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Uptake of Protein Bound Vitamin B_{12} by Canine Organs[1] (38214)

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Transcobalamin II (TC II), a transport protein of vitamin B_{12} (B-12) in the plasma, promotes B-12 uptake by HeLa cells, immature erythrocytes, murine leukemia L1210 cells, and transformed lymphocytes (1–4). Proteins of that group of B-12 binders sometimes called the "R" binders (5) do not enhance B-12 uptake by HeLa cells (1, 5).

Transport proteins *in vivo* do not, however, deliver B-12 to free cells in suspension but across capillary walls to tissue cells organized into structures. In order to study uptake under more physiologic circumstances we introduced a system of isolated, perfused, canine organs (6). When applied to the liver and kidney (6) the results were somewhat surprising. The kidney took up 82 ± 8% of 100 pg of free $^{57}CoB_{12}$ and 48 ± 6% bound to TC II. The liver took up 34 ± 12% of free B-12 and 67 ±15% bound to TC II. B-12 bound to "R" binders was poorly taken up by both organs. These observations were in partial disagreement with studies with isolated cells where almost no free B-12 was taken up.

The present study was an expansion of the measurement of uptake by isolated organs to include spleen and heart and to differentiate between cortical and medullary uptake by the kidney.

Methods. The conditions of perfusion were the same as described previously (6). The spleen was dissected free except for the attachment by the splenic artery and vein while the dog was alive. Lesser vessels such as those coming from the gastric circulation were ligated. The main vessels were cut and a canula was immediately inserted into the splenic artery above the bifurcation. The organ was flushed with ice cold Tyrode's solution. It was then perfused under pressure as described for the kidney (6). The perfusate contained 30% washed, canine erythrocytes as well as leukocytes and platelets but was essentially free of plasma. As soon as respiration ceased but before exsanguination was complete, the chest was entered and the heart removed with great vessels *en bloc*. The base of the aorta was cleared and opened. A canula was placed in the left coronary artery but only rarely could the tie be placed proximal to the branching of the left circumflex artery. The organ was flushed with ice cold Tyrode's solution and the perfusion started. Cardiac contractions ceased during canulation but resumed for a time during perfusion in about ⅓ of the organs. Perfusion was performed under pressure. Rates of flow through the heart and spleen varied but were greater than those through the kidney but less than through the liver (6).

The system of injection of $^{57}CoB_{12}$, the source and specific activity and the counting system have been described (6). The amount injected was always 100 pg in 2 ml either free or bound to a B-12 binding protein. The latter was either contained in whole serum or isolated from other B-12 binding proteins by gel filtration on Sephadex G-200. The injection was always continued over a 45 sec period. The collection of output of the organs encompassed 2 min timed from the start of the injection. Up-

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TABLE I. Percent Uptake of a 100 pg Injection of Free or Bound $^{57}CoB_{12}$ by Canine Spleen and Heart.

	Free B_{12}	Serum bound B_{12}	TC II** bound B_{12}	RBC bound B_{12}	TC I** bound B_{12}
Spleen					
mean %	40	41	50	18	30
±1 SD	±16	±15			
*n**	29	25	6	2	2
Heart					
mean %	49	47	43	20	23
±1 SD	±22	±23			
*n**	18	21	3	7	1

* Number of organs recciving the injection indicated.

** Preparation using complexes of $^{57}CoB_{12}$ plus a binding protein were separated from other B-12 binders by gel filtration but only semi-purified in respect to toal plasma proteins.

take was calculated by the difference between the amount of ^{57}Co injected and the output of ^{57}Co as collected within the 2 min. The system measured the amount of free or bound B-12 removed by a single passage. An individual perfused organ received 2–5 injections of $^{57}CoB_{12}$ in various forms and in this way served as its own control.

Perfusion and injection were as before (6) for the study of uptake by the renal medulla and cortex. The perfusion was discontinued after a single injection of $^{57}CoB_{12}$ and the usual 2 min collection of output. Cortex and medulla were then separated by dissection, weighed and the content of $^{57}CoB_{12}$ measured.

Results. The uptake of B-12 by spleen and heart is given in Table I. Since B-12 added to canine serum is virtually all bound to TC II (7), serum-bound B-12 was equivalent to TC II-B-12 in these studies. The erythrocyte binder is a member of the "R" group, as is TC I which is found in all human sera and to a much lesser extent in canine sera (7). Two-fifths to one-half of the injected B-12 was taken up by either organ whether injected in the free state or bound to TC II. Expressed as mean pg B-12 uptake per gram of perfused tissue, the spleen took up 0.45 pg as free B-12 and 0.54 pg bound to serum. The heart took up 0.51 pg as free B-12 and 0.59 pg as serum bound. Vitamin B-12 bound to an "R" binder, either erythrocyte 'R' or TC I, was not well taken up by either organ. There was considerable variation in uptake between individual hearts or spleens; however, each individual organ took up highly consistent amounts of repeated injections of any one form of $^{57}CoB_{12}$.

The distribution of $^{57}CoB_{12}$ taken up by the kidney is shown in Table II. Serum (TC II) bound B-12 was taken up almost equally by cortex and medulla. The cortex took up twice as much B-12 injected in the free form as the medulla. As noted previously (6) free B-12 taken up by the kidney remained there as evaluated by external counting of the organs, by counting the minced organ, and by searching for $^{57}CoB_{12}$ in the urine and the effluent perfusate.

TABLE II. Regional Distribution of Renal Uptake* of 100 pg** $^{57}CoB_{12}$.

Binding of injected B_{12}	Uptake by	
	Medulla	Cortex
Free	1.6	3.4
Serum (TC II)	0.85	0.95

* As mean uptake by the two regions from 5 kidneys expressed as pg of B-12 per gram of wet tissue.

** Previously (6) it was observed that 82 ± 8% of this amount of free B-12 was taken up by the whole kidney.

Discussion. Perfused canine organs took up from one third to more than three-fourths of 100 pg of injected free $^{57}CoB_{12}$ during a single passage. There were consistent differences in uptake between organs. From one half to two thirds of $^{57}CoB_{12}$ bound to TC II was taken up by the same organs. Binding to TC II enhanced uptake of B-12 by the liver but renal uptake of TC II-B-12 was less than that of free B-12. TC II did not enhance uptake by heart and spleen. $^{57}CoB_{12}$ bound to binders other than TC II was poorly taken up.

The observations reported here differ from those made with systems of isolated cells where uptake of free B-12 is nil and is enhanced by binding B-12 to TC II. Possibly the differences reflect the ability of canine liver, kidney, heart and spleen to produce TC II (8) which could bind to the passing free B-12 and promote its uptake. The kidney which took up the largest amount of free B-12 had the greatest capacity to produce TC II (6, 8). An alternative explanation would be that tissues vary in their dependence upon TC II as a transport protein.

Whatever the mechanism, both free B-12 and that bound to TC II were taken up rapidly by the isolated organs. Moreover, TC II could be attached to injected free B-12 as it passed once through the organs and some of the TC II-B-12 complex could be recovered in the effluent (6, 8). It follows, then, that the cell receptors for free B-12 and for TC II-B-12 and the location of the TC II of the organ must be directly exposed to the circulating blood. One structure meeting these conditions and to be found in all of the organs studied is the vascular endothelium. It could be significant that the tissue most active in its uptake of B-12, either free or bound to TC II, was the renal cortex which is rich in capillaries.

Summary. The isolated, perfused, canine spleen and heart took up 40–50% of vitamin B-12 labeled with ^{57}Co and bound to canine transcobalamin II (TC II). Other proteins that bind vitamin B-12 depressed uptake. In contrast to previous experience with the liver, binding to TC II did not enhance uptake as compared to uptake of free $^{57}CoB_{12}$. In contrast to previous experience with the kidney, very high uptake of free B-12 was not observed. The transport of B-12 into whole organs differed in some aspects from transport into suspension of cells. Possibly the ability of the organs to produce transport proteins accounted for these differences.

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