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Release of Transcobalamin II by Canine Organs (38717)

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The sites of biosynthesis, distribution, and storage of transcobalamin II (TC II) have never been fully explored. Tan and Hansen (1) observed production by the liver in mice. Sonneborn *et al.* (2) demonstrated that in the dog, the liver was not the only place of synthesis. England *et al.* (3) observed output of TC II from the perfused liver of the rat. We previously detected TC II coming from the isolated kidney and liver of the dog during studies of protein-mediated uptake of vitamin B_{12} (B-12) (4). The present work is an extension of our previous approach to include four organs, a more direct approach, and confirmation of the identity of the TC II by multiple techniques.

The study has been published in abstract form (5).

Materials and Methods. The system of perfusion of the isolated organs was that described previously for canine liver and kidney (4). The spleen was perfused through the main splenic artery and the heart through the left coronary artery. The total of seven organs studied were taken from four dogs.

Prior to perfusion each organ was flushed with cold Tyrode's solution until the wash was clear and the organ chilled (4). Each organ was perfused 30–60 min at 37° before study in order to further wash out traces of blood and to restore aerobic metabolism. The system was drained and the perfusate analyzed for B-12 binding proteins; it was replaced with fresh perfusate which was allowed to circulate for 2 hr. Ten- to twelve-milliliter samples were taken for analysis at intervals; fresh perfusate was added to compensate for the samples removed. In some studies 100 pg of nonradioactive (cold) B-12 was introduced into the organ after the 60-min sampling. For five studies the perfusate consisted of washed canine erythrocytes suspended in an artificial plasma as described (4). For two studies, one liver and one kidney, oxygen was carried by a fluorocarbon emulsion[1] which consisted of 10% perfluorodecalin in 5% pluronic F68 (6). The same ions and glucose used in the regular perfusate (4) were added but not polyvinylpyrrolidone. In a preliminary study it had been found that gas exchange was adequate with the emulsion and the organs were well maintained.

Two milliliters of the aliquots of the supernate from each sample of perfusate were labeled with 2000 pg (2.0 ng) of $^{57}CoB_{12}$, (sp act 16 μCi/μg), dialyzed, and fractionated by gel filtration on Sephadex G-200. Reaction with rabbit anti-canine TC II, uptake by HeLa cells, or retention by carboxymethyl (CM) cellulose were used to confirm the identity of TC II. The anti-canine TC II was made by the method used to make anti-human TC II and the reaction with TC II was detected as before (7–8). Canine TC II was found to have the physicochemical properties of human TC II (9) and HeLa cells were shown to take up canine TC II-B-12 but not as effectively as human TC II-B_{12} (10).

Results. Figure 1 shows in the lower part a typical gel filtration of a sample of renal perfusate. The binding protein assumed by its molecular size to be TC II reacted with anti-canine TC II, (Fig. 1, upper).

Figures 2–4 show the amount of TC II-B_{12} in the total perfusate at the times indicated. Note the differences in ordinates among the three figures. In no case was any TC II detected in the perfusate before circulation. In each case the amount of TC II in the reservoir increased with time.

Three transient declines between samplings are unexplained. It should be noted that the TC II recovered could have been a net expression of release and uptake of TC II; both processes could have been taking place

[1] Kindly supplied by Dr. Leland C. Clark, Jr., College of Medicine, University of Cincinnati.

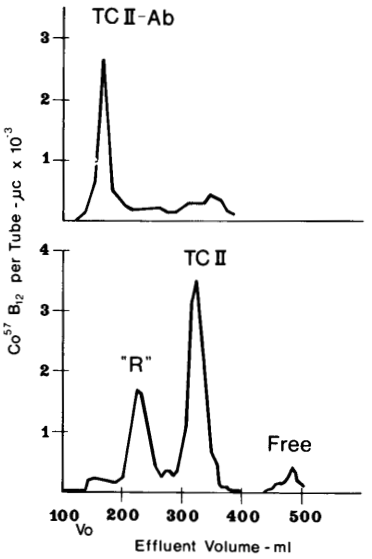

FIG. 1. Lower. Gel filtration of perfusate circulated through the kidney for 120 min and labeled with $^{57}CoB_{12}$ to above capacity. Binding proteins and free B-12 elute characteristically as marked. The amounts in the peaks as pg B-12/2 ml were: "R" binders 843 pg, TC II 1355 pg, and free 120 pg. Upper. The TC II-B_{12} from the lower figure study was concentrated, dialyzed, and combined with 1 ml of rabbit anti-canine TC II. Ninety-seven percent of the TC II-B_{12} reacted with the antibody and the antibody-TC II-B_{12} complex eluted with the void volume.

simultaneously. The studies using an emulsion of fluorocarbon as a perfusate (Fig. 4), where no biological material was introduced into the system clearly showed output of TC II.

The identification of TC II was confirmed by one or more systems of measurement in addition to gel filtration for at least one sample from each organ (Figs. 2–4).

A binder of B-12 corresponding in molecular size to the "R" binders[2] of human blood, body fluids, and cells (10) was observed in the perfusates from all four organs. Binder of this size was found in all perfusing fluid prior to use. Presumably it came from erythrocytes or granulocytes since a similar binder could be observed in preparations of canine blood cells. The amount of "R" binder in the perfusates increased with circulation through organs. There was in-

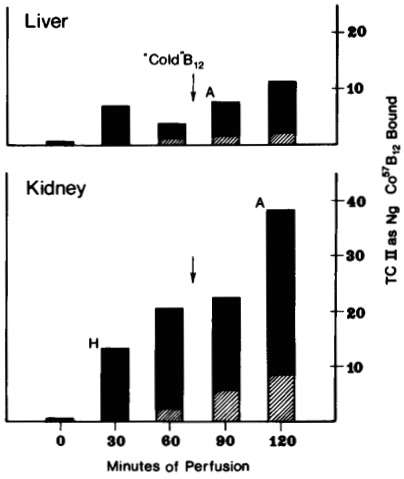

FIG. 2. TC II as detected by gel filtration of samples of labeled perfusate. The full height of each bar represents the total TC II-^{57}Co B_{12} released by that time. The hatched part of the bar represents the amount removed in prior sampling. One hundred picograms of nonradioactive B-12 were injected after the 60 min sample. Symbols indicate which TC II's were checked by a second technique to confirm identity. A reacted with anti-canine TC II. H taken up by HeLa cells. CM retained by CM-cellulose.

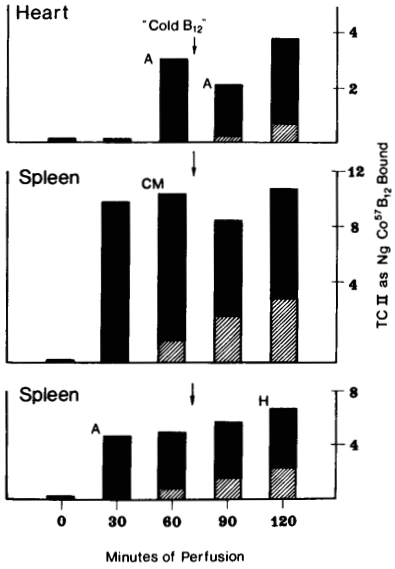

FIG. 3. TC II output of two spleens and one heart. Symbols as in Fig. 2. Note the change in ordinate from Fig. 2.

[2] The "R" type binders of B_{12} constitute a family of isoelectrically heterogenous glycoproteins which are close in molecular size, all reacting to the same antibodies, and clearly distinct from TC II. They are found in many body fluids and cells but their functions are unknown.

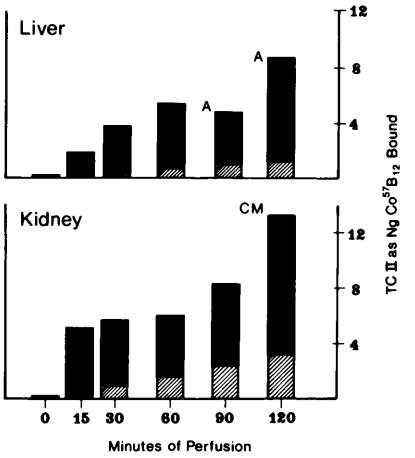

FIG. 4. TC II output of liver and kidney when fluorocarbon was used as a carrier of blood gases instead of erythrocytes. Symbols as in Fig. 2.

creasing hemolysis during perfusion although the amount of "R" binder in the perfusates did not correlate with the measured hemoglobin.

Discussion. TC II, as identified by four independent techniques was observed coming from all four organs. The study does not settle the issue of synthesis and/or storage-release; the term "production" as used here was not considered synonymous with "synthesis" but implied only a bringing forth of TC II. The study was designed to identify the output not to quantitate it. It does not seem likely that the TC II coming from the organs represented simply that from trapped plasma. The total protein was measured in each sample of perfusate and could give an indication of the amount of washout of sequestered plasma. Even by assuming that all of the released total protein came from sequestered plasma and none was synthesized by the organs, the amount of TC II was far greater than could be accounted for by trapped plasma. Moreover, the amount of TC II increased with time and renewal of perfusate. With a washout effect, the amount of released TC II should have declined with washing. Were there merely a release of trapped plasma, the B-12 binding pattern of the output would have been the same for all organs, 1.0–1.5 ng of TC II-B-12/ml of whole plasma and little if any "R" binding (9). Instead, the binding patterns varied consistently among the outputs of the four organs, in some instances the "R" binders predominating. If trapping were the source of TC II, the spleen and liver with their larger mass should have produced more TC II than the smaller kidney but the converse was observed.

The kidney had a perfused mass of about ⅓ that of the liver (4) yet the TC II output was greater (Figs. 2 and 4). The outputs of heart and spleen were also less than renal output. The present findings are in agreement with previous evidence of the liver as one source of TC II (1, 3). We have demonstrated, however, that TC II can be produced by other organs as well. Possibly the sources of the observed outputs of TC II differed among organs. In some cases there may have been storage with release while there was synthesis in others.

The study may also have shown release of "R" type B-12 binders from the organs. The material observed could have come from blood cells although blood cells were absent in the fluorocarbon studies. We believe, however, that studies of the origins of "R" binders by the present approach must await development of techniques suitable for identification of their individual types.

The widespread location of TC II in tissues, however derived, may be important functionally. Organs seem to take up free B-12 better than isolated cells (4, 10, 11) perhaps because they have their own TC II. We have shown previously that when B-12 passes from an organ of high TC II output to one responsive to TC II-B_{12}, uptake is enchanced in the second organ (4). Binding to an "R" binder as B-12 passes through an organ, however, seems to depress B-12 uptake by the next organ in line (4).

Summary. The output of vitamin B_{12} transport proteins by canine tissues was determined from isolated, perfused organs. Transcobalamin II as identified by four techniques was released by the spleen, heart, liver, and kidney. When related to perfused weight, the greatest output occurred from the kidney.

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