SHORT COMMUNICATION

PLASMA AND HEPATIC COBALAMINS IN TROPICAL ATAXIC NEUROPATHY


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SUMMARY

1. Chromato-bioautographic analysis of cobalamins in Nigerian subjects showed that in patients with tropical ataxic neuropathy as in normal control subjects methylcobalamin was the major form of vitamin B₁₂ in plasma. Plasma cyanocobalamin was significantly higher in patients than control subjects. The concentration of plasma adenosylcobalamin in patients was about twice that in control subjects.

2. Adenosylcobalamin was the predominant liver cobalamin in all subjects. Cyanocobalamin was not detected in liver from normal subjects, but a small proportion of cyanocobalamin was detected in the liver in the majority of the patients.

3. The raised cyanocobalamin levels may have resulted from the chronic cyanide intoxication known to exist in these patients. There was no evidence of tissue vitamin B₁₂ deficiency or that conversion of metabolically active cobalamins into cyanocobalamin plays a part in the pathogenesis of tropical ataxic neuropathy. However, the increase in plasma adenosylcobalamin does indicate some disturbance of bodily handling of cobalamins in this disease.

Key words: tropical neuropathy, methylcobalamin, adenosylcobalamin, vitamin B₁₂, cyanocobalamin, hydroxocobalamin, cyanide metabolism, chromatography.

Tropical ataxic neuropathy (TAN) and variants of it have been described in many tropical regions and also in prisoner-of-war camps, and the available evidence suggests that chronic cyanide intoxication of dietary origin from consumption of the staple food cassava is an important aetiological factor (Monekosso & Wilson, 1966; Osuntokun, 1968, 1971; Makene & Wilson, 1972).

One pathway for detoxicating cyanide in man is by its incorporation into the 1-carbon metabolic pool, a reaction in which vitamin B₁₂ is probably an intermediary, with the formation of cyanocobalamin from hydroxocobalamin and indirectly from the two coenzyme forms...
of vitamin B\textsubscript{12}, methylcobalamin and adenosylcobalamin (Matthews \& Wilson, 1971). The reaction of cyanide with hydroxocobalamin is a potentially valuable pathway as hydroxocobalamin can protect against cyanide poisoning in experimental animals (Mushett, Kelly, Boxer \& Rickards, 1952; Smith \& Duckett, 1965). In Nigerians with TAN, it has been suggested that inactivation of metabolically active forms of vitamin B\textsubscript{12} by conversion into cyanocobalamin might play a part in the pathogenesis of the disorder (Osuntokun, 1971). The evidence for this is meagre and based on the results of microbiological estimation of serum vitamin B\textsubscript{12} with and without added cyanide, now known not to provide a reliable indication of the concentration of serum cyanocobalamin (CN-B\textsubscript{12}) or to give any information about the concentrations of the other cobalamins in plasma or serum: hydroxocobalamin (OH-B\textsubscript{12}), methylcobalamin (Me-B\textsubscript{12}) and adenosylcobalamin (Ado-B\textsubscript{12}) (Matthews \& Linnell, 1971; Matthews \& Wilson, 1971).

We have now estimated directly the cobalamins in plasma and liver from control subjects and patients with TAN.

**MATERIALS AND METHODS**

**Subjects**

The subjects, who were all Nigerians, were normal members of hospital staff and patients with TAN. Normal volunteers and patients gave free and informed consent to the investigations. Control samples of liver were taken from Nigerian patients who died as a result of automobile accidents.

**Collection of samples**

Blood was obtained from subjects in a darkened room by using syringes wrapped in aluminium foil or carbon paper. The blood was ejected into heparinized light-proof tubes and the plasma was separated immediately and stored deep-frozen in light-proofed containers. (Plasma cobalamins are rapidly photolysed to hydroxocobalamin within 2–3 min of exposure to daylight.)

Liver samples were obtained from patients within 12 h of taking blood, by percutaneous biopsy with a de Menghini needle, and from control subjects not more than 24 h after death, by a similar technique. The specimens were transferred to light-proof containers and stored deep-frozen. Plasma and liver samples were transferred frozen from Ibadan to London for analysis.

**Determinations**

Total plasma vitamin B\textsubscript{12} was estimated by radioisotopic assay (Matthews, Gunasegaram \& Linnell, 1967). Liver samples were weighed wrapped in a tared square of foil and homogenized in water (4 ml) in a Potter–Elvehjem homogenizer. The homogenate was extracted by heating at 100°C for 20 min with acetate–cyanide buffer, pH 4.6 (Matthews, 1962), and total vitamin B\textsubscript{12} estimated by radioisotopic assay (Matthews \textit{et al}., 1967). Cobalamins were estimated in liver homogenates and plasma by chromatography and bioautography of ethanol extracts as described by Linnell, Hussein \& Matthews (1970) and Linnell, Hoffbrand, Peters \& Matthews (1971). The significance of differences between means was assessed by the \( t \)-test.

**RESULTS**

The results (Table 1) show that Me-B\textsubscript{12} was the predominant plasma form of the vitamin in
### Table 1. Plasma and liver cobalamins in patients with tropical ataxic neuropathy and in control subjects

Me-B₁₂, Methylcobalamin; CN-B₁₂, cyanocobalamin; Ado-B₁₂, adenosylcobalamin; OH-B₁₂, hydroxocobalamin.

<table>
<thead>
<tr>
<th></th>
<th>Total vitamin B₁₂</th>
<th>Me-B₁₂</th>
<th>CN-B₁₂</th>
<th>Ado-B₁₂</th>
<th>OH-B₁₂</th>
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<tbody>
<tr>
<td></td>
<td>pg/ml</td>
<td>pmol/l</td>
<td>%</td>
<td>pmol/l</td>
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<tr>
<td><strong>Plasma</strong></td>
<td></td>
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<tr>
<td>Patients (n = 13)</td>
<td>1062±109 ±199</td>
<td>784 ±58</td>
<td>389 ±8-5</td>
<td>116 ±58</td>
<td>14-8 ±26</td>
</tr>
<tr>
<td>Control subjects (n = 15)</td>
<td>709 ±70</td>
<td>523 ±52</td>
<td>348 ±6-5</td>
<td>18-3 ±74</td>
<td>3-5 ±0-9</td>
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<tr>
<td><strong>Liver</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Patients (n = 15)</td>
<td>- ±0-5</td>
<td>- ±0-5</td>
<td>- ±0-5</td>
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<td>- ±0-5</td>
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<tr>
<td>Patients (n = 4 )</td>
<td>1580 ±381</td>
<td>1166 ±281</td>
<td>12-8 ±2-7</td>
<td>1-1 ±0-05</td>
<td>0-0</td>
</tr>
<tr>
<td>Control subjects (n = 4)</td>
<td>1236 ±224</td>
<td>912 ±165</td>
<td>12-8 ±2-8</td>
<td>1-4 ±0-1</td>
<td>0-0</td>
</tr>
<tr>
<td>Normal Caucasians (n = 7)</td>
<td>1048 ±161</td>
<td>773 ±119</td>
<td>10-8 ±0-5</td>
<td>1-4 ±0-7</td>
<td>0-0</td>
</tr>
</tbody>
</table>

(1) Mean ± SEM.  (2) Total vitamin B₁₂ was not estimated.  (3) Range 0-12-5%.  

Cobalamins in tropical neuropathy
patients and the healthy controls. Total plasma vitamin B$_{12}$ appeared to be slightly higher in patients than controls but the difference was not significant at the 5\% level (0.1 > P > 0.05). Concentrations of Me-B$_{12}$ and OH-B$_{12}$ did not differ significantly between groups but the mean plasma Ado-B$_{12}$ was almost twice as high in the patients as in the control subjects (P < 0.02). In patients with TAN, the percentage of CN-B$_{12}$ in the plasma exceeded 8\% in nine of the thirteen patients, whereas it did not exceed 8\% in any of the control subjects. The mean plasma CN-B$_{12}$ concentration in the patients was more than six times that in the control subjects (P < 0.001).

Liver cobalamins were estimated in nineteen patients and four control subjects (Table 1). In fifteen of the patients results are expressed as percentages only, since the biopsies were very small and the estimation of total vitamin B$_{12}$ was not carried out. Control values from seven Caucasian subjects (Linnell, Hoffbrand, Hussein, Wise & Matthews, 1974) are included for comparison. In all samples from Nigerian subjects Ado-B$_{12}$ was the predominant form of the vitamin. No CN-B$_{12}$ was detected in any control sample but a small proportion (mean 3.1\%) was found in samples from the majority of the patients. Proportions of Me-B$_{12}$, Ado-B$_{12}$ and OH-B$_{12}$ were very similar in both patients and controls.

DISCUSSION

As in Caucasians (Linnell et al., 1971), Me-B$_{12}$ is the predominant plasma form of cobalamin in Nigerians. We are unable to explain the relatively high plasma concentration of Ado-B$_{12}$ in patients compared with healthy controls, a finding similar to that described in Leber's hereditary optic atrophy (Wilson, Linnell & Matthews, 1971), a disease like TAN, believed to be causally related to chronic cyanide intoxication. The plasma concentrations of CN-B$_{12}$ in normal Nigerians are similar to those reported in healthy Caucasians in whom CN-B$_{12}$ is either undetectable or detected only in traces (Linnell et al., 1971). In the Nigerians with TAN, the relatively high concentration of CN-B$_{12}$ in the plasma supports the presence of chronic cyanide intoxication.

The distribution of hepatic cobalamins in both Nigerian patients and control subjects is similar to that found in Caucasians (Linnell et al., 1971, 1974). Although in the Nigerian patients there is a definite increase in plasma CN-B$_{12}$ and also an increase in liver CN-B$_{12}$, the latter is small and this suggests that it is unlikely that inactivation of tissue cobalamins by conversion into CN-B$_{12}$ plays any part in the pathogenesis of TAN.

ACKNOWLEDGMENTS

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REFERENCES


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