PRELIMINARY COMMUNICATIONS

Role of Third Serum Vitamin B₁₂ Binding Protein in Vitamin B₁₂ Transport

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Summary

A third vitamin B₁₂ binding protein present in normal serum has been shown to participate in transport of labelled vitamin B₁₂ absorbed from the gut. All three vitamin B₁₂ binding proteins in serum were labelled at the same time after oral administration of vitamin B₁₂, implying that "free" vitamin B₁₂ reached the portal blood from the gut mucosa.

Introduction

Two proteins in serum, transcobalamins I and II (T.C. I and II), are implicated in the transport of vitamin B₁₂. In the fasting state T.C. I carries almost all of the vitamin B₁₂ present in plasma. Vitamin B₁₂ entering the plasma is largely thought to be carried by the second binder, T.C. II. Hall and Finkler (1965) reported that after oral vitamin B₁₂ T.C. II was the first vitamin B₁₂ binding protein to be labelled. The T.C. II and vitamin B₁₂ complex is rapidly cleared from plasma in a manner similar to haptoglobin (Hall and Finkler, 1965), and in conjunction with the short half-life of the T.C. II and vitamin B₁₂ complex it has been suggested that T.C. II is concerned primarily with transport of vitamin B₁₂ from gut to tissues. There is a lag of several hours before vitamin B₁₂ in the gut appears in blood, and a possible explanation is that the delay is due to the synthesis of carrier protein (?T.C. II) in the intestinal cells.

Under certain conditions a third protein in plasma showing different chromatographic behaviour from either T.C. I or T.C. II is demonstrable. Hom (1967) and Cooper (1969) suggested that such a third vitamin B₁₂ binding protein was a polymer of T.C. II. The monomer has a M.W. of about 35,000 and the polymer a M.W. above 120,000. Lawrence (1969) also identified three vitamin B₁₂ binders, T.C. II being separable into low and high M.W. components. Gizis, Dietrich, Ohoi, and Meyer (1970) eluted three vitamin B₁₂ binding fractions from serum on DEAE cellulose. The third binder linked to labelled vitamin B₁₂ was injected intravenously and was cleared from plasma at a somewhat slower rate than T.C. II. A third vitamin B₁₂ binder was described by Hall and Finkler (1967) in a patient with polycythaemia and a similar binder was also described by Carmel (1972) and Bloomfield and Scott (1972). It remains uncertain whether the third binder is a polymer of T.C. II or whether it is an antigenically distinct protein.

This study aimed to determine the physiological role of the third vitamin B₁₂ binding protein, particularly in relation to the intestinal absorption of vitamin B₁₂, and to see whether this would shed any light on its nature.

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Materials and Method

⁵⁷Co-labelled vitamin B₁₂ (20 μ Ci and 0.3 μ g) was given by mouth to two volunteers over the age of 60. The procedure was explained and informed consent to the procedures given. Twenty-five ml of blood was obtained at the start of the procedure and at various times thereafter up to 48 hours. Plasma radioactivity was measured in all samples. Each plasma sample was concentrated twofold with an Amicon Diaflo ultrafilter before column chromatography.

T.C. I on the one hand and T.C. II and binder III on the other were separated on DEAE cellulose and the fractions containing T.C. II and binder III then placed on Sephadex G-200 (Bloomfield and Scott, 1972) to separate these two binders. Between 84% and 100% of the material put on the column was eluted from DEAE cellulose and between 56% and 96% from Sephadex G-200. (Figs. 1 and 2).

Results

No radioactivity was detected in plasma during the first two hours but was present at three hours. The distribution of the orally-administered dose among T.C. I, T.C. II, and binder III at this time is shown in the Table. The absorbed dose of vitamin B₁₂ was attached to all three vitamin B₁₂ binding proteins.

FIG. 1—Separation of vitamin B₁₂ binding proteins on DEAE cellulose. T.C. II and a third binder were eluted with 0.06M phosphate at pH 6.3 followed by elution of T.C. I with 1.0M NaCl.

FIG. 2—Further chromatography of fractions containing T.C. II and Binder III on Sephadex G-200. Elution is with 0.04M phosphate in 0.5M NaCl at pH 7.4.

Thereafter the amount attached to these fractions increased and was still present at 48 hours (see Table). Essentially similar results were obtained in both patients.

Relative Distribution of Vitamin B₁₂ Absorbed from the Gut among Serum Vitamin B₁₂ Binding Proteins

Time after Oral Dose of ⁵⁷ Co Vitamin B ₁₂ (Hours)	Percentage of Oral Dose per Litre of Plasma			
	Whole Plasma	Transcobalamin		Binder III
		I	II	
1	0	0	0	0
2	0	0	0	0
3	0.44	0.23	0.13	0.13
4	1.80	0.78	0.64	0.17
5	3.26	1.32	0.68	0.28
6	3.25	1.58	0.73	0.15
24	2.58	0.85	0.21	0.18

Discussion

All three vitamin B₁₂ binding serum proteins took up vitamin B₁₂ absorbed from the gut and all three appeared to take it up at the same time. Thus this study failed to confirm the claim that T.C. II was the first binder to take up vitamin B₁₂ absorbed

from the gut. This finding makes it unlikely that the delay in transport of vitamin B₁₂ from gut to blood is due to synthesis of a carrier protein and more likely that in the intestinal absorption of vitamin B₁₂ the free vitamin passes to the blood. The absorbed vitamin B₁₂ links equally to all the carrier proteins available and the total uptake by these proteins is related to their unsaturated vitamin B₁₂ binding capacity at the time.

The third vitamin B₁₂ binder participates in vitamin B₁₂ transport under physiological conditions. Its plasma clearance when bound to vitamin B₁₂ is not very dissimilar to T.C. II (Gizis *et al.*, 1970) and it remains to be shown that it is not a polymer of T.C. II.

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MEDICAL MEMORANDA

Successful Treatment of Candidiasis with Transfer Factor

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Chronic mucocutaneous candidiasis without lethal immunological deficiencies is an infection with *Candida albicans* involving the skin, nails, scalp, and buccal and vaginal mucosa (Kirkpatrick *et al.*, 1971). Many of these patients exhibit anergy to intradermal challenge with extracts of candida, indicating a defect of cellular immunity against the organism, although humoral antibodies to the organism may be present (Turk, 1970; Chilgren *et al.*, 1967).

Of the several currently available approaches for reconstitution of cellular immune function the use of dialyzable transfer factor as described by Lawrence (1969) seems to be the most benign. The risk of graft-versus-host disease invariably accompanying bone marrow transplantation is avoided and sensitization to HL-A and other antigens is excluded, since transfer factor is dialyzable whereas tissue antigens are not (Lawrence, 1969). The reported success of Schulkind *et al.* (1971) with transfer factor and amphotericin B further highlighted its potential therapeutic usefulness.

This report describes results of intensive therapy with transfer factor in a patient previously shown to be refractory to a small dose of this preparation (Rocklin *et al.*, 1970) and to bone marrow transplantation (Meuwissen *et al.*, 1971).

Case Report

The patient, a 9-year-old white girl, was the subject of a previous report of defects of cellular immunity associated with chronic mucocutaneous candidiasis (Rocklin *et al.*, 1970). Skin sensitization for 2,4-dinitrofluorobenzene was attempted but challenge did not lead to a positive reaction. She had never reacted to candida extract skin tests with positive delayed hypersensitivity, there was no demonstrable release of migration inhibition factor by her lymphocytes in the presence of candida antigen, and the phytohaemagglutinin response of her lymphocytes was erratic (Rocklin *et al.*, 1970). Lymphopenia had been present since the age of 4½ years, with total lymphocyte counts ranging from 400 to 1,000 cells/mm³. After her first presentation with severe cutaneous and oral moniliasis at 16 months of age several major therapeutic attempts were made. Amphotericin B 80 mg intravenously over six weeks was given at 16 months of age (body weight 8 kg). Transfer factor prepared from 3 ml of packed leucocytes was injected by Rocklin *et al.* (1970) at age 7 without indication of clinical or in-vitro lymphocytic improvement. One month later intravenous amphotericin B 330 mg was given over three weeks with definite signs of clearing of candida lesions.

Immediately after this last course a bone marrow transplant was undertaken in September 1969 with an HL-A non-identical sister as donor (Meuwissen *et al.*, 1971). Initial disappearance of candida infection after this combined treatment lasted four to six weeks. New candida lesions then appeared, and three months after the transplant a severe phase of cachexia began, characterized by diarrhoea, anorexia, raised liver enzymes, and splenomegaly, which was believed to be a graft-versus-host reaction (Meuwissen *et al.*, 1971). Severe mucocutaneous candidiasis recurred, leading to a further course of therapy with amphotericin B 230 mg intravenously over 19 days in April 1970 (body weight 14 kg). The lesions showed no sign of clearing when the drug was stopped. 5-Fluorocytosine 25 mg given by mouth in May and June was fruitless.

TRANSFER FACTOR THERAPY

In November 1970 dialyzable transfer factor was prepared after the method of Lawrence (1969). The lyophilized material was dissolved in distilled water and kept frozen at -20°C until use. On two occasions in December transfer factor from 600 ml of blood taken from highly candida-sensitive donors was given intramuscularly. No change in lesions was noted. In June 1971 a course of amphotericin B 176 mg

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