Effect of Human Serum B_{12} Binders on Uptake of Vitamin B_{12} by Isolated Perfused Rat Liver[1] (35442)

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It has been reported that three binders of ^{57}Co B_{12} are separated by DEAE-cellulose column chromatography following addition of labeled vitamin to normal serum (1, 2). These binders are known as transcobalamin II (TCII), transcobalamin I (TCI) and main protein peak binder (MPPB). TCII and MPPB have an electrophoretic mobility of β-globulin, while TCI has the mobility of α-globulin (2). The molecular size of MPPB is approximately 120,000, similar to that of TCI, and larger than TCII (36,000) (2).

Further observations reveal that intravenously administered TCII ^{57}Co B_{12} and MPPB ^{57}Co B_{12} are cleared rapidly from plasma while TCI ^{57}Co B_{12} shows a much slower pattern (2–4). Finkler and Hall (5) reported that HeLa cells take up TCII but not TCI bound ^{57}Co B_{12}. Retief *et al.* (6) observed that TCII delivers ^{57}Co B_{12} to erythrocytes at a faster rate than TCI. Haught *et al.* (7) recently reported that rat TCII has no function in B_{12} uptake by perfused rat liver.

In this study uptake by isolated perfused rat liver of ^{57}Co B_{12} bound to the three binders was investigated.

Materials and Methods. Preparation of ^{57}Co B_{12} binders. The binder used in each perfusion experiment was prepared from 30 ml of serum, as follows. Serum from normal subjects or pernicious anemia (PA) patients was kept at −20° before use. 300 pg of ^{57}Co B_{12} (sp act around 200 mCi/mg; purchased from Philips-Duphar, Holland) were added/ml to 30 ml of thawed serum. The solution was allowed to stand at 37° for at least 15 min, then was dialyzed in the cold room against 0.0175 *M* sodium phosphate buffer pH 6.3, for 24 hr.

DEAE-cellulose, Schleicher and Schuell, No. 70, of ion exchange capacity between 0.90 to 0.95 mEq/g, was packed after proper preparation, into 3 × 6-cm columns (2). 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). Buffer solutions were made in distilled water containing 0.09% methylparaben and 0.01% propylparaben (Tenneco Chemicals). TCII was eluted with the 0.04 *M* buffer, MPPB with the 0.1 *M* buffer and TCI with the 0.4 *M* buffer (2). Eluates containing binders were dialyzed and freeze dried. No further attempt at purification was made.

Liver perfusion. Rat liver perfusions were carried out as 2 hr experiments according to the procedure described by Miller *et al.* (9), with minor changes as previously noted (8). Livers were removed from normal, male Wistar rats weighing approximately 200 g. Blood was obtained from larger normal, male animals of the same strain.

In a typical experiment, the total blood volume (usually 90 ml) to be used as perfusion medium, containing heparin, essential and nonessential amino acids and glucose, was added to the reservoir of the perfusion apparatus. After circulation was started the excised liver was connected to the perfusion system (8). When satisfactory flow of blood through the liver was established (2–3 ml/g of liver/min under pressure of 21 cm of blood), and flow of bile approximated 0.3–0.6

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ml/hr/liver, the binder under study was made up to 5 ml in Ringer's solution and added to the perfusing blood (total volume = 95 ml). The experiment was terminated 2 hr later. Perfusing blood was recovered from apparatus, and liver was flushed five times with Ringer's solution (total 51 ml). The organ was homogenized in a Teflon glass grinder with fresh Ringer's solution. The latter was made up to 10–15 ml depending on size of liver.

Radioactivity determination. All samples were counted for ^{57}Co activity in a well-type scintillation counter, Nuclear Chicago model SP-5. An aliquot (0.1 ml) of prepared binder was removed and measured for determination of total dose of radioactivity. Two ml of recovered perfusate (total usually 88 ml), total bile collected (usually 0.6 to 1.2 ml), 3 ml of Ringer wash, and 2 ml of homogenized liver were examined for radioactivity. Approximately 85–95% of the total dose was accounted for in each experiment.

Progress of the experiments was monitored by circulating perfusing blood through the scintillation detector by means of an Abbott Radicoil inserted in the well, and registering radioactivity on a Heath recorder. By this means, it was found that in experiments where liver uptake was observed, it was essentially completed in 30–60 min.

TABLE I. Uptake of ^{57}Co B_{12} Bound to Human Serum Binders by Isolated Perfused Rat Liver.

Sample	Donor	Liver wt (g)	Percentage uptake of ^{57}Co B_{12}		
			Liver	Bile	Per g of liver
TCII					
9-29-69	MEL[a]	9.5	35.0	0.2	3.7
11-12-69	SHA[a]	7.3	20.1	6.1	3.6
1-20-70	ALP[a]	6.6	29.5	4.1	5.1
9-15-69	ING[b]	6.9	25.3	2.7	4.1
6-11-69	HEB[b]	6.4	42.9	6.7	7.8
TCI					
5-26-69	ROS[a]	7.3	5.1	1.0	0.8
1-20-70	ALP[a]	6.6	6.9	0.6	1.1
9- 3-60	JAC[b]	10.8	8.6	0.2	0.8
Main peak binder					
7- 7-69	SHA[a]	7.6	24.8	3.3	3.7
1- 7-70	ROS[a]	9.4	53.3	8.4	6.6
1-20-70	ALP[a]	7.8	47.7	8.8	7.2

[a] Normal.
[b] P.A.

Results and Discussion. Table I shows results obtained when ^{57}Co B_{12} bound to the three binders was added to rat liver perfusing medium. These are presented in terms of total percentage uptake in liver and bile per experiment, or per gram of liver. A trend for high uptake of ^{57}Co B_{12} bound to TCII prepared from normal and PA serum and to MPPB is observed. The uptake of ^{57}Co B_{12} bound to TCI is significantly lower than those of the two other binders.

Results obtained in this study correlate with observations that plasma clearance of B_{12} bound to TCII and MPPB is faster than that of B_{12} bound to TCI when administered to normal human subjects (2–4). It is possible that uptake of ^{57}Co B_{12} from binders by liver is an important factor in plasma clearance of the vitamin in human beings.

In an attempt to correlate known properties of binders with uptake by rat liver it is suggested that some property related to electrophoretic mobility of TCII and MPPB may be a factor in the uptake. Molecular size does not appear to be related to uptake, however, since MPPB is similar in weight to TCI, but sugnificantly larger than TCII.

It is accepted that initial rate of entry of a substrate through a membrane system depends on its concentration (10). Although total amount of sample was prepared from 30 ml of serum in each perfusion study, concentration of pure binder could not be ascertained, since a direct analytical method is not available.

It is noteworthy that free ^{57}Co B_{12} is not recognized for uptake by rat liver unless bound to human TCII and MPPB. It can be speculated that human TCII and MPPB carry ^{57}Co B_{12} across the membrane. Evidence for this possibility was obtained in a previous investigation in which vitamin B_{12} associated with hog intrinsic factor related fractions was absorbed by perfused rat livers and transferred to bile (11). When this vitamin B_{12}-containing bile was introduced into the perfusing blood of a second isolated rat liver, vitamin B_{12} was again absorbed by the liver,

indicating that at least some of the factor involved in the uptake of B_{12} by the liver had passed through liver cell membranes and into bile.

Summary. ^{57}Co B_{12}-labeled TCII and MPPB prepared from normal human donors and pernicious anemia patients, exhibited a higher uptake by perfused rat liver than ^{57}Co B_{12}-labeled TCI. This is consistent with faster plasma clearance of radioactivity following intravenous injection of ^{57}Co B_{12} bound to TCII and MPPB in normal human subjects, compared to a slower clearance of TCI B_{12}.

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