Dynamics of Serum Binding of Intravenously Administered ^{57}Co Vitamin B_{12}[1] (35624)

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Following the addition of ^{57}Co B_{12} to normal serum, three binders have been isolated by DEAE–cellulose column chromatography. The order of elution of these binders in phosphate buffer of increasing ionic strength and decreasing pH is transcobalamin II (TCII), a binder eluted with the main protein peak, and transcobalamin I (TCI), according to the terminology used by Hall and Finkler (1). Differences in clearance rates of intravenously administered ^{57}Co B_{12} have been reported from several laboratories for normal subjects and patients with pernicious anemia (P.A.) in relapse and untreated chronic myelocytic leukemia (C.M.L.) (1–9). Hall and Finkler (1) reported that following intravenous administration of ^{57}Co B_{12} to two normal subjects the percentage of residual ^{57}Co B_{12} in the serum bound to TCI increased with time as measured by DEAE–cellulose column chromatography. Maximal labeling of both binders was seen in the first sample which was drawn 1.5 min after administration of the radioactive vitamin. Hom *et al.* (8) showed that 24 hr after intravenous administration of ^{57}Co B_{12} to one normal subject, 55 to 72% of the residual radioactivity calculated on the basis of recovered TCII and TCI, was bound to TCI depending on which of three techniques was employed, *viz.*, DEAE Sephadex chromatography, G-200 Sephadex gel filtration, and ammonium sulfate precipitation. Plasma clearance analyses of ^{57}Co B_{12} bound to TCII and TCI have been reported from several laboratories (1, 3, 10). In all studies, the clearance rate of TCII-bound ^{57}Co B_{12} was faster than that of TCI-bound ^{57}Co B_{12}. Hom and Olesen (10) reported that ^{57}Co B_{12} bound to TCII reappeared bound to TCI, following intravenous injection of ^{57}Co B_{12}-labeled TCII. In this study, clearance rates of intravenously administered ^{57}Co B_{12} were measured in 17 normal subjects, nine P.A., and four C.M.L. patients. Distribution of ^{57}Co B_{12} into TCII and TCI at various time intervals after the intravenous administration of ^{57}Co B_{12} was determined by DEAE–cellulose column chromatography. Purpose of the study was the investigation of the plasma clearance rates of *in vivo*-labeled TCII and TCI in normal subjects, P.A. patients, and C.M.L. patients. Observation that absolute amounts of ^{57}Co B_{12} bound to TCI increased with time would provide a proof that ^{57}Co B_{12} bound to TCII reappears as bound to TCI.

Materials and Methods. Clearance. Clearance studies were performed as described by Meyer *et al.* (9). Two μCi of ^{57}Co B_{12}, sp act, 10–18 μCi/μg (supplied by Squibb Co.) in a volume of 2.0 ml were rapidly injected. At intervals of 1, 2, 5, 15, 30, 45, and 60 min and 2, 4, 8, 12, 24, and 48 hr thereafter, 20 ml of blood were withdrawn. Ten ml were added to sodium and potassium oxalate and rotated thoroughly to prevent clotting. Four ml of plasma from each specimen were counted in a well-type scintillation spectrometer. Serum was separated from the remaining 10 ml of clotted blood and stored at —20° before chromatographic separation. Plasma volume was calculated by estimating total blood volume based on height, weight, and sex of the subjects, using plasmacrit observed on first day of clearance study.

DEAE–cellulose column Chromatography To 1.0 ml of serum obtained from a blood sample drawn at zero time (prior to injection of labeled vitamin), 200 pg of ^{57}Co B_{12} were added and allowed to incubate for 15

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min. All serum samples were treated with hemoglobin-coated charcoal prior to application on the column to remove free vitamin B_{12} (11). One-ml serum aliquots from samples obtained during clearance studies were chromatographed on 5.0-ml DEAE–cellulose columns as described by Retief *et al.* (11). The method was modified in that eleven 2.0-ml samples were collected with the 0.06 *M* phosphate buffer, pH 6.35, and six 2.0-ml samples with 1.0 *M* NaCl. DEAE–cellulose (purchased from Schleicher and Schuell, Keene, N.H.) of ion-exchange capacity between 0.90 and 0.95 meq/g, was used. The information obtained from the serum sample drawn at zero time was used as an indication of how much of the injected ^{57}Co B_{12} was bound to each of the two binding proteins.

Results. Clearance of ^{57}Co B_{12} in normal subjects and distribution to TCII and TCI. Table I (a) shows the percentage of residual radioactivity at zero time, 1 min, and 1,24, and 48 hr; percentage distribution of ^{57}Co B_{12} bound to TCII and TCI at the same time intervals, and ^{57}Co B_{12} bound to TCII and TCI as percentage of injected activity. Values in the last two columns are the products of percentage residual values and percentage distribution of TCII and TCI. Values in parenthesis in the last two columns express circulating ^{57}Co B_{12} bound to TCII and TCI as percentage of values at zero time.

An initial decrease in circulating and radioactivity to 55% of injected dose was observed. Circulating radioactivity decreased as clearance time advanced but at a slower rate per unit time than in the first minute.

A good agreement was observed between values of TCII and TCI at zero time and 1 min. It appeared that the binding pattern of injected ^{57}Co B_{12} was identical to that of the 200 pg of ^{57}Co B_{12} added per ml of serum *in vitro*. The percentage of ^{57}Co B_{12} bound to TCI increased, and consequently, the percentage bound to TCII decreased as clearance time advanced. Due to decrease in residual radioactivity, the absolute amount of ^{57}Co B_{12} bound to both TCI and TCII decreased at all time intervals studied.

Clearance of ^{57}Co B_{12} in pernicious anemia patients and distribution as TCII and TCI. Table I (b) shows clearance data in 9 P.A. patients to whom tracer doses of ^{57}Co B_{12} were administered.

Circulating radioactivity decreased to 60% during the first minute. This continued for the remainder of the 48-hr period of study. Percentage of TCI-bound ^{57}Co B_{12} increased, but because of decrease in total circulating radioactivity the absolute amount of ^{57}Co B_{12} bound to TCI and TCII decreased at all time intervals studied. Plasma disappearance of ^{57}Co B_{12} bound to TCII is clearly faster than ^{57}Co B_{12} bound to TCI.

Clearance of ^{57}Co B_{12} in chronic myelocytic leukemia patients and distribution as TCII and TCI. Table I (c) shows clearance data in four C.M.L. patients to whom tracer doses of ^{57}Co B_{12} were administered.

TCI content at zero time is higher than in normal and P.A. patients. Decrease in residual activity after the first minute was less than in the other two groups studied. Percentage of ^{57}Co B_{12} bound to TCI increased at all time intervals except at 48 hr (it is possible that this discrepancy is due to the high standard deviation). The last two columns in Table I (c) indicate an absolute decrease in the percentage of ^{57}Co B_{12} bound to TCII and TCI. As in normal and P.A. patients, disappearance of ^{57}Co B_{12} bound to TCI was slower than disappearance of ^{57}Co B_{12} bound to TCII.

Discussion. A rapid decrease in residual radioactivity in the first minute of the clearance was observed when ^{57}Co B_{12} was injected to normal subjects.

Clearance rate of *in vivo*-labeled TCII after injection of free ^{57}Co B_{12} resembled closely that of *in vitro*-labeled TCII when administered to normal subjects (3). Clearance rate of TCI labeled *in vivo* was faster in normal persons than that of *in vitro*-labeled TCI prepared from normal serum and injected into normal subjects (3). In the latter case, less than 20% of the administered dose was cleared in the first minute, while in the former instance 50% had been cleared. It is possible that injected free ^{57}Co B_{12} was removed from the circulation before it was bound to TCI and TCII. Instantaneous absorption of cyanocobalamin on cell mem-

TABLE I. TCI and TCII Levels in Serum Following Intravenous Injection of ^{57}Co B_{12} into 17 Normal Subjects, 9 P.A. and 4 C.M.L. Patients.

Time	Res. activity[a] (%)	TCII[b] (%)	TCI[b] (%)	TCII % inj. act[c]	TCI % inj. act[c]
			(a) Normal		
0[d]		88.7 ± 3.4	11.3 ± 3.4		
1 min	54.6 ± 16.9	87.9 ± 3.8	12.8 ± 3.8	48.2 ± 15.8 (53.0)[e]	6.4 ± 2.8 (56.3)
1 hr	13.6 ± 3.0	80.8 ± 7.6	19.2 ± 7.6	11.0 ± 2.6 (12.1)	2.6 ± 1.2 (22.9)
24 hr	4.5 ± 1.2	66.5 ± 12.1	33.5 ± 12.1	3.0 ± 0.9 (3.3)	1.5 ± 0.8 (13.2)
48 hr	3.5 ± 1.0	60.1 ± 12.0	39.9 ± 12.0	2.0 ± 0.8 (2.4)	1.4 ± 0.6 (12.4)
			(b) P.A.		
0		80.9 ± 5.1	19.1 ± 5.1		
1 min	60.9 ± 4.7	88.7 ± 2.4	11.3 ± 2.4	53.9 ± 4.0 (63.6)	6.9 ± 1.7 (36.0)
1 hr	17.4 ± 4.2	71.8 ± 6.3	28.3 ± 6.3	12.3 ± 2.3 (14.8)	5.0 ± 2.3 (26.1)
24 hr	8.1 ± 2.5	60.9 ± 6.6	39.1 ± 6.6	5.0 ± 1.9 (6.0)	3.1 ± 0.8 (16.0)
48 hr	6.4 ± 2.3	55.4 ± 7.2	44.6 ± 7.2	3.5 ± 1.1 (4.2)	2.9 ± 1.3 (15.2)
			(c) C.M.L.		
0		59.9 ± 15.3	40.1 ± 15.3		
1 min	63.7 ± 37.3	66.4 ± 13.3	33.6 ± 13.3	39.5 ± 19.7 (65.9)	24.2 ± 20.2 (60.3)
1 hr	45.9 ± 13.3	56.1 ± 14.0	43.9 ± 14.0	25.8 ± 9.3 (43.1)	20.5 ± 7.2 (51.1)
24 hr	29.8 ± 19.9	41.9 ± 21.0	58.1 ± 21.0	12.0 ± 6.4 (20.0)	17.8 ± 9.6 (44.4)
48 hr	23.1 ± 10.9	45.3 ± 23.7	54.7 ± 23.7	10.6 ± 6.6 (16.7)	12.5 ± 6.2 (31.2)

[a] Res. activity shows percentage injected activity in plasma at indicated time interval.

[b] TCII% and TCI% represent percentage distribution of residual activity of these two binders, determined by DEAE–cellulose chromatography.

[c] Values in columns marked TCII% inj. act and TCI% inj. act are derived by multiplying values of Res. activity by values of TCII% and TCI%.

[d] Zero-time values are calculated by chromatographing patient's serum to which ^{57}Co B_{12} has been added (200 pg/ml).

[e] Numbers in parentheses were derived by dividing "% inj. act" by corresponding zero-time activity and multiplying by 100.

branes of Ehrlich ascites cells has been reported by Paranchych and Cooper (12).

Clearance rates of *in vivo*-labeled TCII and TCI after injection of ^{57}Co B_{12} into P.A. patients were slower than those of labeled TCII and TCI after injection of ^{57}Co B_{12} to normal subjects. The difference does not appear to be significant at the 5% level as estimated by the student *t*-test. High standard deviations were observed in the data from C.M.L. patients, probably due to the smaller number of cases studied or to differ-

ent stages of disease. TCI content was found markedly elevated in accordance with previous reports (1, 13). Plasma clearances of ^{57}Co B_{12} and those of *in vivo*-labeled TCII and TCI were strikingly delayed. Rate of plasma clearance of *in vivo*-labeled TCI was similar to that observed when *in vitro*-labeled TCI was administered to normal subjects.

High TCI content probably hindered clearance of ^{57}Co B_{12}-labeled TCII. The findings in this study differ from those of Hom and Olesen (10). It is possible that these differences are due to the fractionation procedures. It has been reported from several laboratories (14, 15, 16) that the 120,000 molecular size fraction from Sephadex columns does not always correspond to TCI. The presence in serum of a binder with beta-globulin electrophoretic mobility and molecular size similar to TCI has been demonstrated (15, 16). Since a continuous decrease in the amount of ^{57}Co B_{12} bound to TCI as determined by DEAE–cellulose column chromatography was found in this study and an increase of the amount of ^{57}Co B_{12} bound to the 120,000 fraction (gel filtration) was observed by Hom and Olesen, ^{57}Co B_{12} possibly reappeared in circulation bound to this binder (10).

Summary. Following intravenous injection of ^{57}Co vitamin B_{12}, clearance rates of endogenously labeled TCII and TCI were delayed in patients with C.M.L., compared to normal subjects and cases of P.A. If ^{57}Co B_{12} from TCII reappeared bound to TCI, it occurred at a rate slower than that of plasma disappearance of ^{57}Co B_{12} bound to TCI.

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