Transcellular Transport of Vitamin B$_{12}$ in LLC-PK1 Renal Proximal Tubule Cells

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Abstract. The transcobalamin-vitamin B$_{12}$ complex is responsible for the transport of B$_{12}$ from plasma and into the tissues. The complex is filtered in the renal glomeruli and is a high-affinity ligand for the endocytic receptor megalin expressed in the proximal tubule. This study shows by the use of the proximal tubule LLC-PK1 cell line that transcobalamin-B$_{12}$ is internalized by megalin-mediated endocytosis. After endocytosis and accumulation in endosomes, transcobalamin is degraded and the B$_{12}$ molecule is released from the cells in complex with newly synthesized proteins. The release is polarized in such a way that vitamin in the apical medium is bound to proteins with the size of haptocorrin, whereas the B$_{12}$ released at the basolateral side is complexed to two different proteins with the sizes of transcobalamin and haptocorrin. Furthermore, transcobalamin mRNA was identified by reverse transcription-PCR in LLC-PK1 cells and human and pig kidney, whereas haptocorrin mRNA was identified only in LLC-PK1 cells. The results strongly suggest that megalin located in the proximal tubule cells is important for receptor-mediated tubular reabsorption followed by transcellular transport and release of vitamin B$_{12}$ complexed to newly synthesized carrier proteins. This mechanism is likely to play a significant role in the maintenance of B$_{12}$ homeostasis by returning filtered B$_{12}$ to the pool of circulating vitamin.

Cellular uptake of vitamin B$_{12}$ is mediated by endocytosis of B$_{12}$ bound to the plasma transport protein transcobalamin. This 43-kD protein carries approximately 30% of plasma B$_{12}$, whereas the remaining 70% is bound to haptocorrin (1–3), a protein of unknown function. Intravenously injected transcobalamin-B$_{12}$ has been identified in the endocytic compartment of the proximal tubule, indicating glomerular filtration and tubular uptake of the complex (4). The filtered amount in humans has been estimated to be approximately 1.1 nmol daily (5). Furthermore, B$_{12}$ accumulates in the kidney of normal and B$_{12}$-loaded rats (6–9) especially concentrating in the convoluted proximal tubule (10).

Transcobalamin-B$_{12}$ is a high-affinity ligand for the multiligand endocytic receptor megalin. Furthermore, this receptor is able to mediate the endocytosis of transcobalamin-B$_{12}$, suggesting that the tubular reabsorption involves megalin (4). Megalin is located in the microvilli and endocytic compartments in all three segments of the proximal tubule, although with different distribution patterns (11). It belongs to the low-density lipoprotein receptor family and binds a number of heterogeneous ligands. Among the ligands are retinol-binding protein, vitamin D–binding protein, and transcobalamin-B$_{12}$, indicating a general role for megalin in the uptake of vitamins (12–15). Tubular uptake of transcobalamin-vitamin B$_{12}$ must be followed by basolateral secretion of the vitamin to return this to the circulating pool of B$_{12}$. High levels of transcobalamin mRNA are present in rat and human kidney homogenates, suggesting that B$_{12}$ is transferred to newly synthesized transcobalamin in the kidney (16).

The mechanisms involved in tubular uptake and processing of vitamin B$_{12}$ have not been elucidated fully. To establish a model for studying this, we examined uptake and secretion of transcobalamin and B$_{12}$ in proximal tubule LLC-PK1 cells. We showed that the transcobalamin-B$_{12}$ complex is endocytosed by megalin in LLC-PK1 cells. The vitamin is released from the cells and associated with B$_{12}$-binding proteins that behave similarly to haptocorrin and transcobalamin. In the apical medium, only haptocorrin-like proteins are present, whereas both transcobalamin- and haptocorrin-like proteins are secreted into the basolateral medium. Furthermore, mRNA for transcobalamin is detected by reverse transcription-PCR (RT-PCR) in human kidney, kidney cortex from pig, and LLC-PK1 cells, whereas haptocorrin mRNA is detected only in LLC-PK1 cells. This suggests that proximal tubule cells are capable of transcellular transport of vitamin B$_{12}$ by a process that involves the binding of released vitamin to newly synthesized B$_{12}$-binding proteins.

Materials and Methods

Cell Culture

Experiments were conducted with the proximal tubulus cell line LLC-PK1, generously provided by Dr. J. Øivind Moskaug (University of Oslo, Oslo, Norway). The cells were grown in Dulbecco’s minimal essential medium (DMEM; BIO Whittaker, Vervier, Belgium; cat. no. 12-614 F) supplemented with 10% fetal calf serum (FCS; Biological
Industries, Kibutz Beit Haemek, Israel; lot. no. 055541), 2 mM L-glutamine (BIO Whittaker; cat. no. 17-605 E), 50 U/ml penicillin, and 50 µg/ml streptomycin (BIO Whittaker; cat. no. 17-603 E). Incubation of the cells was carried out at 37°C in 5% CO₂/95% air. Experiments were performed with confluent monolayers cultured on Transwell inserts (Costar, Acton, MA; cat. no. 3450; diameter, 24 mm; pore size, 0.4 µm) or in 24-well multidishes (Nunc, Roskilde, Denmark; cat. no. 143982; diameter, 16 mm). Trypsination (Trypsin Versene; BIO Whittaker; cat. no. 17-161 E) or phosphate-buffered saline (PBS)/0.5 mM ethylenediaminetetraacetate treatment induced detachment of the monolayers from multidishes and Transwell inserts, respectively.

**125I-Transcobalamin-B₁₂ Uptake by LLC-PK1 Cells**

LLC-PK1 cells were cultured in 24-well multidishes for 3 to 4 d. The confluent monolayers were washed once with serum-free medium (DMEM + glutamine [see above] and 5 × 10⁻⁸ M dexamethasone) containing 0.1% bovine serum albumin (referred to as “medium”). Medium was added to 125I-transcobalamin-B₁₂ or 125I-transcobalamin-B₁₂ + 1 µM unlabelled receptor-associated protein (RAP (17); recombinant RAP was kindly provided by Morten Nielsen, Department of Medical Biochemistry, University of Aarhus, Aarhus, Denmark) and transferred to the wells. Rabbit transcobalamin-B₁₂ was iodinated with the use of the chloramine-T method (18) to a specific activity of 5.9 × 10⁶ cpm/µg (2.6 × 10⁵ cpm/pmol). To each well was added approximately 100,000 cpm (0.4 pmol) dissolved in 400 µL of medium. Incubation was carried out at 37°C in 5% CO₂/95% air for 1, 2, and 3 h. Incubation was stopped by aspiration of the medium, washing of the cells in PBS, and addition of 200 µL of Trypsin Versene. The samples were counted in a γ counter (Cobra 5003, Packard, Meriden, CT).

Degradation was measured by precipitation of the medium by 10% TCA and the TCA-soluble fraction, defined as the degraded fraction. To correct for liberation per se of iodine from 125I-transcobalamin-B₁₂ during the experiment, we incubated the medium in control wells without cells. Degradation was calculated as the TCA-soluble fraction in the incubation medium minus the TCA-soluble fraction in the medium from control wells and divided by the total amount of added tracer. The medium from control wells contained a TCA-soluble fraction of approximately 10 to 14%. Degradation was expressed as a percentage. Cell-associated 125I-transcobalamin-B₁₂ was calculated as bound and internalized tracer divided by the total amount of tracer added in each well.

**Visualization of Transcobalamin-B₁₂ Uptake in LLC-PK1 Cells**

57CoB₁₂ (specific activity was 3.3 × 10⁵ cpm/pmol) labeled transcobalamin was prepared from partly purified human seminal transcobalamin (19) by incubating approximately 3.7 pmol of unlabelled transcobalamin with 3.7 pmol of 57Co-B₁₂ (Amersham, Horsholm, Denmark). The fraction of 57Co-B₁₂ that bound transcobalamin was 85%.

In the experiments with iodinated transcobalamin, the protein originated from rabbit, whereas in the experiments with labeled B₁₂, transcobalamin originated from man. The reason for these species differences is purely practical and is unlikely to convey any significance as transcobalamin from different species binds cobalamin and its receptors with comparable affinity. After growth for 4 d on Transwell inserts, the monolayers were incubated with transcobalamin-57CoB₁₂ (60,000 cpm/ml, 0.18 pmol/ml) for 24 h to obtain a satisfactory level of grains for autoradiography or 125I-transcobalamin-B₁₂ (133,000 cpm/ml, 0.5 pmol/ml) for 2 h at 37°C because of the fast metabolism of transcobalamin. After incubation, the medium was aspirated and the cells were washed twice in PBS. The fixative, which consisted of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 3.7% sucrose (pH 7.2), then was added at both sides of the cells, and fixation continued overnight at 4°C. Subsequently, the cells were processed as follows: the filters were cut out of the inserts, washed 2× in 0.1 M cacodylate buffer (pH 7.2), postfixed in 1% osmium in the same buffer for 30 min, washed as above followed by 3× wash in maleate buffer (pH 5.2). Then en bloc staining with 0.5% uranylacetate in maleate buffer was accomplished for 1 h. The cells were washed again, dehydrated in graded alcohols, transferred to propyleneoxide 3 × 10 min, and finally infiltrated with Epon (TAAB, Berkshire, England).

Sections with a thickness of 50 to 70 nm were cut for electron microscopy and autoradiography with a Reichert Jung UltraCut S microtome (Leica, Vienna, Austria). Ultrastuctural analysis was performed with a Phillips 100 CM electron microscope, and autoradiography was conducted as described by Christensen et al. (20), modified from Maunsbach (21).

**Secretion Studies of Transcobalamin-57CoB₁₂ and Iodinated Transcobalamin-B₁₂**

The LLC-PK1 cells were cultured on Transwell inserts and used after 3 to 4 d when they had reached confluence. The cells were washed in warm DMEM. Medium containing 2 mM L-glutamine, 10⁻⁶ M dexamethasone and transcobalamin-57CoB₁₂ (1.5 ml, app. 60,000 cpm/ml, 0.18 pmol/ml) or 125I-transcobalamin-B₁₂ (133,000 cpm/ml, 0.5 pmol/ml) then was added to the apical side of the cells (see above for preparation of tracers). The same medium that was used on the apical side was added to the basal side of the cells except that the tracer was omitted (2.5 ml). The cells were incubated for 24 h with transcobalamin-57CoB₁₂ and 2 h with 125I-transcobalamin-B₁₂ at 37°C in 5% CO₂/95% air.

The media then were aspirated, the cells were washed, and DMEM + L-glutamine + penicillin/streptomycin were added for up to 48 h of incubation. At different time points, the medium was removed and the cells were washed and counted. After 24 h of chase in the transcobalamin-57CoB₁₂ study, the media were counted and concentrated in Centricon 10 tubes (Millipore, Bedford, MA). The concentrated media (approximately 100 µl) were examined by gel filtration on a SMART system (Pharmacia a/s, Hørsholm, Denmark) with the use of a Superdex 200 column (Amersham, 10 × 300 mm) and run with 0.1 mol/L Tris, 1 mol/L sodium chloride, 0.02% sodium azide, 0.05% human albumin (pH 8.0). The flow rate was 400 µl/min, and optical density at 280 nm was monitored continuously. Fractions of 400 µl were collected and counted in an automatic γ counter (Wallac 1470 Wizard, Turku, Finland). The column was calibrated with the use of Dextran blue (Sigma, St. Louis, MO), void volume and 22 sodium (Amersham), total volume. Normal serum saturated with 57Co-cobalamin was used as marker for elution of transcobalamin and haptocorrin.

**RT-PCR**

Total RNA was purified from kidney cortex and LLC-PK1 cells by the use of The Purescript kit (Genta, Minneapolis, MN). The purity and concentration thereafter was determined by optical density at wavelengths of 260 nm and 280 nm. Purified RNA (0.1 µg) was incubated in a reverse transcriptase reaction mixture containing 6.25 mM MgCl₂, 50 mM KCl, 1 U/µl RNase inhibitor, 1 mM deoxyribonucleoside triphosphates (dATP, dTTP, dGTP, dCTP), 2.5 µM of a 16mer d(T) oligonucleotide primer, and 2.5 U/µl reverse transcriptase (10 mM Tris-HCl [pH 8.3]; all reagents were from Perkin Elmer,
PCR

Because only the human and bovine sequence for transcobalamin were known, we applied primers with sequences that were homologous for these two species (22,23). After optimization of an RT-PCR with this primer set and pig RNA, the PCR product from pig was sequenced, and on the basis of this sequence, new pig specific primers were designed and applied.

The reverse transcription reaction (2.5 μl) was added to PCR reaction mixtures consisting of 1.5 mM MgCl₂, 50 mM KCl, 0.2 mM deoxyribonucleoside triphosphates (dATP, dTTP, dGTP, dCTP), 1.25 U Taq polymerase (Pharmacia), 5 pmol of sense and antisense primers, and 10 mM Tris-HCl (pH 9.0; all reagents were from Perkin Elmer). After the PCR reaction, the products were visualized by running a 1.5% agarose gel stained with ethidium bromide (0.5 μg/ml).

PCR Programs. Human Transcobalamin. Denaturation was conducted for 3 min at 94°C and followed by 35 cycles of PCR, 60 s at 94°C, 30 s at 68°C, and 90 s at 72°C; after cycling, the reaction ended with 7 min at 72°C. Human transcobalamin primers had the following sequences: sense 5′ TACCTGCACAGCCTCAAGCT 3′ and antisense 5′ GGGTGCTGTAGACATTCCC 3′. They were located at position 251-270 and at position 777-758 in the human transcobalamin sequence respectively (22).

Pig Transcobalamin. The same as above, except only 27 cycles were performed; the primer concentration was 5 pmol, and the annealing temperature was 62°C. Pig-specific primers had the following sequences: sense 5′ CCCTCTATCTGCTCGCTC 3′ and antisense 5′ GGAGTCTCTCAGTTGAGCA 3′. They were located at position 354-372 and at 633-615 (numbers correspond to the human sequence).

Pig Haptocorrin. As above but with annealing temperature of 58°C, primer concentration of 10 pmol, and 35 cycles. Primers were as follows: sense 5′ TCTTGACCTGTGGTAAGAGGA 3′ and antisense 5′ TCTCCGTCATGGTGAAACCA 3′. Their positions in the haptocorrin sequence were 582-601 and 1108-1089, respectively (24).

Pig Actin. The same basic program as above with annealing temperature at 60°C, primer concentration of 10 pmol, and 30 cycles. Primers were as follows: sense 5′ GGAGTCTCACAAGCAGGAGT 3′ and antisense 5′ GCAGCATGAAACAGATGGCT 3′. Their positions were 4328-4347 and 4637-4619 in the pig actin gene (25).

DNA Sequencing

To make pig-specific transcobalamin primers and to ensure that the PCR products observed originated from transcobalamin and haptocorrin, we sequenced the PCR products. PCR reactions were performed as described above, and the products were cut out of an agarose gel, frozen for 15 min at −20°C in a Costar Spin-X column (cat no. 8160), and extracted from the gel by centrifugation at 14,000 × g for 15 min. The PCR products were precipitated by the addition of 1/10 vol of 4 M NaCl and 2.5 vol of 96% EtOH and placed for 30 min at −20°C. This was followed by centrifugation for 15 min at 14,000 × g. The pellet was washed in 70% EtOH and centrifuged again as above and finally resuspended in H₂O. The PCR product was then mixed with Big Dye Terminator mix (Perkin Elmer), and either the sense or the antisense primer was added. The reactions were run on the following program for 27 cycles: 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. The reactions were precipitated with ethanol as described above and then solubilized in template suppression reagent (Perkin Elmer). Before sequencing, the DNA was denatured at 95°C for 2 min and immediately placed on ice. Sequencing reactions were analyzed on an ABI PRISM 310 Genetic Analyzer (Perkin Elmer).

**Results**

**Uptake of Transcobalamin-B₁₂ in LLC-PK1 Cells**

The uptake of ¹²⁵I-transcobalamin-B₁₂ as well as transcobalamin-⁵⁷CoB₁₂ was visualized by autoradiography (Figure 1). After 2 h, the ¹²⁵I-transcobalamin was present mainly in the endocytotic apparatus. After 24 h, ⁵⁷Co-B₁₂ was identified in the endocytotic apparatus as well as in the cytoplasm and mitochondria.

Internalization and degradation of ¹²⁵I-transcobalamin-B₁₂ reached 10% of total added tracer after 3 h of incubation (Figure 2). In the presence of 1 μM RAP, a protein known to inhibit the binding between megalin and transcobalamin, the degradation was totally abolished and the uptake was inhibited by approximately 70 to 75% at all time points (Figure 2).

The results show that transcobalamin-B₁₂ is internalized by a RAP-sensitive receptor. After endocytosis, the protein part of the complex is degraded, most likely in lysosomes, and the vitamin is transferred to the cytoplasm and mitochondria.

**Cellular Release of Internalized Transcobalamin-B₁₂**

To study the processing of internalized cobalamin, we incubated the LLPC-K1 cells for either 2 h with iodinated transcobalamin or 24 h with transcobalamin-⁵⁷CoB₁₂, and the release of labeled protein or cobalamin was estimated during the next 48 h. After 48 h of chase, most of the transcobalamin that had been internalized during the pulse was degraded and released from the cells, whereas approximately 60% of internalized ⁵⁷CoB₁₂ was associated with the cells (Figure 3). Twenty to 25% of total ⁵⁷CoB₁₂ was released into the apical medium, compared with 8 to 12% into the basal medium. The majority of the released vitamin was associated with protein (80 to 98%). The nature of the B₁₂-binding proteins from samples recovered after 24 h of chase was analyzed by gel filtration. Proteins that eluted similar to serum haptocorrin were identified in both the apical and the basal media, whereas proteins that eluted similar to serum transcobalamin were observed only in the basal medium (Figure 4).

**Identification of mRNA for Transcobalamin and Haptocorrin**

To examine *de novo* synthesis of transcobalamin and haptocorrin in the LLC-PK1 proximal tubule cells and in proximal tubule cells in *vivo*, we developed porcine-specific RT-PCR for the two proteins. We initially amplified approximately 526 bp (human size) porcine transcobalamin with the use of porcine kidney cortex RNA and primers that covered homologous sequences for human and bovine transcobalamin. The RT-PCR product was sequenced, and in comparing 357 bp, we found 84.4% homology to human and 86.3% to cow transcobalamin (Figure 5). Several amino acids, which are conserved in transcobalamin, haptocorrin, and intrinsic factor from different species (23), also were conserved in pig. To optimize the
Figure 1. (A) Electron microscope autoradiography on sections of LLC-PK1 cells incubated with $^{125}$I-transcobalamin-B$_{12}$ for 2 h. Grains are observed over the endocytotic compartments. (B) Electron microscope autoradiography on sections of LLC-PK1 cells incubated with transcobalamin-$^{57}$CoB$_{12}$ for 24 h. Grains are observed over endosomes (E) and mitochondria (M) and in the cytoplasm (arrow). E, endosomes; L, lysosomes; MV, microvilli. Magnifications: x34,000 in A, left; x35,000 in A, upper right; x46,000 in A, lower right; x36,425 in B; x32,550 in B, inset.
mRNA analyses, we chose a new set of primers specific for pig transcobalamin. With the use of this primer set and the primer set specific for human, transcobalamin was detected both in the LLC-PK1 cells and in kidney from human and kidney cortex from pig (Figure 6A). Haptocorrin mRNA was identified in LLC-PK1 cells but not in adult porcine kidney cortex (Figure 6B). The use of actin primers resulted in approximately the same band intensity in pig cortex and LLC-PK1 cells (not shown), indicating that the observed difference in haptocorrin mRNA levels was not due to differences in RNA integrity or total RNA concentration. The LLC-PK1 cell line originates from a juvenile pig, and the expression of haptocorrin mRNA therefore was examined in kidney cortex from fetal pig. At RNA concentrations above 3 μg/μl, a weak expression of haptocorrin mRNA was detected in fetal cortex by RT-PCR but still not in cortex from adult pig (not shown).

Discussion

The kidney has been suggested to be important for the maintenance of B12 homeostasis of the organism and thereby for its metabolic associates, i.e., increased homocysteine levels in nephropathic conditions (5,6). The transcobalamin-B12 complex is filtered in the kidney glomeruli and in resemblance with 25-(OH) vitamin D3 in complex with vitamin D–binding protein, tubular reabsorption is important to prevent urinary loss of B12 (4,14). Reabsorbed vitamin must be transported through the tubular cell to reenter the circulating pool of B12.

The present study shows that transcobalamin-vitamin B12 is internalized in proximal tubule LLC-PK1 cells by endocytosis, which can be inhibited by RAP, suggesting the involvement of megalin. The internalized B12 is released by basal secretion coupled to transcobalamin synthesized in the proximal tubular cells. Finally, our data suggest that haptocorrin is synthesized and secreted bidirectionally in the LLC-PK1 cell line.

The proximal tubule LLC-PK1 cell line has been characterized previously with regard to morphology, megalin expression, and activity (26). The RAP-inhibitable uptake and degradation of transcobalamin-B12 in the LLC-PK1 cell line...
supports its role as a suitable in vitro model for megalin-mediated endocytosis in the kidney proximal tubule. Uptake and degradation of transcobalamin have been studied previously in primary culture of isolated kidney cells and in a rat yolk sac carcinoma cell line, as well as in proximal tubules of rats that had received a microinjection of transcobalamin-\(^{57}\)CoB\(_{12}\) (4,5). Inhibition of uptake by RAP has been demonstrated in both proximal tubule and yolk sac cells (4). RAP is a specific inhibitor of ligand binding to megalin in the proximal tubule (27), and inhibition by RAP strongly supports the involvement of megalin in transcobalamin-\(^{57}\)CoB\(_{12}\) binding and uptake. Using OK cells, Ramanujam et al. (28) showed uptake of B\(_{12}\) in complex with the intrinsic factor, but no uptake was seen when B\(_{12}\) was complexed to other B\(_{12}\) binding proteins. However, because intrinsic factor is not present in any significant amounts in plasma, it is not likely to be involved in renal tubular B\(_{12}\) reabsorption.

Our data show degradation of endocytosed transcobalamin and slow release of B\(_{12}\) from the LLC-PK1 cells. The released B\(_{12}\) recovered from the medium was bound to proteins. In the

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**Figure 4.** Size chromatography of concentrated apical and basal media recovered from LLC-PK1 monolayers incubated for 24 h with transcobalamin-\(^{57}\)CoB\(_{12}\) followed by 24 h of chase. The figures show the elution profile of the proteins associated with \(^{57}\)CoB\(_{12}\) secreted during the chase period (thick line) and the protein profile in the sample (thin line). (A) Normal plasma incubated with \(^{57}\)CoB\(_{12}\). The first peak corresponds to haptocorrin, the second peak corresponds to transcobalamin, and the third peak is free \(^{57}\)CoB\(_{12}\) (thick line). (B) Apical medium. The apically secreted \(^{57}\)CoB\(_{12}\) is associated with proteins with the size of haptocorrin (thick line). (C) Basal medium. The \(^{57}\)CoB\(_{12}\)-associated proteins in the basal medium are of sizes corresponding to both transcobalamin and haptocorrin (thick line).
apical medium, the B_{12}-binding proteins were of the size of haptocorrin, whereas basally it was complexed to proteins with the sizes of both transcobalamin and haptocorrin. Polarized secretion of B_{12}-binding proteins from OK proximal tubule cells also was demonstrated by Ramanujam et al. (29). However, the distribution was different from the present results. Transcobalamin was identified in both the apical and the basal media, whereas haptocorrin was demonstrated in the apical medium only. Different methods were used for identification of the secreted proteins in the two studies. Ramanujam et al. used metabolic labeling and immunoprecipitation to identify the proteins, whereas we identified the proteins by their binding ability of \(^{57}\text{CoB}_{12}\) and size. Both apo- and holoproteins were detected in the study of Ramanujam et al., whereas only holoproteins were identified in our study. It is therefore possible that fragments of transcobalamin that were not able to bind B_{12} were identified in the work of Ramanujam et al. However, both cell types release transcobalamin into the basal medium, and in our study, associated B_{12} is demonstrated. This basal secretion of the vitamin coupled to transcobalamin is the important factor for recirculation of cobalamin by the kidney.

Secretion of transcobalamin also has been reported from perfused dog kidneys (30).

The identification of transcobalamin mRNA in LLC-PK1 cells, as well as the finding of slowly released B_{12} associated with transcobalamin in combination with rapid degradation of endocytosed transcobalamin, strongly supports the notion that vitamin B_{12} released from the tubular cells is coupled to newly synthesized transcobalamin.

Haptocorrin mRNA was not identified in adult porcine kidney cortex. The LLC-PK1 cell line is derived from a juvenile porcine kidney (31), and when cortex from fetal pigs was investigated by RT-PCR at RNA concentrations above 3 \(\mu g/\mu l\), we observed haptocorrin mRNA in this tissue, suggesting an expression of haptocorrin restricted to the early life stages of the pig.

The present study establishes a mechanism by which filtered vitamin B_{12} may be internalized and released from the renal tubular cells. It is not known whether this process only prevents the loss of B_{12} in the urine or whether the vitamin is metabolized within the proximal tubule cell as has been reported to be the case for vitamin D (14). The finding by autoradiography that \(^{57}\text{CoB}_{12}\) endocytosed from the apical medium is transported to the mitochondria combined with the slow release of the vitamin suggests the latter. In the mitochondria, cobalamin is converted to 5'-deoxyadenosyl-cobalamin, serving as a cofactor for the methylmalonyl CoA mutase (32). The role of the proximal tubule can be compared with the ileum with regard to absorption and secretion of vitamin B_{12}. In this tissue, it has been shown that even though B_{12} is supplied as 5'-deoxyadenosyl-cobalamin, it is localized in the mitochondria during transcytosis (33). Still, B_{12} supplied as cyanocobalamin is secreted both as 5'-deoxyadenosyl-cobalamin and cyanocobalamin to the blood (34). Thus, in this tissue, it also is not clarified how transcytosis is associated with metabolism of apically endocytosed cobalamin.

In the present study, vitamin B_{12} seems to accumulate in the proximal tubule cell as a result of an uptake, which is faster than the release. The slow release can be explained by the handling time of the vitamin in the cell. This means that besides preservation of the vitamin, the kidney might play a role in the metabolism of B_{12}. Thus, further studies are needed to elucidate whether the mechanism described in the present study is dependent on other factors than glomerular filtering of the vitamin and whether the transcytosis through the proximal tubule cell is important for the metabolism of the vitamin.

Acknowledgments
The authors thank Inger Blenker Kristoffersen, Hanne Sidelmann, Anna Lisa Christensen, Birgit Mortensen, and Alice Willemoes for excellent technical assistance. The work was supported by grants from the Danish Biotechnology Program, the Danish Medical Research Council, the Aarhus University Foundation, and the Novo Nordisk Foundation.
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