Heat Lability of Transcobalamin II[1] (35758)

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Normal serum contains at least three ^{57}Co B_{12} binders which can be separated by DEAE-cellulose column chromatography (1, 2). Transcobalamin II (TCII) is eluted in one method of separation with 0.04 *M* phosphate buffer, pH 5.9; the main protein peak binder (MPPB) with 0.1 *M* sodium phosphate buffer, pH 5.8; and transcobalamin I (TCI) with 0.4 *M* sodium phosphate buffer, pH 5.2 (2).

It has been reported that plasma disappearance of ^{57}Co B_{12} prebound to serum heated at 56° for 30 min was slower than that of ^{57}Co B_{12} bound to unheated normal serum (3). Since TCII is the principal binder of ^{57}Co B_{12} when small amounts of the labeled vitamin are added to serum (1, 2), it was postulated that heat may have altered this binder. To investigate this hypothesis ^{57}Co vitamin B_{12} was added to normal serum. An aliquot was heated to 56° for 30 min or 2 hr. Heated and unheated samples were chromatographed on DEAE-cellulose columns and elutates were compared for bound ^{57}Co B_{12}. Molecular sizes of isolated binders from normal and heated sera were compared by gel filtration on Sephadex G-200 columns. In addition, TCII ^{57}Co B_{12} prepared from normal serum was heated at 56° for 30 min and then chromatographed on Sephadex G-200 columns. The bound forms of vitamin B_{12} eluted from the DEAE-cellulose columns with 0.04 *M* buffer from normal and heated serum, and heated TCII solution, were administered intravenously to normal subjects. Plasma disappearance of injected radioactivity was examined at intervals as described below.

Materials and Methods. DEAE-cellulose column chromatography. Details of this separation were reported elsewhere (2). Three hundred pg of ^{57}Co B_{12} (sp act 150 mCi/mg; Philips-Duphar, Holland) were added per milliliter to approximately 30 ml of serum. After incubation at 37° for 15 min the reaction mixture was dialyzed for 48 hr against the initial buffer in the cold room before application on 3 × 60-cm DEAE-cellulose columns. The percentage of free ^{57}Co B_{12} was calculated from the recovery in the dialysate. Serum samples were heat treated prior to dialysis as described in the following section. The following buffers were used for elution at 4° and flow rate of 30 ml/hr:0.0175 *M* sodium phosphate buffer, pH 6.3 (600 ml); 0.04 *M* sodium phosphate buffer, pH 5.9 (1000 ml); 0.1 *M* sodium phosphate buffer, pH 5.8 (500 ml); and 0.4 *M* sodium phosphate buffer, pH 5.2 (700 ml).

Heat treatment. Approximately 30 ml of serum, to which 300 pg ^{57}Co B_{12} had been added per milliliter, were placed in a 35-ml vial and agitated in a water bath at 56°. After 30 min, or in one case 2 hr, the sample was removed from the water bath, dialyzed against the initial buffer, and chromatographed on a DEAE-cellulose column as described in the previous paragraph.

TCII prepared by DEAE-cellulose column chromatography from 30 ml of normal serum was dissolved in 100 ml of normal saline and sterilized by Millipore filtration (2). Half of the sterile solution was used as control and the remainder was heat-treated at 56° for 30 min.

Gel filtration. Fractions of bound ^{57}Co B_{12} in normal and heated serum prepared by DEAE-cellulose column chromatography were dialyzed and freeze-dried. These fractions were chromatographed on Sephadex-G-200 columns, 2 × 60 cm, using 0.005 *M* sodium phosphate buffer, pH 7.4, containing

[1] This work was partly supported by U.S. Public Health Service Grant No. CA 08279-06.

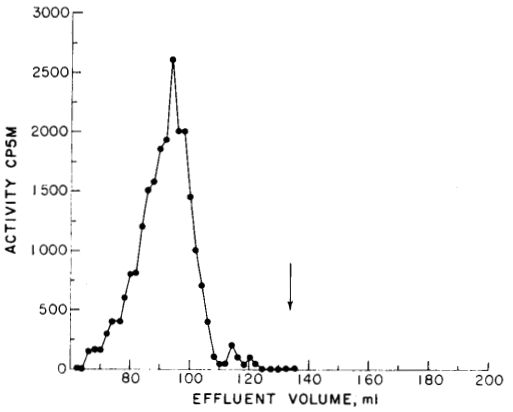

FIG. 1. Gel filtration on Sephadex G-200 column of ^{57}Co B_{12} eluted with 0.04 *M* buffer (DEAE-cellulose column) from serum heated at 56° for 30 min. Arrow indicates the elution volume of ^{57}Co B_{12} bound to normal TCII.

1.0 *M* NaCl for elution (2). A sample of heat-treated TCII solution was also chromatographed on Sephadex column. Two-ml samples were collected at a flow rate of 12 ml/hr and counted for radioactivity.

Plasma clearance. Solutions of TCII in normal saline were sterilized by filtration through Millipore 0.45-μ filter (2). Sterility of solution was determined by culture on blood agar plates and thioglycollate media. Fifty ml of TCII were administered intravenously into normal subjects. Blood samples were drawn at 1, 2, 5, 15, 30, and 45 min and 1, 2, 4, 8, 12, 24, and 48 hr. Aliquots of 4 ml of plasma were counted in a scintillation counter and the percentage of residual activity was calculated from the estimated plasma volume and the radioactivity of the injected sample (4).

Results. DEAE-cellulose column chromatography. Table I shows the amount of radioactive vitamin eluted with each buffer, and unbound ^{57}Co B_{12} in normal serum, and serum that was heated at 56° for 30 min or 2 hr. The percentage of labeled vitamin in heat-treated serum eluted from the DEAE-cellulose column with 0.04 *M* buffer was significantly less than that from normal serum. The percentage of the ^{57}Co B_{12} bound form eluted from heated serum with the 0.1 and 0.4 *M* buffers increased significantly over unheated serum.

Gel filtration. Figure 1 shows the radioactive profile obtained by gel filtration of the bound form of the labeled vitamin eluted with 0.04 *M* buffer from serum which had been heated at 56° for 30 min. The molecular size is clearly larger than that of the binder obtained from unheated normal serum (TCII) Figure 2 shows the radioactive profile obtained by gel filtration of heated TCII solution prepared from normal serum. It appears that the radioactivity was dissociated from the binder as a result of heat treatment. A portion of the free ^{57}Co B_{12} had a molecular size slightly larger than that of cyanocobalamin, but this observation was not investigated further. It could be speculated that this ^{57}Co B_{12} is bound to a fragment of TCII similar to a ^{57}Co B_{12} binding peptide that was isolated by electrodialysis of serum (7). The radioactive profile (Fig. 3) obtained by gel filtration of the labeled vitamin in heated serum eluted with 0.1 *M* buffer was identical with that of the MPPB binder, while the binder eluted with 0.4 *M* was identical with the major peak of TCI from normal serum (Fig. 4). A minor component accompanying TCI was not detected in the preparation from heated serum.

Plasma clearance. Table II shows plasma

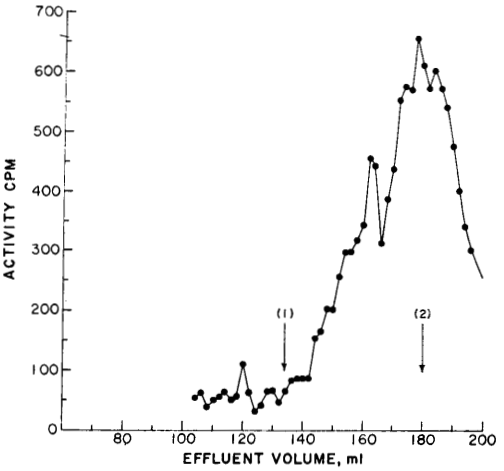

FIG. 2. Gel filtration on Sephadex G-200 column of ^{57}Co B_{12} which was bound to TCII prior to heating the binder solution at 56° for 30 min. Arrows at 134 ml indicate the elution volume of ^{57}Co B_{12} bound to TCII and at 180 ml of free ^{57}Co B_{12}.

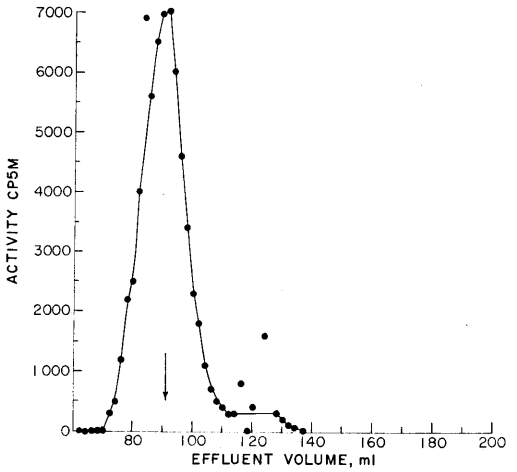

FIG. 3. Gel filtration on Sephadex G-200 column of ^{57}Co B_{12} eluted with 0.1 *M* buffer (DEAE-cellulose column) from serum heated at 56° for 30 min. Arrow indicates the elution volume of ^{57}Co B_{12} bound to MPPB prepared from normal serum.

clearance rates after intravenous administration of heat-treated TCII, normal TCII, and the binder in heat-treated serum eluted from DEAE-cellulose column with the 0.04 *M* buffer. In all instances, normal values were observed.

Discussion. Table I shows that heating labeled normal serum at 56° for 30 min is followed by a decrease in the amount of ^{57}Co B_{12} bound to TCII with corresponding increase in binding of radioactivity to TCI and MPPB. In serum heated for 2 hr, the binding capacity of all 3 binders was decreased and 80% of added ^{57}Co B_{12} remained free. The binder derived by gel filtration of the eluate with 0.04 *M* buffer from the DEAE-cellulose column was larger than TCII (Fig. 1) and probably represents an aggregate of the normal binder. It has been reported previously, that TCII exhibited the tendency to aggregate at low ionic strength (5) and in preparations from some pernicious anemia patients (6). As previously mentioned, no alteration in size of ^{57}Co B_{12}, TCI, or MPPB was noted after heating of normal serum. If this binder is an aggregate of TCII, the observation suggests that size of TCII does not affect plasma clearance.

Following intravenous administration of heat-treated TCII, the clearance pattern was normal (Table II). It has been shown in the section on "gel filtration" that heating this labeled binder is followed by an almost complete liberation of ^{57}Co B_{12} so that, in effect, measurement of clearance rate was that of free ^{57}Co B_{12}. Slow plasma clearance of ^{57}Co B_{12} prebound to heated serum (3) can be explained on the assumption that ^{57}Co B_{12} bound to TCII is released as free ^{57}Co B_{12} (or bound to a small fragment of TCII) and is subsequently attached to MPPB and TCI. Since the percentage of labeled vitamin bound to the slow clearing TCI (2) has increased, plasma clearance of ^{57}Co B_{12} prebound to heated serum is slower than that of ^{57}Co B_{12} bound to normal serum. Similar changes in the binding of vitamin B_{12} to serum due to heating as determined by gel filtration on Sephadex columns were reported by Gullberg (8).

Summary. ^{57}Co B_{12} labeled normal serum was heated at 56° for 30 min and chromatographed on DEAE-cellulose columns, using the following sodium phosphate buffers for elution: 0.0175 *M*, pH 6.3; 0.04 *M*, pH 5.9; 0.1 *M*, pH 5.8, and 0.4 *M*, pH 5.2. The amount of bound ^{57}Co B_{12} eluted with 0.04 *M* buffer decreased and that eluted with 0.1 and 0.4 *M* buffer increased in heat-treated

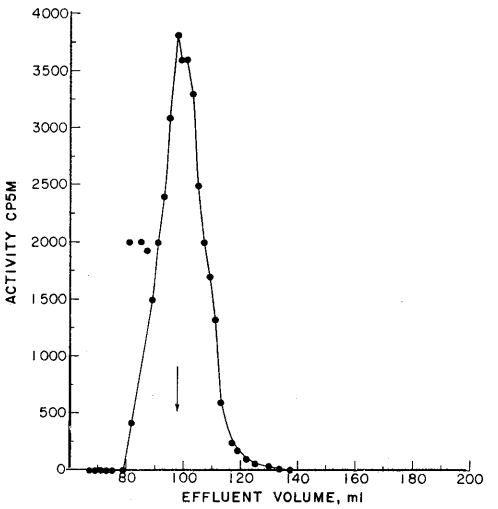

FIG. 4. Gel filtration on Sephadex G-200 column of ^{57}Co B_{12} eluted with 0.4 *M* buffer (DEAE-cellulose column) from serum heated at 56° for 30 min. Arrow indicates elution volume of ^{57}Co B_{12} bound to TCI prepared from normal serum.

TABLE I. Yield (%) of ^{57}Co B_{12} Eluted with Indicated Buffers from DEAE-Cellulose.

Name	Treatment	Buffer (*M*) 0.04	0.1	0.4	Free ^{57}Co B_{12}
B.D.	Normal	71	17.8	3.3	7.9
	Heated 30 min, 56°	46	36.5	10.5	7.0
K.M.	Normal	68.1	20.5	5.4	6.0
	Heated 30 min, 56°	28.3	43.5	20.2	8.0
M.E.	Normal	68.8	18.8	6.8	5.6
	Heated 30 min, 56°	32.7	41.5	17.8	8.0
	Heated 2 hr, 56°	1.4	10.7	7.4	85.2

TABLE II. Plasma Clearance of Heated TCII ^{57}Co B_{12}.

Preparation	Residual radioactivity (%) 1 hr	24 hr	48 hr
1[a]	12.5	5.5	3.2
2[b]	15.1	2.6	2.0
3[b]	11.8	3.4	2.6
4[c]	12.0	3.8	3.5
Normal TCII (15 subj)	13.7 ± 1.3	4.2 ± 1.6	3.2 ± 1.3

[a] TCII ^{57}Co B_{12} prepared from normal serum heated at 56° for 30 min; administered to one subject.

[b] TCII ^{57}Co B_{12} heated at 56° for 30 min after preparation from normal serum; administered to one subject.

[c] Unheated TCII ^{57}Co B_{12}; administered to same subject as in preparation 3.

serum. Gel filtration indicated that 0.04 *M* eluate was possibly an aggregate of transcobalamin II (TCII). Plasma disappearance rate of this fraction was similar to that of TCII. Gel filtration of normal TCII, heated in solution at 56° for 30 min, indicated that ^{57}Co B_{12} was in free form. Following heating of ^{57}Co B_{12} labeled serum at 56° for 2 hr, 80% of the vitamin was unbound. It appeared that TCII is a heat labile substance.

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Received Feb. 2, 1971. P.S.E.B.M., 1971, Vol. 137.