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Histochemical analysis of carbohydrate moieties and sugar-specific acceptors in the kidneys of the laboratory mouse and the golden spiny mouse *(Acomys russatus)*

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Summary. The aims of this work were to histochemically compare the pattern of lectin binding and endolectin expression in different portions of nephrons of two rodent species producing either normal hyperosmotic urine (the laboratory mouse) or highly concentrated urine *(Acomys russatus*, the golden spiny mouse). A panel of biotinylated lectins and neoglycoproteins and the avidin-biotin-peroxidase complex technique were used on Bouin's fixed, paraffin-embedded sections. Various segments of the uriniferous tubule in both species showed differential affinity for labelled lectins and neoglycoproteins. Significant differences were also evident between comparable tubular segments in laboratory and golden spiny mouse kidneys. Whether the histochemical expression of sugar moieties of glycoconjugates as well as endolectins, thus both sides of presumed protein-carbohydrate interactions, may be correlated to the various glycoproteins which would include constituents of the glycocalyx and domains of a variety of transport enzymes deserves further studies.

Key words: Rodent kidney, Lectins, Neoglycoproteins, Glycohistochemistry

Introduction

The functional unit of the mammalian kidney is the uriniferous tubule composed of a long tortuous segment, the nephron, and intrarenal collecting ducts (Bulger, 1988). Specialized structures that perform various functions including ultrafiltration, reabsorption of macromolecules and fluid as well as secretion and reabsorption of electrolytes are commonly described (Bulger, 1988; Dantzler, 1989; Kim et al., 1992). All these transport processes are supposed to be driven by specialized glycoproteins of, as yet, largely not well-defined structures; at any rate, glycans are thought to play a key role (Kanwar et al., 1983).

In recent years, lectins with their specific affinities for sugar residues have been extensively used as histochemical probes for the characterization and assessment of distribution of complementary sugar sequences in the nephron of various species (Holthöfer, 1983; Murata et al., 1983; Schulte and Spicer, 1983; Holthöfer et al., 1987, 1988; Laitinen et al., 1989) including man (Holthöfer et al., 1981; Faraggiana et al., 1982; Truong et al., 1988; Laitinen et al., 1990). In addition to analysis of normal types of kidney, comparison of parameters with kidney of animals that have adapted to special environmental conditions may provide clues for a correlation to special functions. Thus, we include such a model into our study.

The golden spiny mouse *(Acomys russatus)* is widely distributed and abundant in deserts in Israel (Shkolnik and Borut, 1969).

Since the kidneys undoubtedly have a role in the adaptation of vertebrates to desert life (Schmidt-Nielsen, 1964, 1991; Dantzler, 1989), the aim of the present work was to compare the expression of defined determinants in different segments of the nephron of both golden spiny and laboratory mice. In addition to lectin histochemistry we initiated the application of carrier-immobilized sugar residues, termed neoglycoproteins, to simultaneously examine the distribution of binding sites with specificity for certain carbohydrate moieties in this type of tissue, technically referred to as reverse lectin histochemistry (Gabius et al., 1993).

To assess binding profiles of both glycohistochemically-detectable carbohydrate residues and endogenous carbohydrate-binding proteins, we used a panel of biotinylated lectins and neoglycoproteins, respectively, on Bouin's-fixed, paraffin-embedded sections (Danguy and Genten, 1990; Danguy et al., 1991).

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Materials and methods

Animals and tissue preparation

Kidneys were obtained from 5 adult laboratory mice (NMRI strain) and 5 adult golden spiny mice *(Acomys russatus)* of both sexes for this study. The animals had been housed individually, given food and water *ad libitum* and maintained on a 12 hr light/12 hr dark regimen. *Acomys russatus* was kept at 26 ± 1 °C. Tissue samples were fixed in Bouin's solution for 24 hr, dehydrated through graded ethanol solutions, cleared in toluene, embedded in paraffin and sectioned at a thickness of 5-6 µm. After dewaxing, lectin and neoglycoprotein histochemical stainings were performed using the avidin-biotin method, following optimized procedures (Danguy and Genten, 1990; Danguy et al., 1991).

Lectin histochemistry

Eleven types of biotinylated lectin, purchased from Vector Laboratories (Burlingame, CA, USA), were used. Their full names, abbreviations, natural sources, saccharide specificities and binding inhibitors are listed in Table 1.

After deparaffination, sections were exposed to a solution of 0.3% H_2O_2 in PBS (phosphate-buffered saline, 10 mM phosphate buffer, pH 7.3 ± 0.1, containing 0.15 M NaCl) for 30 min at room temperature to abolish the activity of endogenous peroxidase. Then, the sections were overlayed with a 5 µg/ml solution in PBS of biotinylated lectins for 10 min. After thorough washes to remove the marker, the sections were incubated for 30 min with avidin-biotin peroxidase complex-containing solution (ABC kit, Vectastain Elite, Vector Labs.). After being extensively washed in PBS, the sections were developed in 3,3'-diaminobenzidine-4HCl (DAB, Sigma)-H_2O_2 medium under microscopical control at room temperature to visualize the activity of bound peroxidase. The sections were rinsed with tap water, counterstained with haematoxylin, dehydrated, cleared and mounted with DPX. Controls included omission of lectin or ABC complex, respectively.

Table 1. Lectins used for histochemical characterization of carbohydrate residues.

LECTIN/LATIN NAME (COMMON NAME) ACRONYM	SUGAR RESIDUES OR SEQUENCES/OLIGOSACCHARIDE STRUCTURES RECOGNIZED BY LECTINS	SUGARS FOUND TO INHIBIT HISTOCHEMICAL BINDING
Phaseolus vulgaris (kidney bean)		
PHA-E erythroagglutinin	-Gal-GlcNAc-Man GlcNAc — Man-GlcNAc-GlcNAc-Asn -Gal-GlcNAc-Man	Complex
PHA-L leukoagglutinin	Gal-GlcNAc Gal-GlcNAc-Man — Man-GlcNAc-GlcNAc-Asn Gal-GlcNAc-Man	Complex
Erythrina cristagalli (Coral tree) ECL	Tetraantennary and triantennary oligosaccharides containing some or three N-acetyllactosamine branches respectively > biantennary and linear oligosaccharides.	Gal
Artocarpus integrifolia (Jack fruit) JAC	Gal ß1-3 GalNAc This structure is bound even in a mono- or disialylated form.	Gal
Dolichos biflorus (horse gram) DBA	GalNAc(α1-3)GalNAc > α GalNAc	GalNAc
Ulex europaeus (gorse seed) UEA-I	L-Fuc	Fuc
Triticum vulgare (wheat germ) WGA	$(ß1\text{-}4\text{-}D\text{-}GlcNAc)_{1\text{-}2}$ > NeuAc	GlcNAC
(succinylated wheat germ) s-WGA	$(ß1\text{-}4\text{-}D\text{-}GlcNAc)_{1\text{-}2}$	GlcNAc
Sambucus nigra (Elderberry bank) SNA	NeuAc(α2-6)Gal/GalNAc	NeuAc
Galanthus nivalis (Snow drop bulb) GNA	Man(α1-3)Man > Man(α1-6)Man > Man(α1-2)Man	Man
Lens culinaris (Lentil) LCA	Man in non-bisected bi- and tri-antennary, complex N-linked sequences[a]	Man

GlcNAc: N-acetylglucosamine; GalNAc: N-acetylgalactosamine; Gal: Galactose; Man: Mannose; Glc: Glucose; NeuAc: Neuraminic acid; [a]: Fuc linked α1-6 to N-glycosidically-bound GlcNAc residues in these oligosaccharides increases their affinities for LCA.

Preincubation of the lectins with their respective inhibitor sugars (0.2 - 0.3 M) (Janssen chemica, Beerse, Belgium) led to a diminution of the reaction (Fig. 6). The localization of carbohydrate determinants in the tissues was examined with a light microscope (Leitz, Wetzlar, FRG). The staining intensities were scored from 0 (no reaction) to 4 (maximal reaction).

Protocol for reverse lectin histochemistry

For detection of the pattern of endogenous sugar receptors with different carbohydrate specificities the following biotinylated neoglycoproteins (chemically glycosylated derivatives of bovine serum albumin; BSA from Biomol, Hamburg, FRG) were used. For ß-galactoside-specific receptors: lactose-BSA-biotin; ß-N-acetyl-D-glucosamine-(BSA-biotin) and ß-N-acetyl-D-galactosamine-(BSA-biotin) for receptors with respective specificity for these two naturally occurring N-acetylated sugars; for mannoside- and fucoside-specific receptors: α-D-mannose-(BSA-biotin) and α-L-fucose-(BSA-biotin), respectively; for receptors specific for negatively-charged sugars that contain a carboxyl group: D-glucuronic acid-(BSA-biotin); for ß-xyloside-specific receptors: ß-D-xylose-(BSA-biotin); for ß-galactoside-specific receptors: lactose-(BSA-biotin); in addition, for α-glucoside-specific receptors, maltose-(BSA-biotin) was used. The batch of BSA, used for neoglycoprotein synthesis, was additionally treated with periodate to destroy any traceable contamination with carbohydrates.

Chemical preparation, quality controls and properties of labelled carbohydrate-protein conjugates are described in detail elsewhere (Gabius and Bardosi, 1991). The various steps of staining are outlined as follows:

- deparaffination and inhibition of endogenous peroxidase was performed as above.
- after rehydration in graded alcohol the sections were preincubated with 0.1% BSA containing 0.1 M phosphate buffer (pH 7.4) solution for 15 min to minimize the unspecific binding of BSA-biotin derivatives used in the following steps.

Incubation was performed using the biotinylated carbohydrate-BSA conjugates dissolved in 0.1% BSA containing 0.1 M PBS (10 µg lyophilized derivative in 1 ml of this buffer solution) at room temperature for 15 min. For control studies sections were preincubated at room temperature with homologous unlabelled carbohydrate-BSA conjugates at a concentration of 20 µg/ml to mask sugar-specific receptors. Incubation was then performed for 90 min with a mixture (in a relation of 1:100) of labelled carbohydrate-BSA conjugate (10 µg) and the corresponding unlabelled neoglycoprotein as competitive inhibitor. Absence of staining with the carbohydrate-free, labelled carrier protein was also ascertained at the same concentration, substantiating lack of binding of the individual probes by protein-protein interaction. To assess the contribution of glycosyltransferases to binding of probes, incubation of the biotinylated markers was performed in the presence of the nucleotides cytidine-5'-diphosphate and uridine-5'-diphosphate, which have been shown to suppress binding to glycosyltransferases (Gabius and Bardosi, 1991). The visualization and evaluation of the intensity of neoglycoprotein binding was carried out, as described in detail for lectin histochemistry.

Results

Overview of binding sites of each biotinylated lectin

Obviously, the patterns of binding of the lectins to the two types of organ were not generally uniform. The localization and intensity of binding sites of each lectin are summarized in Table 2.

Red kidney bean erythroagglutinin (PHA-E) exhibited a faint staining of glomerular structures in the two species. In distal tubules and collecting ducts of the laboratory mouse the luminal parts of the tubuli were moderately to strongly stained. In both species the brush border of proximal tubules was strongly reactive. Henle's loop reacted faintly, if at all, in both species. Similarly, the cytoplasm of tubular epithelial cells of both species exhibited a faint diffuse staining. Neither the luminal part nor the cytoplasm of collecting tubules in *Acomys russatus* were labelled with this lectin.

The binding pattern, seen with PHA-L, was somewhat different when compared to that of PHA-E. In laboratory mice, glomerular structures including the glomerular basement membranes, also seen in *A. russatus* were moderately stained. The luminal part and the cytoplasm of Henle's loop of laboratory mice exhibited a moderate reaction. The distal tubule was unlabelled except for the luminal part in *Acomys russatus*, where a slight reaction was evidenced. In this species PHA-L bound weakly to the collecting duct. In both rodents PHA-L had strong affinity to the proximal tubule brush border (Fig. 1).

ECL expressed a similar binding pattern in both species. Only Henle's loop and the luminal part of the distal tubule were heavily stained with this lectin. Staining of the other nephron components was weak or doubtful.

JAC showed a quite similar binding pattern in both species. Glomerular structures and the brush border of the proximal tubule were moderately labelled (Fig. 2). The luminal part of all tubuli and the cytoplasm of epithelial cells of Henle's loop expressed a strong binding. The epithelial cells of the distal tubule and the collecting duct were weakly stained. In the golden spiny mouse no binding to the proximal epithelial cells persistently occurred with this lectin.

Notably, the DBA-binding pattern was very dissimilar in the proximal tubule of the two rodents. A moderate to strong staining was seen in the golden spiny mouse (Fig. 3), whereas reaction to DBA was persistently negative in the laboratory mouse (Fig. 4). In

Table 2. Semiquantitatively determined intensity of lectin binding to the nephron of laboratory mice (LM) and golden spiny mice (GS).

LECTINS	PHA-E		PHA-L		ECL		JAC		DBA		UEA-I		s-WGA		WGA		SNA		GNA		LCA	
SPECIES	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS
STRUCTURES																						
Glm	1	1	2	1(2)	0-1	0	2	2	0	0(1)	0-1	0-1	0	0	1(3)	1(2)	1(3)	1(3)	0	0	1(3)	1
PT[a]																						
bb	3	3	3	3	0-1	0	2	2	0	3	0-1	0-1	1	0-1	2	1	0-1	0	1	1	2-3	2-3
cyt	0-1	1[2]	1	2[3]	0-1	0	1[3]	0[3]	0	2[3]	0-1	0-1	1[2]	0-1	1[3]	1[3]	0-1	0	2[3]	2[3]	1[3]	1[3]
HL[a]																						
lum	1	0-1	2	0-1	3	2-3	3	3	2	2	0-1	2	3	3	4	4	3	3	2	2	3	2-3
cyt	1	0-1	2	0-1	3	2	3	2	2	2	0-1	2	1	1	1	1	3	2	2	2	3	2-3
DT[a]																						
lum	3	2	0	1	3	3	3	3	3	4	0-1	0-1	3	4	4	3	0	4	1	1	1-2	1-2
cyt	0-1	0-1	0	0	0-1	1	1	1	2	1-2	0-1	0-1	1	2	3	2	0	4	1-2	1-2	0-1	0-1
CD[a]																						
lum	2	0	2	1	1	1	2	3	0	0	0-1	0-1	0	3	4	3	0	3	1	1	0-1	0-1
cyt	0-1	0	1	1	1	1	1	1	0	0	0-1	0-1	0	1	1-2	2-3	0	1-2	1-2	1-2	0-1	0-1

( ): staining intensity of glomerular basement membrane; [ ]: staining intensity of vesicle-like structures within the cytoplasm; n-n: variable reactivity in the same structure; [a]: see discussion for the nomenclature used; Glm: glomerulus; PT: proximal tubule; HL; Henle's loop; DT: distal tubule; CD: collecting duct; bb: brush border; cyt: cytoplasm of tubular epithelial cells; lum: luminal part of the tubule. For the names of the lectins see Table 1. 0, 1, 2, 3, 4 denote negative, faint, moderate, strong and very strong reaction, respectively.

tubular areas moderate to strong DBA-reactive sites were located in Henle's loops and distal tubules (Fig. 4) of both species.

There was no affinity of DBA to the epithelial cells of collecting ducts.

UEA-I was a good marker for Henle's loop in *Acomys russatus*. The other structures were labelled rather faintly, if at all, in both species.

Except for Henle's loop (Fig. 5) and the glomerulus, the intensity of reaction obtained with s-WGA varied in both species. The intensity of labelling was stronger in the distal tubules and collecting ducts of *A. russatus* than in those of the laboratory mouse (Fig. 5).

In both species WGA exhibited a prominent labelling in the basement membranes of the capillary network in the glomerulus and to the apical areas of Henle's loop, distal tubules and collecting ducts. A faint or moderate staining was produced in the brush border of proximal tubules of laboratory and golden spiny mice, respectively.

SNA bound strongly to basement membranes in the glomerulus. Epithelial cells of proximal tubules unequivocally failed to react. In contrast, Henle's loop in both species showed a strong labelling. The distal tubules and collecting ducts of *A. russatus* were only stained with SNA.

An almost identical binding pattern was seen with GNA in both species. Only the glomerulus failed to present any reaction.

LCA reacted in the same manner in both species. This lectin bound intensively to the brush border of proximal tubules and the luminal part of Henle's loops. Remarkably, LCA strongly labelled basement membranes in the glomeruli of the laboratory mouse.

Using JAC, WGA, GNA and LCA as probes, granular or vesicular structures within the proximal epithelial cells were heavily labelled in both species. The same components were also revealed in *A. russatus*

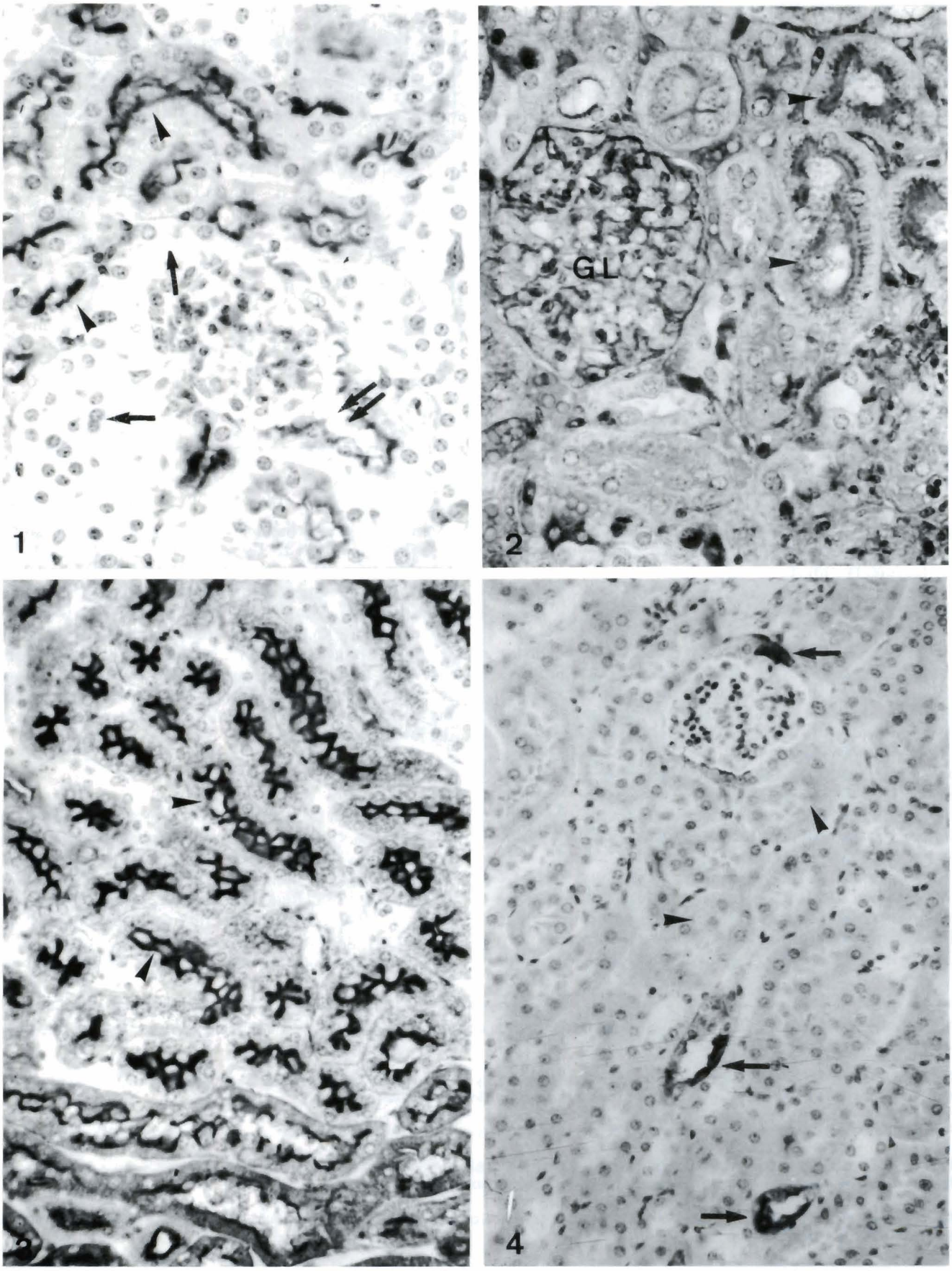
Figs. 1-5. Representative photomicrographs of the laboratory and golden spiny mouse kidney illustrates the distribution of lectin binding sites (dark areas).

Fig. 1. Binding sites of PHA-L in the laboratory mouse. Especially marked reaction products are found at the brush border of proximal cells (arrowheads). Distal tubules are unlabelled (arrows). Confluence of Bowman's space with the proximal tubule lumen at the urinary pole is seen (double arrow). x 400

Fig. 2. Binding sites of JAC in the golden spiny mouse. Glomerular structures (GL) and the brush border of the proximal tubule are moderately labelled (arrowheads). x 400

Fig. 3. Binding sites of DBA in the golden spiny mouse. The brush border and the apical cytoplasm of the proximal tubules are strongly stained (arrowheads). x 256

Fig. 4. Binding sites of DAB in the laboratory mouse. The brush border and the cytoplasm of the proximal tubule cells are unreactive (arrowheads). Distal tubule cells are distinctly labelled (arrows). x 256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GL
2
4

Table 3. Semiquantitatively determined intensity of neoglycoprotein binding in the nephron of laboratory mice (LM) and golden spiny mice (GS).

NEOGLYCOPROTEIN	LAC-BSA		FUC-BSA		GlcNAc-BSA		Gluc Acid-BSA		Man-BSA		GalNAc-BSA		Mal-BSA		Xyl-BSA	
SPECIES	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS	LM	GS
STRUCTURES																
Glm	0	0	0	0	0	0	1	1	1	0-1	0	0	0	0	1	2
PT[a]																
bb	1	0-1	0	1	0	0	1	1	1-2	1	0	0	0	0	1	1
cyt	2	1	1-2	1	0-1	0-1	2-3	3	2	0-1	1	0-1	0-1	0-1	2-3	2
HL[a]																
lum	1	0	1	1	0	0	1-2	2	1	1-2	0	1	1	1	2	3
cyt	1	0	1	1	0	0	1-2	2	1	1-2	0	1	1	1	2	3
DT[a]																
lum	2-3	2	2	2	0	0	3	3	2	2-3	1	1-2	1	1	3	3
cyt	2-3	2	2	2	0	0	3	3	2	2-3	1	1-2	1	1	3	3
CD[a]																
lum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyt	2	2	2-3	2-3	1	2	3	3	3	3	1-2	2	1-2	2	3	3

Same abbreviations as in Table 2.

using PHA-E, PHA-L and DBA. s-WGA revealed this granular material only in the proximal epithelial cells of the laboratory mouse.

Reverse lectin histochemistry

Whereas the lectins visualize presence of certain carbohydrate moieties, such ligands, attached to a labelled carrier, can be employed to localize accessible binding sites in the tissue that are not impaired by fixation. Similes to the application of lectins, specificity of staining was proven by adequate controls. These probes clearly showed that respective binding sites were present in the animal kidney. Focussing on the comparative aspect, most of neoglycoproteins expressed similar binding patterns in the segments of the nephron in both species (Table 3). GlcNac-BSA was the only compound which failed to bind to any structure except to the cytosol of the collecting duct. None of the neoglycoproteins bound to the luminal part of this duct. The strongest binding intensity was obtained with GlucA-BSA, Man-BSA (Fig. 7) and Xyl-BSA (Fig. 8).

In both species there was a strong reaction of all the neoglycoproteins in the distal tubules except with GlcNAc-BSA. Using Lac-BSA, GalNAc-BSA and Xyl-BSA as probes, species differences were seen in Henle's loop. Lac-BSA and Man-BSA exhibited a stronger affinity to the cytosol of proximal tubular cells in the laboratory mouse than in the golden spiny mouse. Using GlcNAc as carrier-immobilized ligand for tissue receptors a stronger binding intensity was observed in the collecting duct cells of *A. russatus* than in those of the laboratory mouse.

Discussion

Carbohydrate moieties are widely distributed in animal tissues and confer to glycoconjugates an essential role in many biological processes (Olden et al., 1982; Edelman, 1985, 1986; Cook, 1986; West, 1986; Gabius and Nagel, 1988; Rademacher et al., 1988; Gabius and Gabius, 1993). During the last decade considerable evidence has been accumulated to relate this physiological significance of glycoconjugates to an

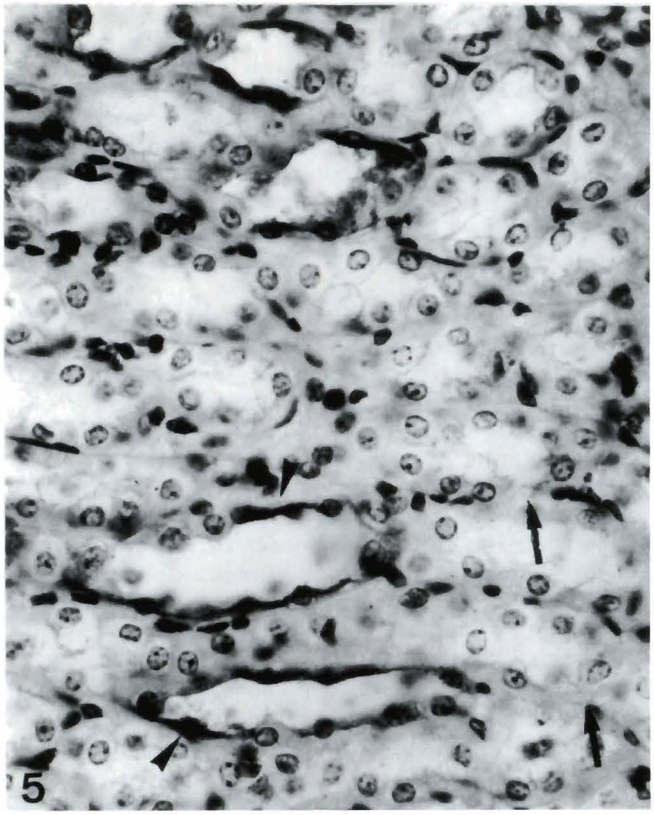
Fig. 5. Binding sites of s-WGA in the laboratory mouse. Cells of Henle's loop have moderate to strong affinity to this lectin (arrowheads). Cells of the collecting tubule lack binding sites (arrows). x 400

Fig. 6. Unlabelled photomicrograph from control section in the golden spiny mouse. This section was incubated with a solution of DBA and 0.3 M of its inhibitory sugar (GalNAc). The nuclei were counterstained with haematoxylin. GL: glomeruli. x 256

Figs. 7-8. Representative photomicrographs of the laboratory and golden spiny mouse illustrate the distribution of neoglycoprotein binding sites.

Fig. 7. Binding sites of Man-BSA in the laboratory mouse. The thin epithelium of Henle's loop (in longitudinal section) is weakly reactive (arrowheads). The distal and collecting tubule cells are moderately to strongly stained (arrows). x 512

Fig. 8. Binding sites of Xyl-BSA in the golden spiny mouse. Henle's loop cells (in longitudinal sections) (arrowheads) and distal tubule cells (arrows) display a strong reaction. x 256



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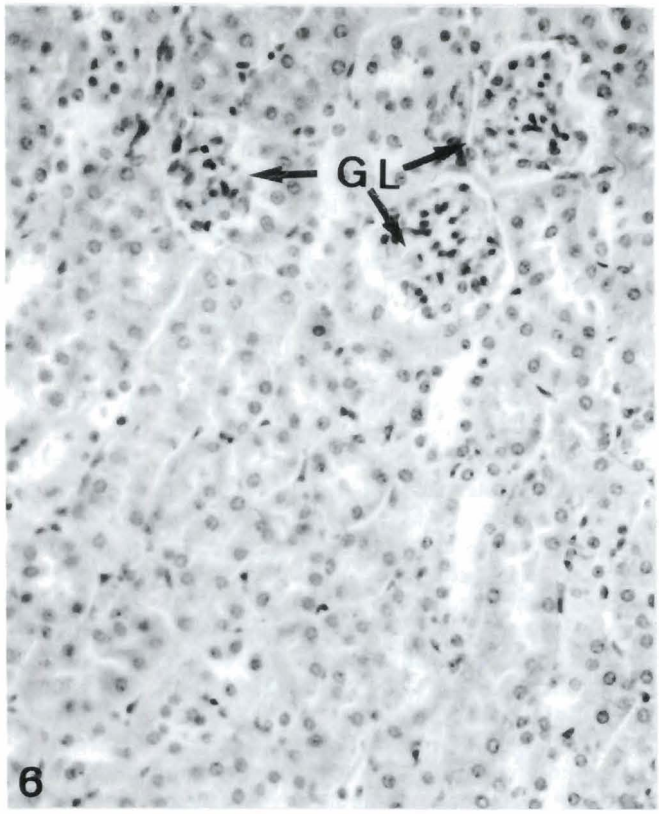

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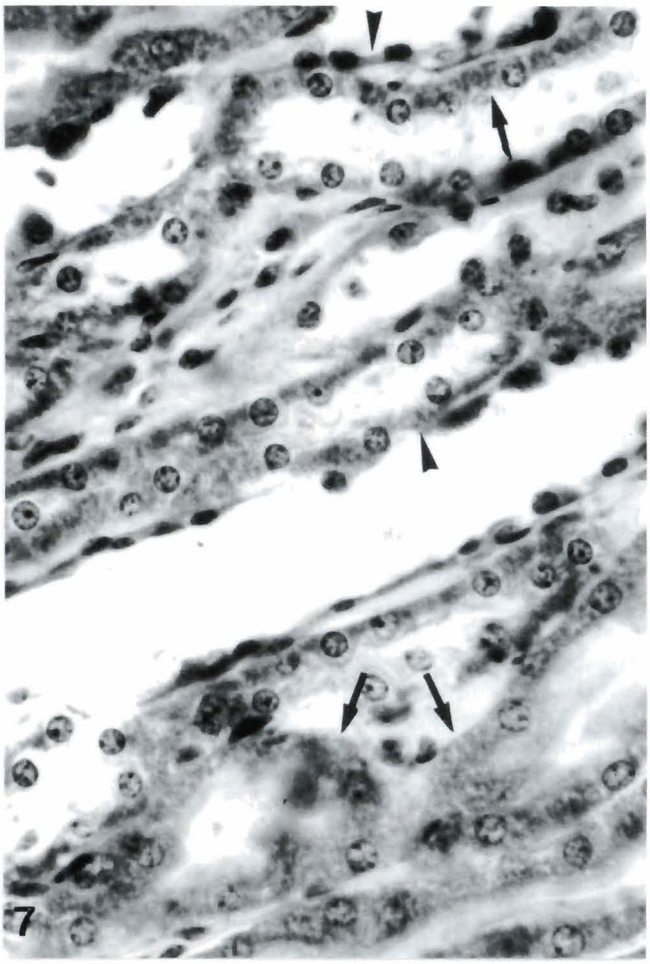

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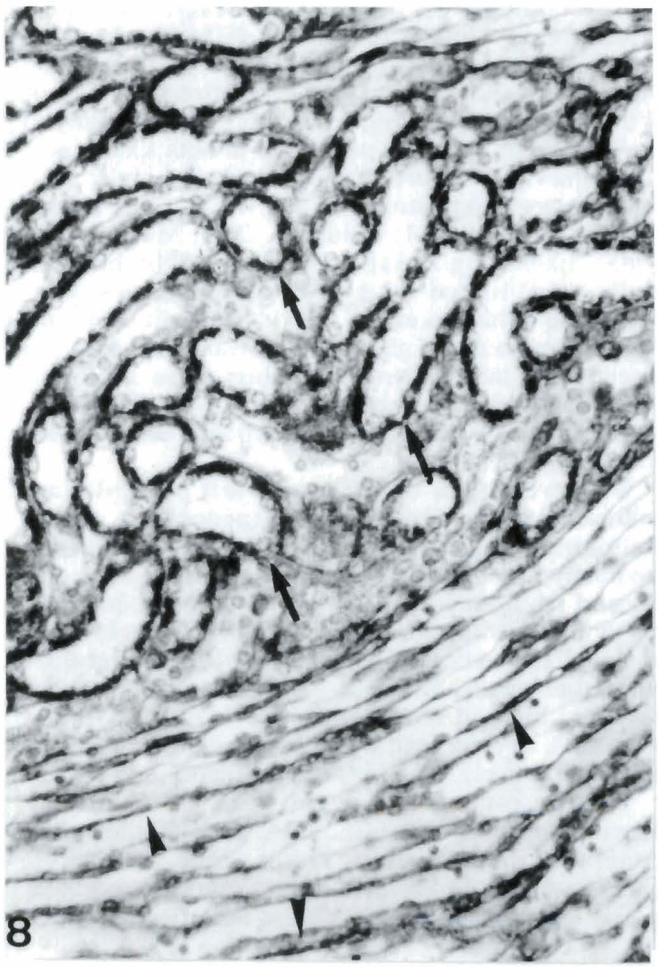

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interplay *in situ* with animal lectins (Barondes, 1984; Kolb-Bachofen, 1986; Gabius, 1987, 1991; Andersen et al., 1991; Harrison, 1991; Zanetta et al., 1992a,b). However, it has to be acknowledged that we are at present only at the beginning of understanding the functional roles of the versatile protein-carbohydrate interaction. In the kidney, lectin-reactive glycoconjugates are present in tubules, interstitium, vessels and glomeruli and are potentially important in several renal functions (Kanwar et al., 1983; Holthöfer et al., 1988; Truong et al., 1988). Consequently, we addressed two issues: glycohistochemical probes, namely lectins and neoglycoproteins, were employed to measure extent of binding for the two types of probes, and their distribution in the nephron of two rodent species living in different habitats was comparatively assessed.

The kidney is an organ of complex structure and the nomenclature, encountered throughout the literature, is somewhat chaotic. In the present study, the following terms were used: the «proximal tubule» includes both the convoluted and straight part of the tubule; the «distal tubule» includes the straight tubule or thick ascending limb and the convoluted part; «Henle's loop» comprises the descending and ascending thin limbs; the «collecting duct» emerges from the confluence of many nephrons and includes cortical and medullary portions.

The comparison of staining reactivities with biotinylated lectins that have different binding specificities to sugar sequences revealed some interesting features. A moderate to strong binding of WGA and SNA to the glomerular tuft and especially to the glomerular basement membrane in both species was seen. Previous reports have shown that sialic acid moieties are ligand structures for WGA in the kidney (Holthöfer et al., 1981; Holthöfer, 1983; Murata et al., 1983). Exhibiting linkage preference, SNA binds to sialic acid, linked α2-6 to D-galactose in glycoconjugates (Shibuya et al., 1987). The sialic acids are major components of glomerular polyanions, which are essential for the glomerular filtration (Blau and Haas, 1973; Latta, 1980), and besides WGA, SNA thus seems to be a marker for the glomerular filtration barrier in the species studied here.

Moreover, it has been reported that podocytes are endowed with a cell surface coat (podocalyx), rich in sialoproteins (Kerjaschki et al., 1984). In both the laboratory and the golden spiny mouse the presence of bi- and tri-antennary complex N-linked sequences, highly branched (in tri-antennary or more) non-bisected complex sequences, D-galactose-(ß 1-3)-N-acetyl-D-galactosamine and D-mannose-containing parts in non-bisected bi- and tri-antennary, complex N-linked sequences, as evidenced with PHA-E, PHA-L, JAC and LCA, respectively, can be ascertained in the glomeruli.

In both rodents the negative or faint binding reactions, obtained with ECL, DBA, s-WGA, allow us to conclude that N-acetyl-lactosamine, N-acetyl-galactosamine and N-acetylglucosamine are not or poorly represented and accessible to the probes.

In the same way, Murata et al. (1983) were unable to detect any DBA binding in the rat glomerulus, but the same authors reported that N-acetylglucosamine residues are also present in glomeruli, as revealed by WGA binding, in contrast to our results. The concomitant use of s-WGA and WGA emphasizes an indication for differential extent of staining between rat and the examined mice.

The brush borders of proximal tubules are abundant in complex oligosaccharide sequences, judging from the binding with *Phaseolus vulgaris* and *Lens culinaris* agglutinins. This is not the case for other carbohydrate determinants. D-galactose-(ß1-3)-N-acetyl-galactosamine, N-acetyl-glucosamine, sialic acid and mannose, which are specifically bound with JAC, s-WGA, WGA and GNA, are weakly to moderately distributed at the brush border of the two species. Remarkably, a pronounce species difference was observed at the proximal structures, using DBA as probe. In *A. russatus*, the brush border and the cytosol including granular structures, interpreted as phagosomes or lysosomes, were heavily labelled with this lectin, indicating a high density of N-acetylgalactosamine residues. In the common mouse no DBA-binding sites could be detected.

In the human kidney, complex N-linked oligosaccharide sequences also seemed to be almost limited to the brush border of proximal tubules with less cytoplasmic staining (Truong et al., 1988; Ivanyi and Olsen, 1991). Concerning the proximal tubule, species differences for DBA staining were previously reported among rodents and the human. In the rat, DBA-binding sites were detected (Holthöfer, 1983; Murata et al., 1983; Schulte and Spicer, 1983), whereas specimens from the mouse (Holthöfer, 1983; Schulte and Spicer, 1983; Laitinen et al., 1987) and man (Holthöfer et al., 1981; Truong et al., 1988) were devoid of specific binding.

These data may suggest differences of proximal tubule functions among species, reflected by the equipment with physiologically relevant glycoproteins. Granular or vesicle-like structures were labelled with some lectins (Table 2). They may represent the endocytotic apparatus of the proximal tubule cells. Indeed, one of the major functions of the proximal tubule is to reduce drastically the volume of glomerular filtrate, accomplished both by carrier-mediated active transport of ions and the prominent endocytotic apparatus including tubular invaginations of the apical cell membrane, a series of several clathrin-coated vesicles, large and condensing vacuoles (Bulger, 1988). Arising by means of invaginations of apical cell membranes, it has been claimed that this vacuolar apparatus displays the same antigens as those evidenced in the brush border (Okot-Opiro et al., 1992). Our lectin histochemical studies are in accordance with such observations.

Most of the sugar-binding probes applied in this study reacted moderately to strongly with the thin segment of Henle's loop, and especially with the luminal part of this

tubule. This indicates a broad distribution of sugar moieties and oligosaccharide sequences in both species. Sialic acid and N-acetylglucosamine were plentifully distributed, judging by the level of WGA, SNA and s-WGA binding.

In the same way, accessible N-acetyllactosamine, branched or in linear form, D-galactose-(ß1->3)-N-acetyl-galactosamine or mannose in linear or in bi-antennary complex N-linked sequences are found in the glycoconjugates of Henle's loop. These observations are partly in accordance with those published previously (Murata et al., 1983; Schulte and Spicer, 1983; Laitinen et al., 1987) but comparisons are sometimes difficult, because the loop of Henle was here considered as both the thin descending and ascending segments, excluding the thick ascending part incorporated in the distal tubule. Differences between Henle's loop of the laboratory and the golden spiny mouse were detected using PHA-L and UEA-I as probes. In this last species, fucosyl residues were found in a moderate amount, whereas a moderate amount of highly branched (in tri-antennary or more) non-bisected complex sequences was found in the former.

It is generally accepted that the effectiveness of a countercurrent multiplier increases with the length of the loop (Dantzler, 1989; Schmidt-Nielsen, 1991). A long papilla was observed in the kidney of *A. russatus* accounting for an eminently efficient mechanism for urine concentration. Indeed, urea concentrations in the urine or spiny mice are among the very highest known in mammals (Schmidt-Nielsen and O'Dell, 1961; MacMillen and Lee, 1967; Shkolnik and Borut, 1969). The differences observed here may be related to specific transport and permeability properties in each of the two limbs of Henle's loop. The principal physiological role of the distal tubules lies in net sodium chloride reabsorption on the basis of active transport of chloride (Burg and Green, 1973). Furthermore, it has been claimed that the distal tubule produces and secretes urinary glycoproteins; the so-called Tamm-Horsfall protein (TH). TH was located in the cytoplasm of epithelial cells lining the distal straight tubules (Hoyer et al., 1979; Peach et al., 1988). The major carbohydrate components of TH glycoproteins are galactose, mannose, N-acetyl-glucosamine and fucose (Fletcher et al., 1970). In most respects, our lectin-binding results are consistent with the above biochemical data in both species, especially at the luminal part of the tubule. Fucosyl residues were not, however, unquestionably detected. The moderate to strong staining pattern with PHA-E suggests that the N-lined oligosaccharides were of the bisected bi- and/or triantennary complex type. N-Acetyl-galactosamine-containing structures were also plentifully distributed in both species, judging by DBA labelling. Interestingly, conflicting results have been previously reported for the laboratory mouse (Holthöfer, 1983; Schulte and Spicer, 1983; Laitinen et al., 1987). These discrepancies may be explained by variability of expression of glycoconjugates among individuals of inbred and outbred species and in interspecific hybrids due to genetic differences, as conclusively shown by Spicer's group (Spicer et al., 1987). A clear species difference was observed, using SNA as probe. A moderate to very strong binding reaction was seen in the distal tubules in *A. russatus*. Thus, it can be concluded that epithelial cells of the distal tubule of this species are rich in sialic acid moieties, linked in α2-6 to D-galactose in the glycoproteins. In *A. russatus* this carbohydrate element was also moderately to strongly expressed in the collecting duct, and SNA thus seems to be a marker for these two segments, also called the distal nephron (Bulger, 1988).

Another interesting difference was the affinity for s-WGA, observed on the apical surface and in the cytoplasm of epithelial cells that line the collecting ducts of the golden spiny mouse, but not of the laboratory mouse. Only in this last species was the luminal part of the collecting duct labelled with PHA-E, emphasizing an additional species difference. In the laboratory mouse the very strong staining of the luminal part of the collecting duct along with the failure of s-WGA and SNA to bind is puzzling. An explanation might be that sialic acid residues other than those linked α 2-6 to galactose were displayed. Some attention must be paid to the lack of staining of the collecting duct in the two species, using DBA as probe. Our results are in sharp contrast with those reported in the rat (Murata et al., 1983; Holthöfer et al., 1987, 1988) whereas marked heterogeneity of staining was observed in the mouse (Schulte and Spicer, 1983). Both species differences and genetic differences among various strains of one species may explain these discrepancies (Spicer et al., 1987). The collecting ducts show a remarkable functional diversity, participating crucially in the final regulation of urinary acid-base balance and electrolyte composition (Bulger, 1988; Dantzler, 1989). In addition to light principal cells, dark mitochondria-rich intercalated cells have been described (Bulger, 1988; Dantzler, 1989), and several reports have appeared emphasizing the selective staining of collecting duct cells with lectins (Holthöfer et al., 1981, 1988; Faraggiana et al., 1982).

Several enzymes, i.e. $(Na^+ + K^+)$-ATPase and carbonic anhydrase, involved in transport functions have been revealed immunocytochemically (Holthöfer, 1987, Holthöfer et al., 1988).

In the present study no attempt has been made to distinguish between intercalated and principal cells of collecting tubules, because none of the lectins used displayed a clear distinction between these cells. In other instances, principal and intercalated cell characteristics have been reported to be coexpressed within one cell type, suggesting that intercalated cells might be derivatives of principal cells (Minuth et al., 1989).

Some previous studies, however, have emphasized such distinction using lectins (Holthöfer et al., 1981, 1988; Schulte and Spicer, 1983).

To take the next step to elucidate the importance of lectin-detectable carbohydrate moieties, it is necessary to

reveal binding capacity for such structures in the tissues. Labelled neoglycoproteins are appropriate tools for this purpose, because they are able to interact specifically with putative receptors for the carbohydrate ligand in the conjugate of carbohydrate structure and carrier molecule (Gabius, 1991; Gabius and Bardosi, 1991; Gabius and Gabius, 1991, 1993). They have already proved to be valuable tools in the detection of endolectins as functionally important molecules *in situ* (Danguy et al., 1991; Gabius and Bardosi, 1991; Gabius and Gabius, 1991, 1993). The present data and those from other laboratories (Holthöfer, 1983; Holthöfer et al., 1981; Truong et al., 1988) have found glyco-histochemically-defined differences between the epithelial cells of proximal and distal kidney tubules. However, to our knowledge, this report initiates a comparative histochemical study on endogenous carbohydrate-binding proteins in rodent kidneys.

Some species dichothomy can be encountered, as far as neoglycoprotein binding patterns are concerned. Accessible sugar receptors with specificity to ß-galactose were expressed in Henle's loop of laboratory mice, while some receptor expression was visualized in the same segment of golden spiny mice, using GalNAc-BSA as probe. Sugar receptors with specificity to ß-galactose and α-mannose were expressed in comparatively high concentration in the cytoplasm of proximal tubule cells of the laboratory mouse. These discrepancies cannot at present be readily explained. In the two species similar neoglycoprotein binding was coarsely observed in cells of the distal tubule and collecting duct. Recently, Kojima et al. (1992) were able to purify 33 kDa and 41 kDa proteins from bovine kidney extracts. These substances are Ca^{++}-dependent carbohydrate-binding proteins of the annexin family. However, knowledge about their physiological functions related to the carbohydrate-binding activities is still limited. Another carbohydrate-binding protein know to occur in kidney is calcyclin (Gabius et al., 1989; Zeng and Gabius, 1991). The lack of comprehensive biochemical structures in this respect notwithstanding, it is reasonable to propose that the concominant monitoring of both sides of a protein-carbohydrate interplay, namely the glycoconjugate structure as well as the sugar-binding protein, is essential to unequivocally provide evidence for any functional implications. Our study thus gives a guideline for further biochemical investigations to elucidate the nature of the detectable binding sites.

In conclusion, the present results show that although some glycans or endogenous sugar receptors of nephrons seem to be located in a similar way in both species, each of these presents a characteristic distribution of some lectin and neoglycoprotein binding sites as well. Whether these differences in the localization and structure of carbohydrate composition are associated with the different physiological activities in the various segments of rodent nephrons is a question that requires further investigation.

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