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Mosaic lectin labelling in the quail collecting ducts

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Summary. Morphological and histoenzymological differences have been observed between intercalated and principal cells of the quail *Coturnix coturnix japonica* collecting ducts. The present study was designed to shed light on the lectin affinity of the collecting duct cells within cortex and medulla by the use of HRP-labelled lectins combined with glycosidase degradation. Binding of PNA and RCA-I lectins consequent to enzymatic release of sialic acid revealed abundant sialylated carbohydrate moieties within the principal cell cytoplasm. This characteristic binding pattern differed considerably from the staining observed in the intercalated cells. Interesting information also emerged about the presence of sialoglycoconjugates having the terminal disaccharide sialic acid-ß-N-acetylgalactosamine originating from the increased SBA binding and the unmodified DBA labelling after removal of sialic acid. Sequential degradation by sialidase/ß-galactosidase followed by incubation with DBA offered the possibility to suspect that the receptor sugar for the penultimate ß-galactose may be N-acetylgalactosamine. Conversely, we were not able to define the acceptor sugar for penultimate ß-GalNAc owing to the lack of availability of ß-N-acetylgalactosaminidase enzyme. When although further studies are clearly needed to elucidate the physiological role of the cellular sialoglycoconjugates detected, the present results already provide valuable insight into the carbohydrate composition of intercalated and principal cells in the quail collecting ducts.

Key words: Quail, Kidney, Sugars, Lectins, Glycosidases, Histophotometry

Introduction

The distribution of carbohydrates in the kidney has been widely explored in many mammalian, reptilian and avian species. Accordingly, it has been proved that renal glycoconjugates show a heterogeneous distribution and seem to be differently involved in the renal function. In the last decade the use of conjugated lectins as histochemical tools has been introduced into carbohydrate histochemistry of the kidney (Holthöfer et al., 1981, 1982; Le Hir and Dubach, 1982a,b; Le Hir et al., 1982; Sato and Spicer, 1982; Murata et al., 1983; Roth et al., 1983; Schulte and Spicer, 1983a; Faraggiana et al., 1984; Kunz et al., 1984; Holthöfer and Virtanen, 1987; Laitinen et al., 1987; Truong et al., 1988) but, with regard to the quail kidney, only a partial characterization has been made. Indeed, the great part of the research has been restricted to the proximal and distal tubules (Holthöfer, 1983).

The present study aimed to investigate the epithelial lining of collecting ducts in quail *(Coturnix coturnix japonica)* kidney by means of lectin histochemistry combined with enzymatic degradation, and supported by histophotometrical evaluation of reactivity patterns. Indeed, an appropriate use of specific lectins and selective exoglycosidases has been shown to represent an useful technique for investigating cell populations, identifying cell surface and cytoplasmic sugar residues, and sequentiating the oligosaccharides that characterize them (Menghi et al., 1985, 1989, 1991, 1992, 1993; Bolognani Fantin et al., 1989; Ito et al., 1988a,b, 1989a,b, 1992; Schulte and Spicer, 1985; Schulte et al., 1985; Menghi and Materazzi, 1994). Previous investigations pointed out the occurrence of cellular heterogeneity within the avian collecting system. Up to date, available data are concerned with morphological and histochemical peculiarities and allow us to discriminate between dark, carbonic anhydrase-rich cells and metachromatic, mucin-secreting cells (Ridderstråle, 1980; Nicholson, 1982; Gabrielli et al., 1990; Laverty and Alberici, 1991). On the basis of an analogy to the well-known heterogeneity of mammalian collecting ducts (Madsen and Tisher, 1986; Holthöfer et al., 1988), the two cell types are here referred to as intercalated cells and principal cells, respectively.

Materials and methods

Tissue collection

Five-month-old quail *(Coturnix coturnix japonica)* were used. After sacrifice, kidneys were dissected out

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and immediately immersed in Carnoy's fluid for 24 h and postfixed in a 2% calcium acetate-4% paraformaldehyde solution (1:1) for 3 h. After routine dehydration, specimens were cleared in xylene and embedded in paraffin wax.

They were then serially sectioned at 5 μm of thickness and mounted on albumin-coated slides.

Lectin histochemistry

Adjacent sections with and without prior exoglycosidase digestion were incubated with horseradish peroxidase (HRP)- labelled lectins (Table 1) as previously detailed (Menghi et al., 1985, 1989). Briefly, after inhibition of endogenous peroxidase activity by immersion with 0.3% H_2O_2/methanol for 30 min, tissue sections were incubated with lectin-horseradish peroxidase (HRP) conjugates (0.02-0.2 mg/ml) dissolved in 0.05M phosphate-buffered saline (PBS), pH 7.2, for 30 min at room temperature. After rinsing in PBS and 0.05M Tris-HCl buffer, pH 7.4, sections were immersed in diaminobenzidine-H_2O_2 medium (Graham and Karnovsky, 1966) for 10 min, dehydrated with graded ethanols and mounted in Eukitt (Bio Optica).

Enzyme digestion

Adjacent sections, before lectin staining, were treated with the following exoglycosidases:

a- Sialidase (neuraminidase, Type V, from *Clostridium perfringens)* was dissolved in 0.1M sodium acetate buffer, pH 5.5, in the presence of 10 mM $CaCl_2$ with a concentration of 0.5 U/ml. Digestion was performed at 37 °C for 16 h (Spicer and Warren, 1960).

b- α–Fucosidase (from bovine epididymis) was dissolved at a concentration of 1 U/ml in 0.1M sodium citrate buffer, pH 6.0, containing 25 mM EDTA. Incubation was carried out at 37 °C for 14 h (Menghi et al., 1985).

c- ß-Galactosidase (from *Escherichia coli*, grade VI) was used at a concentration of 3 U/ml. Enzyme was dissolved in 0.05M citrate buffer, pH 3.9, containing 25 mM EDTA (Menghi et al., 1989).

Table 1. Occurrence of lectin labelling in quail kidney collecting ducts.

LECTINS	NOMINAL SPECIFICITY	OUTER CORTEX				CORTEX-MEDULLA				INNER MEDULLA	
		Intercalated cells		Principal cells		Intercalated cells		Principal cells		Principal cells	
DBA	α-D-GalNAc	(1)	+		-	(1)	+		-		-
Sial/DBA		(1)	+		-	(1)	+		-		-
Sial/ß-Gal/DBA		(1)	+	(1,4)	+/++	(1)	+	(1,4)	+/++		-
α-Fuc/DBA		(1)	+		-	(1)	+		-		-
SBA	D-GalNAc>D-Gal		-	(3,4)	+		-	(3)	+	(2,3)	+/++
Sial/SBA			-	(1,3,4)	+/++		-	(1,3,4)	+/++	(2,3,4)	++
Sial/ß-Gal/SBA			-	(1,3,4)	+/++		-	(1,3,4)	+/++	(2,3,4)	++
PNA	ß-D-Gal(1-3)-D-GalNAc	(1)	+		-	(1)	+		-	(3,4)	+/++
Sial/PNA		(1,2)	+	(3,4)	++/+++	(1)	+	(3,4)	++/+++	(3.4)	+++
α-Fuc/PNA		(1)	+		-	(1)	+		-	(3,4)	+/++
RCA-I	ß-D-Gal(1-4)-D-GlcNAc	(3,4)	-/±	(3,4)	±	(3,4)	±/+	(3,4)	±/+	(3,4)	+
Sial/RCA-I		(3)	-/±	(1,3,4)	+/++	(3)	±/+	(1,3,4)	+/++	(3,4)	++
α-Fuc/RCA-I		(3,4)	-/±	(3,4)	±	(3,4)	±/+	(3,4)	±/+	(3,4)	+
ECA	ß-D-Gal(1-4)-D-GlcNAc		-		-		-		-		-
Sial/ECA			-	(3)	-/±		-	(3)	-/±	(3)	±/+
α-Fuc/ECA			-		-		-		-		-
WGA	ß-D-GlcNAc>>sialic acid	(3,4)	-/±	(3,4)	-/±	(3,4)	±	(3,4)	+	(3,4)	+/++
Sial/WGA		(1,3,4)	-/±	(3)	-/+	(1,3,4)	±	(3,4)	+	(3,4)	+/++
α-Fuc/WGA		(3,4)	-/±	(3,4)	-/±	(3,4)	±	(3,4)	+	(3,4)	+/++
GSA-II	α-D-GlcNAc; ß-D-GlcNAc		-		-		-		-		-
Sial/GSA-II			-		-		-		-		-
Sial/ß-Gal/GSA-II			-		-		-		-		-
α-Fuc/GSA-II			-		-		-		-		-
LCA	α-D-Man>α-D-Glc>α-D-GlcNAc		-		-		-		-		-
α-Fuc/LCA			-		-		-		-		-
Con-A	α-D-Man>α-D-Glc>>α-D-GlcNAc	(1)	++		-	(1)	++		-		-
α-Fuc/Con-A		(1)	++		-	(1)	++		-		-
UEA-I	α-L-Fuc	(1)	±/+		-	(1)	±/+		-		-
Sial/UEA-I		(1)	±/+		-	(1)	±/+		-		-
LTA	α-L-Fuc	(1)	±/+		-	(1)	±/+		-		-
Sial/LTA		(1)	±/+		-	(1)	±/+		-		-

(1): cytoplasm; (2): lumen; (3): apical surface; (4): supranuclear cytoplasm.

Table 2. Histophotometrical evaluation of lectin binding patterns with and without prior glycosidase digestion.

LECTINS	OUTER CORTEX		CORTEX-MEDULLA		INNER MEDULLA
	Intercalated cells	Principal cells	Intercalated cells	Principal cells	Principal cells
DBA	0.1440±0.0089	0.0622±0.0095	0.1537±0.0122	0.0671±0.0260	0.0642±0.0117
Sial/DBA	0.1510±0.0107	0.0619±0.0173	0.1504±0.0087	0.0662±0.0108	0.0631±0.0130
Sial/ß-Gal/DBA	0.1492±0.0129	0.2034±0.0127	0.1518±0.0212	0.1980±0.0083	0.0654±0.0215
α-Fuc/DBA	0.1472±0.0132	0.0618±0.0125	0.1523±0.0133	0.0658±0.0092	0.0637±0.0118
SBA	0.0752±0.0183	0.1552±0.0122	0.0721±0.0205	0.1581±0.0217	0.1580±0.0172
Sial/SBA	0.0744±0.0167	0.1788±0.0219	0.0737±0.0272	0.1790±0.0189	0.1692±0.0220
Sial/ß-Gal/SBA	0.0729±0.0218	0.1769±0.0204	0.0729±0.0218	0.1772±0.0203	0.1718±0.0211
PNA	0.1407±0.0125	0.0618±0.0062	0.1414±0.0289	0.0613±0.0102	0.2528±0.0193
Sial/PNA	0.1424±0.0134	0.4440±0.0457	0.1403±0.0215	0.4250±0.0382	0.5530±0.0758
α-Fuc/PNA	0.1413±0.0082	0.0603±0.0083	0.1422±0.0083	0.0622±0.0110	0.2621±0.0203
RCA-I	0.0921±0.0070	0.1027±0.0087	0.1172±0.0127	0.1191±0.0070	0.1472±0.0125
Sial/RCA-I	0.0897±0.0129	0.1648±0.0127	0.1201±0.0210	0.1714±0.0129	0.1621±0.0083
α-Fuc/RCA-I	0.0913±0.0084	0.1041±0.0122	0.1188±0.0118	0.1215±0.0082	0.1452±0.0073
ECA	0.0463±0.0079	0.0487±0.0128	0.0471±0.0118	0.0511±0.0073	0.0570±0.0127
Sial/ECA	0.0422±0.0108	0.0911±0.0072	0.0435±0.0070	0.0792±0.0083	0.0622±0.0125
α-Fuc/ECA	0.0472±0.0131	0.0493±0.0081	0.0485±0.0120	0.0523±0.0131	0.0585±0.0087
WGA	0.0927±0.0079	0.1081±0.0112	0.0940±0.0128	0.1092±0.0089	0.1180±0.0092
Sial/WGA	0.0970±0.0122	0.1092±0.0128	0.0983±0.0072	0.1129±0.0212	0.1220±0.0182
α-Fuc/WGA	0.0932±0.0127	0.1056±0.0123	0.0918±0.0085	0.1120±0.0079	0.1169±0.0127
GSA-II	0.0498±0.0103	0.0594±0.0077	0.0632±0.0149	0.0580±0.0156	0.0570±0.0081
Sial/GSA-II	0.0482±0.0076	0.0613±0.0113	0.0642±0.0194	0.0587±0.0158	0.0593±0.0129
Sial/ß-Gal/GSA-II	0.0485±0.0135	0.0597±0.0107	0.0638±0.0220	0.0572±0.0087	0.0577±0.0093
α-Fuc/GSA-II	0.0493±0.0072	0.0589±0.0118	0.0627±0.0217	0.0579±0.0124	0.0583±0.0077
LCA	0.0474±0.0120	0.0562±0.0083	0.0593±0.0232	0.0549±0.0079	0.0582±0.0081
α-Fuc/LCA	0.0479±0.0138	0.0580±0.0128	0.0572±0.0120	0.0561±0.0087	0.0593±0.0117
Con-A	0.2870±0.0163	0.0642±0.0078	0.2930±0.0132	0.0653±0.0118	0.0587±0.0128
α-Fuc/Con A	0.2720±0.0182	0.0635±0.0130	0.2780±0.0079	0.0644±0.0131	0.0579±0.0083
UEA-I	0.1151±0.0100	0.0658±0.0100	0.1160±0.0125	0.0609±0.0094	0.0664±0.0125
Sial/UEA-I	0.1146±0.0113	0.0638±0.0152	0.1168±0.0087	0.0618±0.0075	0.0673±0.0089
LTA	0.1182±0.0134	0.0620±0.0127	0.1177±0.0219	0.0611±0.0103	0.0626±0.0094
Sial/LTA	0.1171±0.0085	0.0623±0.0135	0.1203±0.0183	0.0618±0.0190	0.0641±0.0103

Controls

Controls for lectin staining were carried out by incubating sections with lectin PBS buffer with 0.2M of appropriate hapten sugar.

Controls for enzyme digestion were effected to check the buffer influence (Plendl et al., 1989), the specificity of the enzymatic activity, as well as the efficacy of the methodology. To this end sections were incubated with enzyme-free buffer solutions, with the lectins recognizing the sugar detached by enzyme pretreatment, as well as with all lectins in order to verify whether the presence of neighbouring oligosaccharides can influence the affinity of lectins for the respective sugars.

Histophotometrical analysis

Stain intensity of each lectin with and without prior glycosidase digestion was evaluated by using a Zeiss 0.1K histophotometer equipped with scanning stage and data station (Zeiss Apamos program). Intercalated and principal cells of the cortical collecting ducts and principal cells of the medullary collecting ducts were scanned on line with 0.25 µm step at a wavelength of 480 nm. Results are referred to as mean absorbance values ±SD of 50-60 scanned fields.

Results

Intercalated and principal cells of the quail collecting ducts reacted differentially towards the lectins tested and the value of using lectins with similar and different selectivity strongly emerged (Table 1).

Table 2 shows the intensity of affinity patterns quantified histophotometrically prior and after enzymatic digestion.

Controls

The addition of the respective hapten sugars did not produce appreciable staining in control sections.

Enzyme digestions resulted in effective and specific cleavage of the selected sugars. Buffer solutions slightly decreased staining in some cases.

Discussion

The main purpose of this study was to establish the structure, distribution and location of individual carbohydrates and specific terminal sequences that occur within the collecting duct cells of quail kidney. We applied the methods available for visualizing and locating individual glycosidic residues and accomplished them with glycosidase digestion for characterizing specific carbohydrate sequences.

In some cases it was difficult to appreciate a faintly increased or decreased staining as well as to decide whether an apparent lack of reaction indicated the absence of the component or was due to the fact that insufficient lectin was linked to the substrate to produce an appreciable staining or, finally, was attributable to the inaccessibility of the sugar due to steric hindrance. However, to better estimate relative changes in amount we performed quantitative analyses by using an objective histophotometrical method of measurement.

On the basis of non-lectin histochemistry, no acid glycoconjugates were found in intercalated cells as defined by their morphology and stainability (Nicholson, 1982; Gabrielli et al., 1990). Conversely, sialidase- and bacterial and testicular hyaluronidase-labile material was demonstrated in both cortex and medulla principal cells (Gabrielli et al., 1994). Actual findings confirmed previous data; in addition it was found that principal cell sialoglycoconjugates are characterized by the terminal dimers sialic acid-ß-N-acetylgalactosamine (Figs. 3, 4)

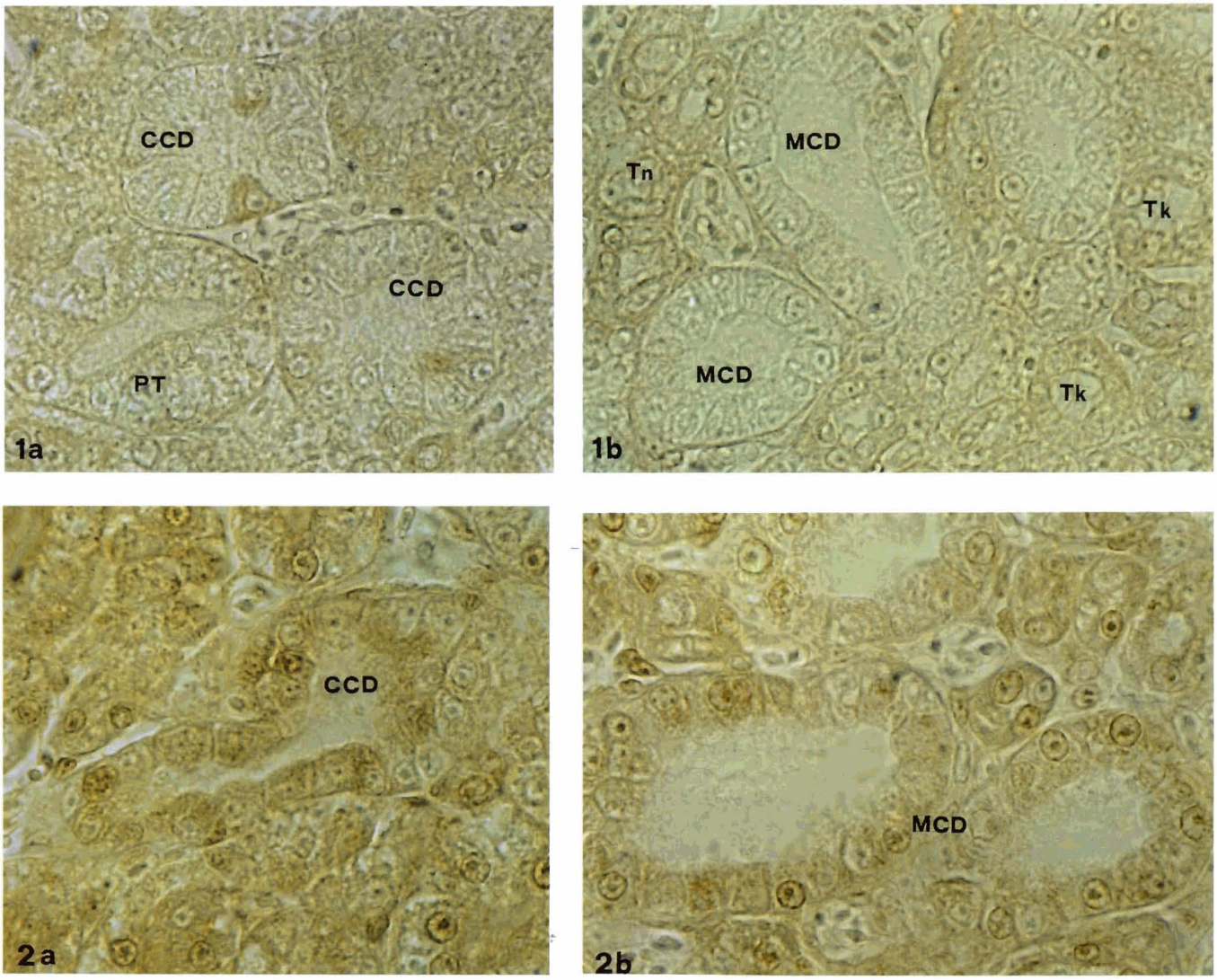

Fig. 1. DBA-HRP staining. **a.** Cytoplasmic staining is only observed at the intercalated cells of cortical collecting ducts (CCD). **b.** Medullay collecting ducts (MCD) do not react. Proximal tubule (PT), thin (Tn) and thick (Tk) segments. x 920

Fig. 2. Sialidase/ß-Galactosidase/DBA-HRP staining. New DBA positive sites are visualized at the principal cell level of both cortex **(a)** and medulla **(b)**. Cortical collecting duct (CCD), medullary collecting duct (MCD). x 920

and sialic acid-ß-galactose (Fig. 5). Sequential degradation of sialoglycoconjugates, mainly located at the principal cell supranuclear region, demonstrated that the acceptor sugar for penultimate ß-galactose is N-acetylgalactosamine (Figs. 1, 2). As far as intercalated cells are concerned, we observed a faint luminal occurrence of sialic acid residues, but we concluded that sialoglycoconjugates occurring at this site could refer to a coherent layer covering the luminal surface of collecting ducts, as previously suggested (Gabrielli et al., 1994).

The sugar which acts as receptor for terminal fucose (Fig. 6) was not identified, probably due to the fact that fucose represents very short lateral chains; accordingly, after enzymatic removal of fucose its subterminal sugar may not be accessible to the respective lectin. However, histological structures such as the medullary thick segments testify for the presence of the terminal dimer fucose-galactose in the quail nephron (Fig. 7). On the other hand, it has been demonstrated in man, by immunohistochemical methods, that a fucosylated N-acetyl-lactosamine [ß-D-galactose-1-4(α-L-fucose 1-3) N-acetyl-D-glucosamine] is localized on different segments of the proximal convoluted tubule in the foetal and adult kidney (Fleming and Brown, 1986).

The heterogeneity of collecting duct cells emerged above all by evaluating lectin labelling correlated with the ability of lectins to disclose only terminal residues or terminal and internal ones. Lectins such as DBA, PNA, Con-A, UEA-I and LTA proved to be markers of the intercalated cells; SBA specifically bound to the principal cells.

Certain lectins have proved to be suitable markers for some types of normal and/or malignant cells (Cooper, 1982; Holthöfer et al., 1982; Nakayama et al., 1985; Suganuma et al., 1985). However, to distinguish betwen

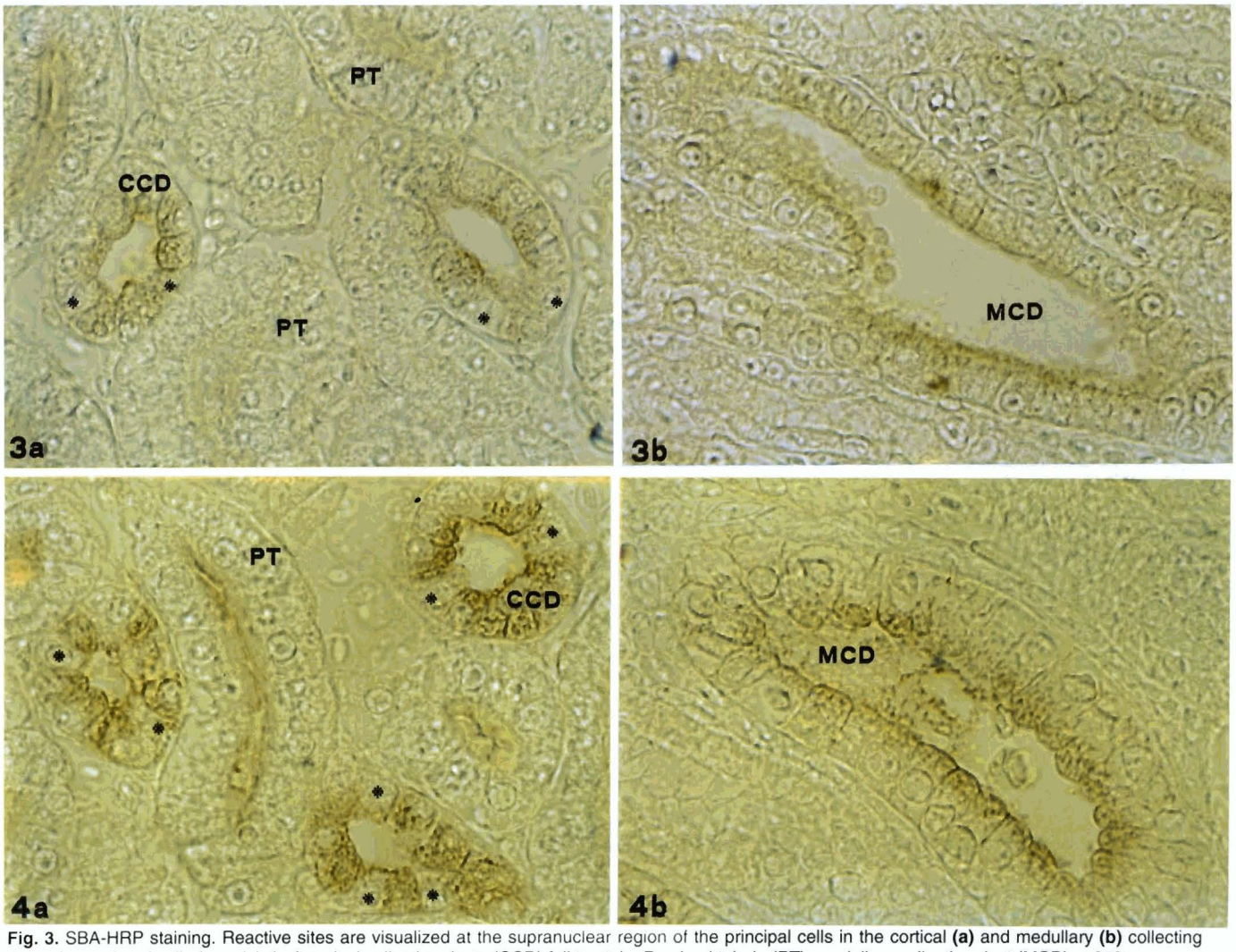

Fig. 3. SBA-HRP staining. Reactive sites are visualized at the supranuclear region of the principal cells in the cortical **(a)** and medullary **(b)** collecting ducts. Intercalated cells (asterisks) of cortical collecting ducts (CCD) fail to stain. Proximal tubule (PT), medullary collecting duct (MCD). x 850

Fig. 4. Sialidase/SBA-HRP staining. Enzymatic removal of sialic acid discloses an increased SBA labelling at the principal cells of cortex **(a)** and medulla **(b)**. Intercalated cells (asterisks) show no affinity. Proximal tubule (PT), cortical collecting duct (CCD), medullary collecting duct (MCD). x 850

different cell populations it is more correct to consider lectins rather than sugars as cell markers. We refer to the possibility of opposite binding, also in the case of very similar nominal specificity, and to the accessibility of lectins towards the respective sugars which could be present but not recognizable.

The best correspondence of staining between two lectins of similar specificity appeared to be that between LTA and UEA-I. It should be considered that Holthöfer (1983) could demonstrate species-dependent differences for UEA-I binding sites in the kidney, in that he found affinity in the distal tubules of the pig, dog, rabbit, and guinea pig kidney but not in the rat or mouse kidney. Conversely, lectins known to have near sugar binding properties, such as DBA and SBA, showed an opposite behaviour; indeed, DBA marked intercalated cells while SBA marked principal ones. DBA may be considered as a specific N-acetylgalactosamine reagent with clear preference for α-linked N-acetylgalactosamine when used as an end-group reagent in structural studies of carbohydrates. In contrast to other lectins, SBA shows no anomeric specificity for N-acetylgalactosamine, and also binds D-galactose (Hammarström et al., 1977; Lis and Sharon, 1986; Ito and Hirota, 1992). Comparison of binding between lectins, thought biochemically to have very similar carbohydrate binding specificity, has repeatedly revealed marked differences in histochemical reactivity (Schulte and Spicer, 1983b, 1984).

A striking contribution of lectin methods, combined with glycosidase digestion, concerns here the possibility to discriminate between sialoglycoconjugates which formerly appeared rather similar on the basis of basic dyes, in contrast to the heterogeneity of sulphated glycoconjugates that were heterogeneously distributed between principal cells of renal cortex and medulla (Gabrielli et al., 1994). In this connection, sial/PNA and sial/ß-gal/SBA binding patterns testified to a marked occurrence of sialoglycoconjugates with terminal sequence sialic acid-ß-galactose-ß-N-acetylgalactosamine in both cortical and medullary principal cells. The

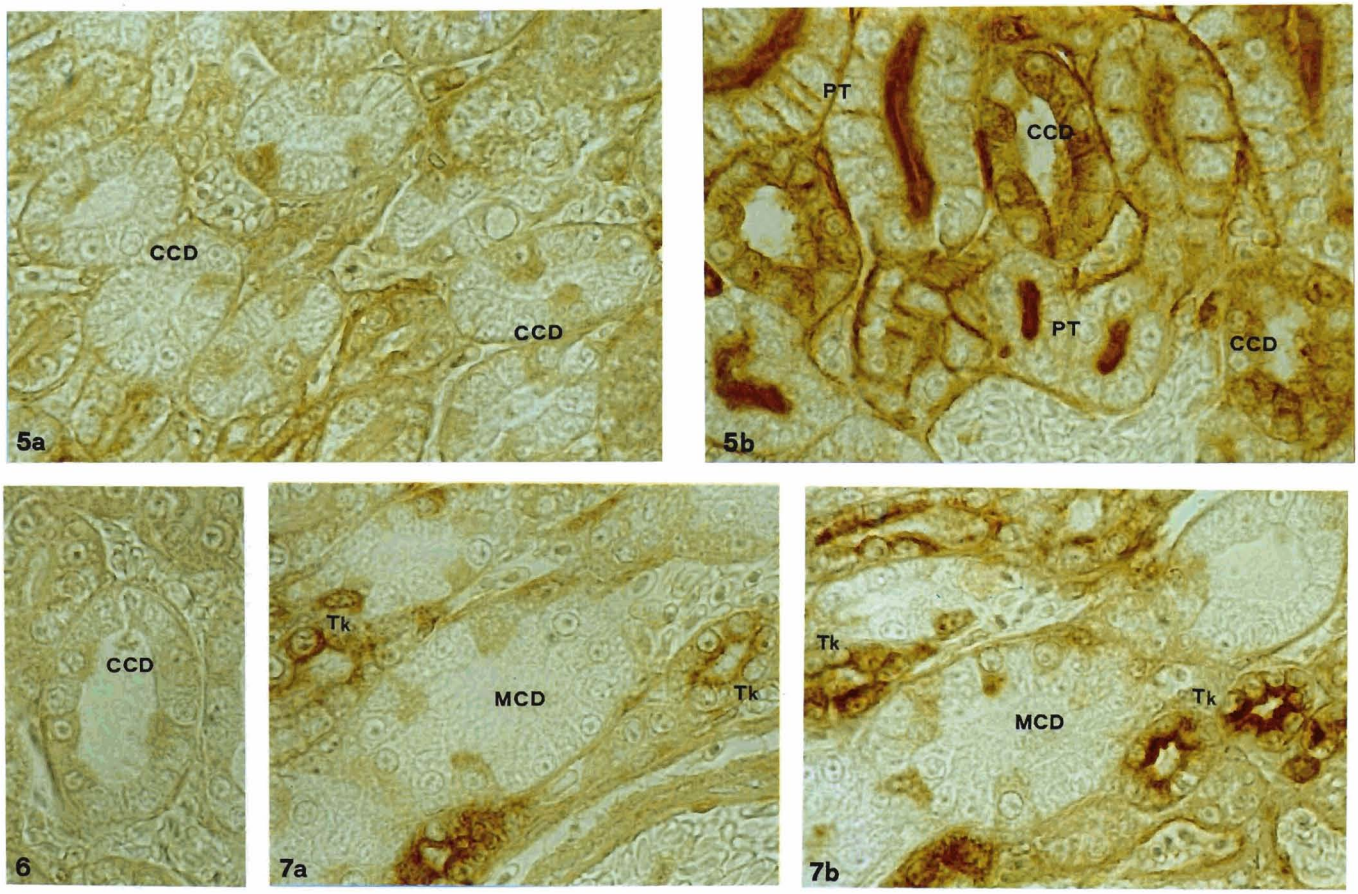

Fig. 5. Renal cortex. **a**. Marked PNA staining is located on the whole cytoplasm of intercalated cells. Principal cells do not react. **b.** Sialic acid cleavage produces the visualization of penultimate ß-Gal residues in the principal cells. Proximal tubule (PT), cortical collecting duct (CCD). x 750

Fig. 6. UEA-I-HRP staining. Renal cortex. Note the faint staining of intercalated cells. Cortical collecting duct (CCD). x 750

Fig. 7. Cortex-medulla. The PNA affinity **(a)** of intercalated cells is found to be unmodified after fucosidase digestion **(b)**. It should be noted that the thick segments (Tk) show an increased binding consequent to the removal of terminal fucose. Medullary collecting duct (MCD). x 750

finding of a general increase in sialic acid-containing molecules on passing from cortex to medulla suggests the association of renal sialoglycoconjugates to functional activities which are increasingly required in the final events of the renal processes resulting in the ureteral urine production. The PNA binding sites, unexpectedly visualized only in the principal cells of inner medulla, besides indicating structural diversity in glycosylated components within collecting duct principal cells, might give further insight into the physiological implications of renal sialoderivatives. In other words, a polarized heterogeneity of principal cells between cortex and medulla may be suspected, probably in relation to an increased synthesis of mucus. Mucoid materials, the so-called «urinary colloids» (Porter, 1963), are in fact reported as common components of both the supernatant and the precipitate fractions of avian urine. They would act as protective colloids to prevent urate precipitation (McNabb et al., 1973; McNabb, 1974) or to lubricate tubular walls when precipitation occurs (Siller, 1971), as well as to participate in the renal control of Na^+ and K^+ (Long and Skadhauge, 1983). In relation to an increasing requirement for mucin secretion, the principal cells of the inner medulla would also expose ß-galactose residues which are transitorily terminal since galactose usually acts as acceptor sugar for sialic acid. Finally, it is worth noting that the principal cells of the inner medulla are lacking of the terminal sequences sialic acid-ß-galactose-α-N-acetylgalactosamine which characterize, conversely, the principal cells of the renal cortex. Probably, these oligosaccharide chains belong to glycoproteins with functions different from those related to mucus production.

Further correlations between specific sugar residues and functional events may be expressed. Thus, fucose has been proposed to be involved in proton pump and sodium chloride transport (Greven and Schreibmüller, 1981; Bradley et al., 1987). Its occurrence in the intercalated cells of the collecting ducts fully fits with the histochemical and physiological data which propose the intercalated cells as an active site for ion transport and urinary pH regulation (Ridderstråle, 1980; Holthöfer et al., 1988; Gabrielli et al., 1990; Laverty and Alberici, 1991; Schuster, 1991).

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