in volumes of 4 ml and the appropriate eluent fractions were pooled to give two fractions, one containing TCII–vitamin B12 and the other containing the other (TCI and TCIII) TC–vitamin B12 complexes. The pooled fractions were stored in foil-wrapped glass containers at -20°C until dialysis in Cuphrophan tubing against distilled water for 15 h to remove buffer salts.

The vitamin B12 in the dialysed fractions was extracted into a concentrated aqueous extract by the method described by Farquharson & Adam (1976) and the forms of vitamin B12 were separated by thin-layer chromatography in the dark by using silica gel sheets (Eastman Chromogram Sheets no. 6061) with a solvent system of butan-2-ol/propanol/water/ammonia, 7:4:3:1, by vol.

The identification of the separated forms was effected in daylight by the bioautographic method described by Linnell, MacKenzie, Wilson & Matthews (1969); the growth zones from the test sample were identified by their position relative to standards of methylcobalamin, adenosylcobalamin, hydroxocobalamin and cyanocobalamin. The amounts of each form of vitamin B12 in the extracts were estimated by comparing the size of the growth zones obtained from the extracts with those yielded by the standards which had been applied in known amounts. Account was also taken of the effect of the diluting and concentrating procedures and the amount of each form of vitamin B12 in the extract was finally expressed as a percentage of the total in both extracts.

**Results**

The results are shown in detail in Table 1. The expression of results in the form used implies a degree of precision which is unrealistic in practice but was dictated by the disproportion in the amounts of vitamin B12 in the two TC fractions. This does not affect the essential features of the results which are that methylcobalamin and adenosylcobalamin were present in both TC fractions in all cases, that hydroxocobalamin was present in all the samples but never in the TCII–vitamin B12 fractions, and that cyanocobalamin was found in three samples, being present in both TC fractions in two samples and in the TCII–vitamin B12 fraction only in the third.

**Table 1. Distribution of the forms of vitamin B12 detected on the transcobalamin–vitamin B12 complexes in normal subjects**

The amount of each form is expressed as a percentage of the total vitamin B12 in serum.

<table>
<thead>
<tr>
<th></th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCII fraction</td>
<td>Other TC fractions</td>
<td>TCII fraction</td>
<td>Other TC fractions</td>
</tr>
<tr>
<td>Methylcobalamin</td>
<td>4 35</td>
<td>8 48</td>
<td>7 55</td>
<td>4 52</td>
</tr>
<tr>
<td>Adenosylcobalamin</td>
<td>4 35</td>
<td>8 24</td>
<td>7 15</td>
<td>8 26</td>
</tr>
<tr>
<td>Hydroxocobalamin</td>
<td>0 10</td>
<td>0 8</td>
<td>0 15</td>
<td>0 8</td>
</tr>
<tr>
<td>Cyanocobalamin</td>
<td>2 10</td>
<td>2 2</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td>All forms</td>
<td>10 90</td>
<td>18 82</td>
<td>14 85</td>
<td>14 86</td>
</tr>
</tbody>
</table>
The predominant form of vitamin $B_{12}$ was methylcobalamin, which accounted for 39–62% (average 54%), adenosylcobalamin accounting for 22–39% (average 32%), hydroxocobalamin for 8–15% (average 10%) and cyanocobalamin for up to 12% (average 4%).

The ratios of methylcobalamin to adenosylcobalamin ranged from 0.5 to 1.0 for the TCI–vitamin $B_{12}$ fraction and from 1.0 to 3.7 for the other TC–vitamin $B_{12}$ fraction.

Discussion

TCII and TCIII participate in intravascular transport of vitamin $B_{12}$ and facilitate entry of vitamin $B_{12}$ into cells. The TCII–cyanocobalamin complex is cleared rapidly from plasma by various tissues; shortly after cell uptake, the TCII moiety is degraded and a significant fraction of the vitamin $B_{12}$ in as yet unidentified form leaves the cell and enters the plasma in the free state. The TCIII–cyanocobalamin complex is also cleared rapidly but exclusively by the liver; part of the complex is excreted in bile, and the TCIII moiety of the remainder is degraded and the vitamin $B_{12}$ in unknown form is excreted into plasma bound to TCII. The fate of the TC–vitamin $B_{12}$ complex which is cleared relatively slowly is not known (Cooksley, Allen, Schneider, Mehlman & England, 1975; Allen, 1975, 1976; Schneider, Burger, Mehlman & Allen, 1976).

Leaving aside the unknown quantities of the function of TCI and the affinities of the TC for the various forms of vitamin $B_{12}$, the absence of hydroxocobalamin from the TCII–vitamin $B_{12}$ complex would therefore suggest that this form, unlike the others which can enter all cells, can enter only hepatocytes and is not excreted from hepatocytes to plasma. Such concepts, however, leave open to question the origins of non-dietary hydroxocobalamin in plasma and do not accord with observations on the uptake of hydroxocobalamin by cells other than hepatocytes both in vivo and in vitro (Linnell, 1975). A more interesting and acceptable concept is that TCI affects the cell entry and exit of hydroxocobalamin and is solely responsible for this function in cells other than hepatocytes.

Acknowledgment

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References


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