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Characteristics of D-galactose transport systems by luminal membrane vesicles from rabbit kidney

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The characteristics of renal transport of D-galactose by luminal membrane vesicles from either whole cortex, pars recta or pars convoluta of rabbit proximal tubule were investigated by a spectrophotometric method using a potential-sensitive carbocyanine dye. Uptake of D-galactose by luminal membrane vesicles prepared from whole cortex was carried out by an Na^+-dependent and electrogenic process. Eadie-Hofstee analysis of saturation-kinetic data suggested the presence of multiple transport systems in vesicles from whole cortex for the uptake of D-galactose. Tubular localization of the transport systems was studied by the use of vesicles derived from pars recta and pars convoluta. In pars recta, Na^+-dependent transport of D-galactose and D-glucose occurred by means of a high-affinity system (half-saturation: D-galactose, 0.15 ± 0.02 mM; D-glucose, 0.13 ± 0.02 mM). These results indicated that the 'carrier' responsible for the uptake of these hexoses does not discriminate between the steric position of the C-4 hydroxyl group of these two isomers. This is further confirmed by competition experiments, which showed that D-galactose and D-glucose are taken up by the same and equal affinity transport system by these vesicle preparations. Uptake of D-galactose and D-glucose by luminal membrane vesicles isolated from pars convoluta was mediated by a low-affinity common transport system (half-saturation: D-galactose, 15 ± 2 mM; D-glucose, 2.5 ± 0.5 mM). These findings strongly suggested that the 'carrier' involved in the transport of monosaccharides in vesicles from pars convoluta is specific for the steric position of the C-4 hydroxyl group of these sugars and presumably interacts only with D-glucose at normal physiological concentration.

Introduction

Despite many attempts to investigate the characteristics of renal transport of sugars by proximal tubule of mammalian kidney, a number of questions concerning the tubular localization and the structural requirements for the reabsorption of these important metabolites have remained unanswered. The opportunity to prepare luminal-membrane vesicles from two different regions of rabbit proximal tubule, namely pars convoluta and pars recta [1,2] has provided exciting new prospects in renal physiology to study at the subcellular level the localization and nature of the transport of various compounds [3–6]. It is now established that the reabsorption of D-glucose along the proximal tubule occurs via two distinct transport systems, with different Na^+/D-glucose coupling ratios in these vesicle preparations: a low-affinity system present in pars convoluta and a high-affinity system localized in luminal-membrane vesicles from pars recta [1,2,7,8]. Whether this is also true in the case of renal reabsorption of other sugars is not clear at present.

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Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

The uptake of D-galactose had been studied earlier in renal cortical slices [9,10]. The results of these studies indicated the presence of a single, relatively low-affinity transport system ($K_m \approx 2$ mM) for D-galactose. The kidney cortex slices have the disadvantage of not allowing discrimination between transport of substances across the basolateral and the luminal membrane. However, the ability to establish an accumulation index greater than 1 between tissue and medium is taken as evidence for active transport (for a review see Ref. 11). Microperfusion experiments carried out by Ullrich [12] indicated that D-galactose is actively transported by rat proximal tubule, but with lower affinity than D-glucose for the reabsorption system.

In the present communication we have studied the characteristics of D-galactose transport system(s) along the rabbit kidney proximal tubule by using luminal-membrane vesicles prepared from pars convoluta and pars recta according to the method recently described from our laboratory. In contrast to previous observations, we have found that the uptake of D-galactose in vesicles from whole cortex is mediated by means of dual electrogenic transport systems, namely a low-affinity system and a high-affinity system. Furthermore, our experiments have revealed that the uptake of D-galactose in vesicles from pars recta is strictly Na^+-dependent and is mediated via a single high-affinity transport system ($K_A \approx 0.15 \pm 0.02$ mM). A low-affinity but Na^+-dependent transport system ($K_A \approx 15 \pm 2$ mM) for D-galactose was found to be localized in vesicles from pars convoluta. Finally, the heterogeneity of D-galactose transport is compared with the localization of D-glucose transport systems.

Methods

Preparation of luminal-membrane vesicles

Luminal-membrane vesicles were isolated from whole cortex, from the pars convoluta and form the pars recta of the proximal tubule of rabbit kidney according to the method previously described [2] and mentioned here only briefly. 'Outer cortical' tissue (cortex corticis) was obtained by taking slices approx. 0.2–0.3 mm thick from the surface of the kidney (containing pars convoluta); strips of 'outer medulla' tissue approx. 1 mm thick (representing predominantly pars recta) were dissected from the outer stripe of the outer medulla, from the same kidneys, and the two preparations were performed in parallel. The purity of membrane preparations from the pars convoluta and the pars recta was examined by electron microscopy [2] and by measuring specific activities of various markers as previously described [13]. The amount of protein was determined by the method of Lowry et al. [14], as modified by Peterson [15], with human serum albumin as standard.

Uptake experiments

Uptake of D-galactose and D-glucose by various vesicle preparations was examined by a spectrophotometric method with potential-sensitive carbocyanine dye as previously described [16], and details of the individual experiments are given in the legends to the figures. Except in experiments performed with ionophores the media used were as follows. Intravesicular medium was 310 mM mannitol and 15 mM Hepes-Tris (pH 7.5), whereas the extravesicular medium was 155 mM NaCl, 103 mM Na_2SO_4, 155 mM sodium D-gluconate or 155 mM KCl. When ionophores were used the media were as follows. Valinomycin (5 μg/mg protein): the vesicles were pre-loaded for 1 h in 103 mM K_2SO_4; the external medium was 103 mM Na_2SO_4. Gramicidin (8 μg/mg protein): the vesicles were pre-loaded and suspended in 103 mM Na_2SO_4. Valinomycin and nigericin (23 μg/mg protein): the vesicles were pre-loaded and suspended in 51.5 mM K_2SO_4/51.5 mM Na_2SO_4. In all these experiments the buffer system in both the intravesicular and the extravesicular media was 15 mM Hepes-Tris (pH 7.5). All solutions used in this study were sterilized before use and all experiments were performed at 20°C.

Materials

Valinomycin, gramicidin, Trizma base, Trizma hydrochloride and Hepes were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Nigericin was supplied by Calbiochem-Behring, La Jolla, CA, U.S.A. D-Galactose was purchased from Fluka Chemical, Switzerland. D-Glucose was obtained from J.T. Baker Chemicals, Deventer, The Netherlands. 3,3'-Diethyloxodicarbocyanine

iodide was a product of Eastman Kodak Co., Rochester, NY, U.S.A. All reagents used in this study were of A.R. grade.

Results

Uptake of D-galactose by luminal-membrane vesicles from whole cortex

Fig. 1 shows the absorbance changes caused by the addition of 5 mM D-galactose to the membrane-dye suspension in the presence of various salt gradients. It appears from the figure that transient uptake ('overshoot') of D-galactose by membrane vesicles occurred only in the presence of Na^+-salt gradients. Furthermore, the magnitude of the 'overshoot' varies with the type of the Na^+-salt anion in the following order: $Cl^- > SO_4^{-2}$ > D-gluconate. Fig. 2A shows the absorbance changes measured at the peak of the 'overshoots' (30 s uptake values) induced by addition of increasing concentrations of D-galactose or D-glucose to suspensions of membrane vesicles and dye. Figs. 2B and C show Eadie-Hofstee analysis of the experimental data. A curvilinear plot was obtained, which indicates the presence of multiple transport systems in vesicles from whole cortex for the uptake of D-galactose and D-glucose. It should be mentioned here that we have also studied the transport of these two hexoses by using the initial rate of uptake (4 s uptake value) instead of measuring the peak value of the 'overshoot'. The approach resulted in more scattered data, but led to the same conclusion.

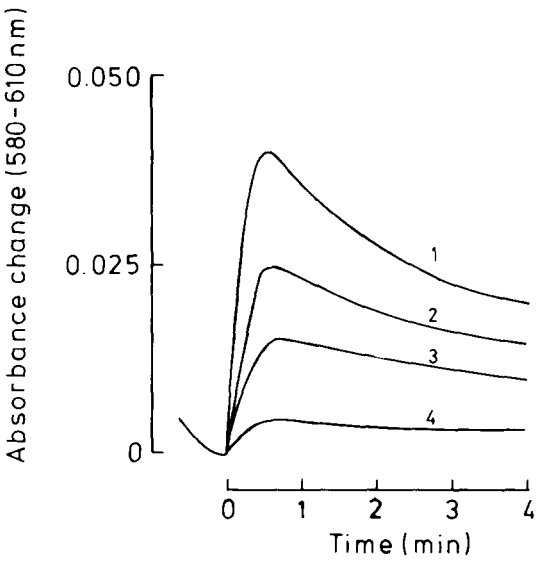

Fig. 1. Uptake of 5 mM D-galactose by luminal membrane vesicles as studied by spectrophotometry; at time zero, 60 μl vesicle suspension (7–9 mg/ml) was added to 2.4 ml dye and 155 mM NaCl (curve labelled 1), 103 mM Na_2SO_4 (2), 155 mM sodium D-gluconate (3) or 155 mM KCl (4). 80 μl D-galactose stock solution was added at the time of maximal hyperpolarization; the dye concentration was 15 μM; the break in the curves, at zero time, indicates addition of solute; all the spectral curves were corrected for the effect of adding 80 μl 15 mM Hepes-Tris buffer alone (the medium of the solute's stock solution); the spectrophotometer was operated in the dual-wavelength mode with 580 nm and 610 nm (reference wavelength).

Uptake of D-galactose and D-glucose by vesicles from pars recta

Fig. 3A compares the Na^+-dependent uptake of

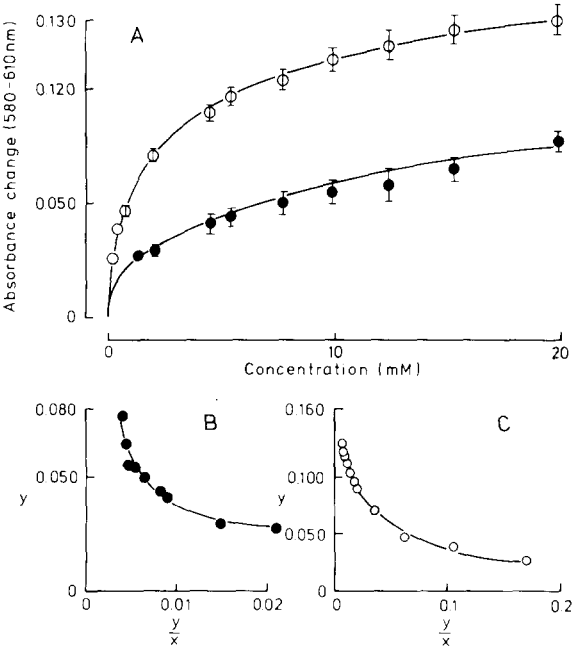

Fig. 2. (A) Uptake of increasing concentrations of D-galactose (●) and D-glucose (○) by luminal membrane vesicles prepared from whole cortex as studied by spectrophotometry. At time zero, 60 μl membrane vesicle suspension (7–9 mg/ml) was added to 2400 μl 155 mM NaCl and dye. 80 μl of a hexose stock solution was added at the time of maximal hyperpolarization. The results are average values of three experiments. (B and C) The same data as in (A) shown in an Eadie-Hofstee plot; D-galactose and D-glucose, respectively. *Y* represents the absorbance change, and *X* stands for the concentrations of the hexoses.

D-galactose and D-glucose by luminal-membrane vesicles derived from pars recta of the same kidneys. The uptake of both hexoses shows a rapid increase at low concentrations in the medium (0.10–1 mM). The rate of uptake of D-galactose and D-glucose is not significantly different and approaches saturation at 5 mM medium concentration, resulting in the same ΔA_{max} value for these compounds. Figs. 3B and C show Lineweaver-Burk analysis of the same data. A straight-line relationship is obtained for both D-galactose and D-glucose, suggesting that the Na^+-dependent uptake of these two hexoses is mediated by means of a single transport system with nearly the same apparent K_A values (i.e., substrate concentration that gives half-maximal uptake): for D-galactose, 0.15 ± 0.02 mM; for D-glucose, 0.13 ± 0.02 mM.

In order to determine that the uptake of D-galactose, like that of D-glucose, is an electrogenic process, a series of experiments was performed by using various ionophores (valinomycin, gramicidin and valinomycin plus nigericin). Fig. 4 shows the influence of the K^+ diffusion potential generated by valinomycin on the uptake of D-galactose by luminal-membrane vesicles from the pars recta. In these experiments the vesicles were preloaded with K_2SO_4 and suspended in Na_2SO_4 [16,17]. This resulted in an outward-directed K^+ gradient and an inward-directed Na^+ gradient. According to expectations, addition of valinomycin under these conditions resulted in approx. 2-fold increase in dye response caused by D-galactose (compare the difference between curves 1 and 2 with curve 3). The effect of gramicidin D on the rate of uptake of D-galactose is shown in Fig. 5. These experiments were carried out with the same initial concentration of Na^+ inside and outside the vesicles in the absence (curve 3) or in the presence of gramicidin D (curves 1 and 2). It is seen that pretreatment of the membrane vesicles with gramicidin D abolished

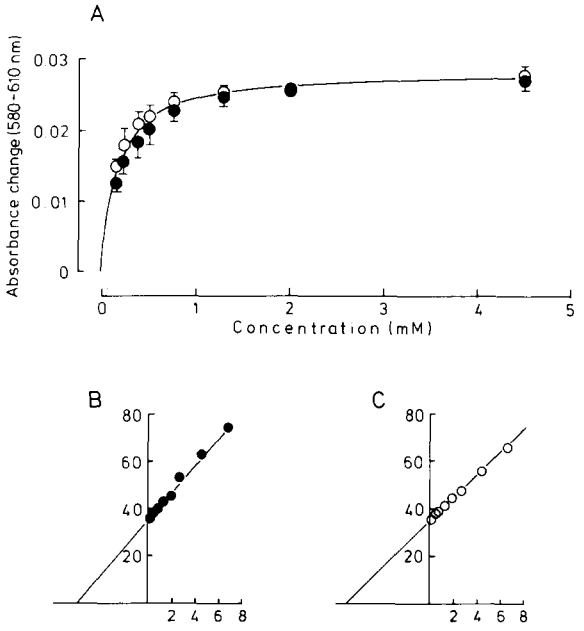

Fig. 3. (A) Uptake of increasing concentrations of D-galactose (●) and -glucose (○) under an NaCl gradient by luminal membrane vesicles prepared from pars recta of the proximal tubule. (B and C) Lineweaver-Burk plot of the results given in (A). The results are average values of three experiments.

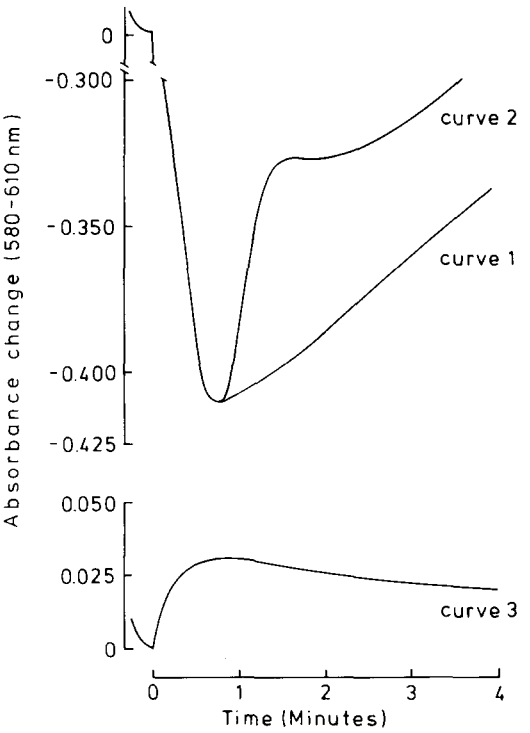

Fig. 4. Effect of valinomycin on the uptake of D-galactose by luminal-membrane vesicles prepared from the pars recta. The membrane vesicles were preloaded for 1 h in 103 mM K_2SO_4, and the spectrophotometric experiments were carried out with an external medium of 103 mM Na_2SO_4. Curve 1 shows addition of 5 μg of valinomycin per mg protein. Curve 2: addition of valinomycin and, about 50 s later, of 10 mM D-galactose. Curve 3: addition of solute in the absence of valinomycin. For further details see legend to Fig. 1.

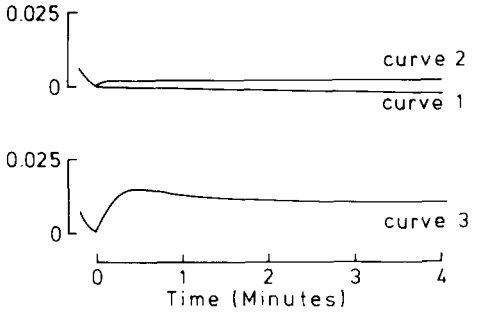

Fig. 5. Effect of gramicidin D on the uptake of D-galactose by luminal-membrane vesicles prepared from pars recta. The membrane vesicles were preloaded and suspended in 103 mM Na_2SO_4. Curve 1 shows the addition of ionophore alone, 38 μg gramicidin D per mg of protein. Curve 2: addition of gramicidin D and, about 30 s later, of 10 mM D-galactose. Curve 3: addition of 10 mM D-galactose. For further details see legend to Fig. 1.

the 'overshoot'. The effect of simultaneous addition of nigericin and valinomycin on the topical response associated with the renal uptake of D-galactose is shown in Fig. 6. These experiments were carried out with the same initial concentration of Na^+ and K^+ inside and outside the vesicles in the absence (curve 3) or in the presence of nigericin and valinomycin (curves 1 and 2). It appears from the figure that pretreatment of the membrane vesicles with these ionophores abolished the optical response. The results of these experiments established that the uptake of D-galactose is a potential-sensitive process.

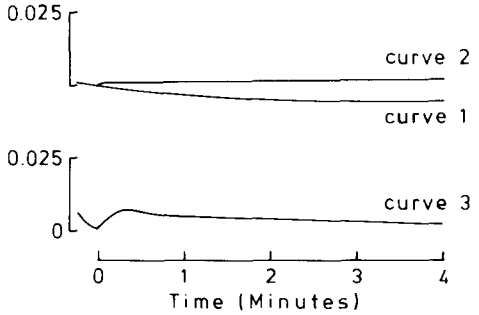

Fig. 6. Effect of valinomycin plus nigericin on the uptake of D-galactose by luminal-membrane vesicles prepared from pars recta. The membrane vesicles were preloaded and suspended in 51.5 mM Na_2SO_4/51.5 mM K_2SO_4. Curve 1 shows the addition of 5 μg valinomycin and 23 μg nigericin per mg protein alone. Curve 2: addition of the ionophores and, about 30 s later, of 10 mM D-galactose. Curve 3: addition of 10 mM D-galactose. For further details see legend to Fig. 1.

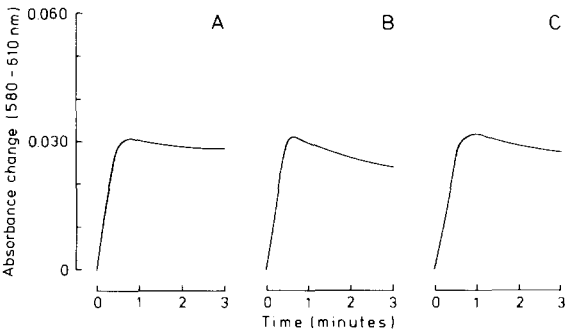

Fig. 7. Absorbance changes caused by addition of 10 mM D-galactose (A), 10 mM D-glucose (B) or 10 mM D-galactose + 10 mM D-glucose to luminal-membrane vesicles prepared from pars recta of the proximal tubules. The intravesicular medium was 310 mM mannitol and 15 mM Hepes-Tris (pH 7.5), whereas the external medium was 155 mM NaCl and 15 mM Hepes-Tris (pH 7.5). For further details see legend to Fig. 1.

The question whether D-galactose and D-glucose are transported in vesicles from pars recta by a common transport system was studied as recently described [3]. Figs. 7A, B, C show the absorbance changes induced either by individual

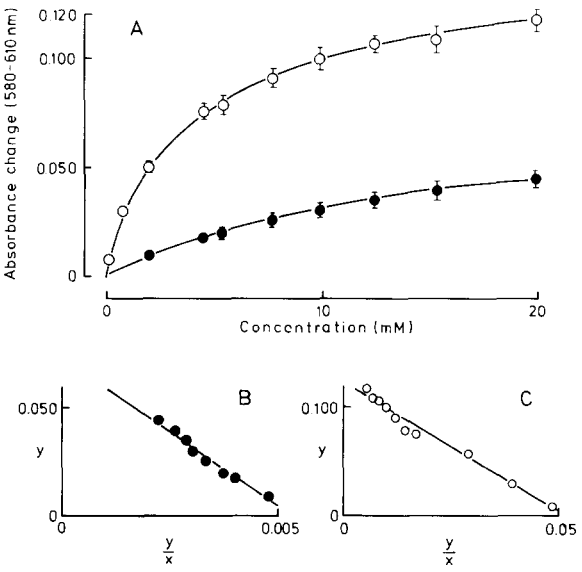

Fig. 8. (A) Uptake of increasing concentrations of D-galactose (●) and D-glucose (○) under an NaCl gradient by luminal membrane vesicles prepared from pars convoluta of the proximal tubule. (B and C) Eadie-Hofstee plots of the data in (A); D-galactose and D-glucose, respectively. y represents the absorbance change, and x stands for the concentrations of the hexoses. The results are average values of three experiments.

application of D-galactose (Fig. 7A) and D-glucose (Fig. 7B) or simultaneous addition of these compounds (Fig. 7C) to vesicle suspension from the pars recta. It can be seen that the magnitude of the maximal optical response induced by simultaneous addition of D-galactose and D-glucose is approximately the same as caused by the addition of D-galactose alone. The results of these experiments showed that D-galactose and D-glucose are reabsorbed by pars recta of proximal tubule via a single common transport system.

Uptake of D-galactose and D-glucose by vesicles from pars convoluta

Figs. 8A, B and C compare the uptake of D-galactose and D-glucose at increasing concentrations of these compounds by luminal-membrane vesicles prepared from pars convoluta of the same kidney cortex. It appears from Fig. 8A that the rate of uptake of D-glucose is much higher as compared to D-galactose. Figs. 8B and C depict Eadie-Hofstee analysis of the data. A straight-line relationship is obtained for both D-galactose and D-glucose, indicating that the Na^+-dependent uptake of these sugars has occurred by means of a single transport system with the following apparent K_A values: for D-galactose, 15 ± 2 mM; for D-glucose, 2.5 ± 0.5 mM.

We have also examined the possibility that the uptake of D-galactose is electrogenic and is mediated by the same system as that of D-glucose in vesicles from pars convoluta. Similar experiments were performed as carried out in the case of vesicles from pars recta. The results of these experiments (not shown) revealed that the uptake of D-galactose is potential-sensitive and occurs via a D-glucose common transport system.

Discussion

Until recently, our knowledge of the structural requirements for the interaction between various monosaccharides and the 'carrier(s)' located at the luminal and basolateral membranes of mammalian kidney proximal tubule was based on multiple indicator dilution techniques [18], microperfusion experiments [12] and the kidney slice technique [19,20]. These studies suggested that a pyranose ring with the confirmational state of D-glucose at C-2, C-3, and C-4 hydroxyl groups are important in the active reabsorption of monosaccharides by the renal tubule. Thus, it has been shown that the renal reabsorption of D-galactose has occurred by means of a single relatively low-affinity ($K_A \approx 2$ mM) common transport system that exists for the transport of D-glucose at the luminal-membrane of renal cells. In contrast to the previous observations, we now present clear evidence for the existence of dual Na^+-dependent electrogenic transport systems for D-galactose which are localized in luminal-membrane vesicles from different regions of the proximal tubule. In the vesicles derived from pars recta, transport of D-galactose and D-glucose was mediated via a common high-affinity transport system. It is of interest to note that both K_A and ΔA_{max} values obtained for D-galactose and D-glucose in these vesicle preparations derived from the same kidneys are nearly the same, indicating that the 'carrier protein' responsible for the reabsorption of these hexoses does not discriminate between the steric position of the C-4 hydroxyl group of the two isomers. On the other hand, the results obtained with the luminal-membrane preparations from pars convoluta revealed that only D-glucose is reabsorbed in this region of the nephron under normal physiological concentrations (approx. 5 mM). The blood D-galactose concentration is almost measureless, and therefore it is reasonable to assume that the very low-affinity transport system for D-galactose ($K_A = 15 \pm 2$ mM) localized in the pars convoluta would be unable to reabsorb efficiently the tracer amount of this hexose that may exist in this region of proximal tubule. These findings strongly point to the existence of various 'carrier protein(s)' for the reabsorption of monosaccharides along the different regions of renal proximal tubule. Further studies are required to characterize the structural requirements for the interaction between various sugars and the 'carrier protein(s)' localized in the luminal-membrane vesicles from pars convoluta and pars recta of proximal tubule.

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