THE BINDING OF METHYLCOBALAMIN AND VITAMIN-B₁₂ COENZYME*  
W. HIFT, M.A., D.M. (OXON), F.C.P. (S.A.), Senior Lecturer and Senior Physician, Department of Medicine,  
University of Natal and King Edward VIII Hospital, Durban

Four forms of vitamin B₁₂ appear to be of importance in the human body. These are cyanocobalamin, hydroxocobalamin, methylcobalamin and vitamin-B₁₂ coenzyme. Various proteins binding cyanocobalamin have been characterized in serum and other body fluids and a large literature has accumulated on the subject. Less work has been done on the binding of hydroxocobalamin and hardly any on the binding of the other two forms. The present study is an attempt to compare the binding of these four forms of the vitamin by serum and gastric juice.

METHODS

Primary binding was measured as previously described using vitamin labelled with ³²Co and protein precipitation with zinc hydroxide. This measures the sum of transcobalamins I and II in serum and intrinsic factor in gastric juice.

Secondary binding (the p-binder) was measured as already described. Recovery of the substances from serum was measured by a radioactive method and by the use of L. leichmannii.

Hydroxocobalamin was prepared from commercial cyanocobalamin by the method of Smith. Methylcobalamin and vitamin-B₁₂ coenzyme, both the radioactive and the cold forms, were kindly prepared by Dr L. Mervyn of Glaxo Research Ltd, England. They were carefully shielded from light throughout the study.

RESULTS

The results are shown in Figs. 1 - 11. The letters CN, OH, ME, COE stand for cyano-, hydroxo- and methylcobalamin and vitamin-B₁₂ coenzyme respectively. The prefix 'pre' stands for preincubation with cold vitamin in the experiments showing competitive inhibition: thus ME - preCN means assay with radio-methylcobalamin after preincubation with cold cyanocobalamin.

In Figs. 1 - 9 total vitamin B₁₂ added to serum is plotted against the amount of it found bound by experiment. The resultant points lie on straight lines, the intersection of which with the 45° line represents primary binding. In some cases the slope is produced by secondary binding and in others by non-specific adsorption. The absolute values so obtained are of not of importance for the purposes of the present study which attempts only to demonstrate similarities and differences between the four forms of the vitamin.

Each diagram is representative of several similar experiments.

Figs. 1 and 2. Primary binding in serum.

Figs 4 and 5. Competitive inhibition by cyanocobalamin (serum).

Fig. 3. Primary binding in gastric juice. There was not enough labelled coenzyme available for testing primary binding in gastric juice.

Fig. 6. Competitive inhibition by methylcobalamin and coenzyme (serum).
Figs. 7 and 8. Competitive inhibition by methylcobalamin and coenzyme (gastric juice).

Fig. 9. Demonstration of secondary binding (serum).

Fig. 10. Measurement of secondary binding (p-binder) in serum.

Fig. 11. Measurement of secondary binding (p-binder) in serum.

**TABLE 1. RECOVERY OF ADDED VITAMIN B₁₂ FROM SERUM**

<table>
<thead>
<tr>
<th></th>
<th>CN</th>
<th>ME</th>
<th>OH</th>
<th>COE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactive method (cyano- and hydroxocobalamin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With cyanide</td>
<td>96%</td>
<td>102%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>96%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radioactive method (methylcobalamin and coenzyme)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With cyanide</td>
<td>94%</td>
<td>79%</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>97%</td>
<td>37%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Leichmanni method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With cyanide</td>
<td>113%</td>
<td>110%</td>
<td>48%</td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>125%</td>
<td>83%</td>
<td>33%</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

Figs. 1 and 2 show that the primary binding is the same for cyano- and hydroxocobalamin though the latter also shows the signs of secondary binding. Methylcobalamin and the coenzyme appear to show secondary, but little or no primary, binding to transcobalamin.

In Figs. 10 and 11 dilution of a plasma incubated with vitamin B₁₂ is plotted against the product: dilution x vitamin remaining in supernatant. The resultant points lie on straight lines, the intersection of which with the x-axis represents the p-binder, the slope being due to adsorption by albumin. "

In Figs. 3 methylcobalamin appears to bind to intrinsic factor to about half the extent of cyanocobalamin. Preincubation with cold cyanocobalamin reduces the binding of hydroxocobalamin but has no effect on the binding of methylcobalamin and coenzyme as shown in Figs. 4 and 5. This was to be expected in view of the lack of primary binding demonstrated above.

In spite of this apparent inability to bind to transcobalamin, Fig. 6 shows that these two forms of the vitamin competitively inhibit the binding of cyanocobalamin; the
methyl compound almost to the same extent as cyanocobalamin itself, the coenzyme to a lesser degree. The same applies to intrinsic factor as shown in Fig. 7, though in this case the inhibition due to methylcobalamin is less than that due to cyanocobalamin. The explanation of this phenomenon is probably as follows: methylcobalamin and coenzyme bind to transcobalamin (and intrinsic factor) but this binding is of a looser nature than that of cyanocobalamin and hydroxocobalamin, so that during protein precipitation it is destroyed. Hence it appears to be absent in Figs. 1-3 but present in Figs. 6 and 7. The binding seems to be only partial in the case of the coenzyme.16

Fig. 9 demonstrates the presence of a secondary binder in serum in that the slope of the graphs increases with prolonged incubation. This obviously does not affect cyanocobalamin. There is no such binder in gastric juice. On measuring the secondary binder it is shown to bind hydroxocobalamin and the coenzyme identically (Fig. 10), but methylcobalamin to a lesser extent (Fig. 11).

The table shows that the radioactive method used gives very good recovery of cyanocobalamin with or without the prior use of cyanide. Hydroxocobalamin is recovered only when cyanide is used. Without cyanide methylcobalamin is recovered poorly and the coenzyme hardly at all. Cyanide improves recovery but not to full levels. The leichmannii method recovers methylcobalamin almost fully, but again falls short of full levels with the coenzyme.

This incomplete recovery of some forms of the vitamin may have a bearing on standard methods of vitamin B₁₂ assay in serum. It might explain the difference between assays with Euglena gracilis and L. leichmannii noted by some workers.13,14 It may also explain the differences found between serum levels by the two methods in liver failure and infection. These were described in a previous study2 when it was shown that in liver failure the microbiological method tended to give higher results, while in acute infections the radioactive method did.

SUMMARY

The binding of methylcobalamin and vitamin-B₁₂ coenzyme in serum and gastric juice are compared with those of cyanocobalamin and hydroxocobalamin.

All four appear to be bound equally to transcobalamin, but the binding is of a looser nature in the case of the former two. There is mutual competitive inhibition between all four forms.

The secondary, or p-binder, reacts to the same extent with methylcobalamin and not at all with cyanocobalamin.

Intrinsic factor binds all four forms but again the binding is looser and only partial in the case of methylcobalamin and the coenzyme. There is no p-binder in gastric juice.

Radioactive assay recovered cyanocobalamin and hydroxocobalamin fully when cyanide was used during the procedure, but only partially recovered the other two.

Microbiological assay with L. leichmannii recovered three forms but also fell short in the case of the coenzyme.

I wish to thank Prof. E. B. Adams in whose department the work was done and who criticized the manuscript, and Miss E. Kleber and Miss A. Rix who very ably performed most of the technical work. Messrs Glaxo Research Ltd of Greenford, Middlesex, England, very kindly provided the methylcobalamin and coenzyme used in this study, both in radioactive form and in the cold state. The work was supported by a grant from the South African Atomic Energy Board and by the South African Institute for Scientific and Industrial Research.

REFERENCES


A NOTE ON THE FOLATE CONTENT OF UNCOOKED MAIZE*

J. METZ, M.D. (RAND), M.C. PATH. (LOND.), A. LURIE, M.B., B.CH. (RAND), F.F. PATH. (S.A.) and M. KONIDARIS, DIP.MED.TECH. (S.A.), Department of Haematology, South African Institute for Medical Research, Johannesburg

Folate deficiency is common in the South African Bantu and occurs particularly in association with pregnancy, lactation and infancy.1 The cause of the deficiency is probably an imbalance between the dietary intake of folate and the physiological requirement.

As maize is the staple item of the diet of the majority of the Bantu, we report the folate content of maize with special reference to the effect of milling procedures.

MATERIAL AND METHODS

Samples of maize products were obtained from the Premier Milling Company, Johannesburg, by courtesy of Mr W. W. Hooper. On arrival at the mill the seed is cleaned and the bran removed, and the seeds are then divided into 2 main lines of processing, depending on whether the maize germ is to be removed or not (Table 1). In the preparation of the degeminated meals the seed is split down the centre and the maize germ is removed in the process. Both the undegerminated and degeminated seeds are progressively ground and sifted, resulting in a finer granular product at each stage. The meals sold commercially as foods are shown in Fig. 1. Samp mealies and samp rice can be bought as foods and are often used as substitutes for rice. Maize grits are used in the production of beer. The most popular products bought by the Bantu are special sifted maize meal ('Impala Special') which is undegerminated and 'Bakers Cones' which is a refined degeminated granular product.

Folate activity in food is present in both a 'free' (unconjugated) and a conjugated form. Assay of crude homogenates represents the 'free' folate activity. Treatment of the crude homogenate with conjugase derived from chicken pancreas renders the conjugated forms of folate available to the test organism and assay of conjugase-treated samples thus represents 'total' folate activity, i.e. unconjugated plus conjugated. In the present study both 'free' and 'total' folate activity were measured.

*Date received: 20 October 1969.