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Monoclonal antibodies against luminal membranes of renal proximal tubules which are kidney-specific

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After immunization with porcine brush-border membrane proteins, 11 monoclonal antibodies were generated which react with proximal tubules. Their antigenic polypeptides were characterized with respect to apparent molecular weight, histochemical localization in porcine and human kidney, and tissue distribution in pig. In porcine kidney, six antibodies bind selectively to the proximal tubule whereas the others also react with other nephron segments. With the exception of one antibody which reacts with the luminal and the basolateral membrane of the porcine proximal tubule, the other antibodies specific for the proximal tubule only stain the brush-border membrane. Four of them react along the entire length of the porcine proximal tubule, whereas one (R1A2) binds to the S3-segment in pig and to the entire length of the proximal tubule in man. This indicates that segment-specific expression may be species-dependent. Testing the antibodies in 21 different extrarenal tissues it was found that three of the antibodies, specific for the brush-border membrane in renal proximal tubules, only react in kidney. Two of these are specific for pig kidney whereas one also reacts with human kidney. This antibody (N4A4) is directed against a polypeptide with an apparent molecular weight of 400 000. Electron microscopic immunohistochemistry showed that N4A4 binds to the intervillus region of the brush-border membrane and to subapical vesicles.

Introduction

Monoclonal antibodies are an important tool to identify, characterize and isolate proteins. Often the histological distribution and prospective function of a protein is defined by monoclonal antibodies before a detailed biochemical investigation of the protein is performed. During our investigations on transport in renal and intestinal epithelial cells [1,2] we tried to raise monoclonal antibodies directed against transport proteins in the luminal cell membrane of porcine renal proximal tubules and hoped to detect an antibody which inhibits one of the Na^+-cotransporters to help in the isolation of the transporter. Another more clinically related line of interest was to produce monoclonal antibodies against defined nephron segments which are kidney-specific and which may be used for the diagnosis of kidney diseases [3]. In our study, mice were immunized with a polypeptide fraction of porcine brush-border membrane vesicles and 200 monoclonal antibodies were identified which bind to brush-border membrane vesicles. None of these antibodies showed inhibition of Na^+-coupled D-glucose-, L-glutamate-, L-lactate- or succinate transport, however, a number of the antibodies reacted with single proteins and showed a specific reaction and tissue distribution in immunohistochemistry. In the present paper, the histochemical localization and the apparent molecular weights of 12 monoclonal antibodies against antigens in proximal tubular cells are described. Six antibodies are specific for the luminal membrane of renal proximal tubules, three of which do not react with extrarenal tissues.

Materials and Methods

Materials. The molecular mass markers IgM (non-reduced, 900 kDa), ferritin (non-reduced, 440 kDa), myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), glyceraldehyde-3-phosphate-dehydrogenase (36 kDa),

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Abbreviations: NHS-LC-biotin, sulfosuccinimidyl 6-(biotinamido)-hexanoate; APAAP, alkaline phosphatase complexed with antibodies against alkaline phosphatase; mAbs, monoclonal antibodies; SDS-PAGE, SDS–polyacrylamide gel electrosphoresis.

carbonic anhydrase (29 kDa) and Triton X-100 were purchased from Sigma (München, Germany), X-Omat S films from Eastman Kodak (Rochester, USA) and immobilized lactoperoxidase from Bio-Rad Laboratories (München, Germany). Reagents for detection of alkaline phosphatase and for counterstaining of tissue sections with hematoxilin were supplied by Dako Diagnostik (Hamburg, Germany). Sulfosuccinimidyl 6-(biotinamido)hexanoate (NHS-LC-biotin) and immobilized streptavidin were obtained from Pierce (Rockford, USA) and protein G Sepharose from Pharmacia (Freiburg, Germany). All other chemicals were obtained as described before [3,4].

Antibodies. Anti-mouse-IgM antiserum and anti-mouse-IgG antiserum from rabbit were provided by Renner (Dannstadt, Germany). Immunoglobulin-heavy-chain-specific antisera from rabbit to mouse IgM, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 and IgA were obtained from ICN Biochemicals (Eschwege, Germany). Alkaline phosphatase-coupled anti-mouse-IgM antiserum from rabbit, anti-mouse-IgG antiserum from rabbit and alkaline phosphatase complexed with mouse antibody against alkaline phosphatase (APAAP-complex) were supplied by Dako Diagnostik (Hamburg, Germany). Rabbit antisera to vinculin, α-actinin, actin, myosin, villin, fimbrin and cytokeratin were provided by Dr. D. Drenckhahn [5,6] and rabbit antisera to proteins PI and PII from porcine intestine by Dr. K. Weber [7].

Monoclonal antibodies (mAbs) against α-tubulin (clone DM1A in Ref. 8) were purchased from Amersham Buchler (Braunschweig, Germany), mAbs against clathrin (clone $C_{HC}5.9$ in Ref. 9) from Progen (Heidelberg, Germany), mAbs against vimentin (clone V9 in Ref. 10) from Boehringer-Mannheim (Mannheim, Germany) and mAbs to Tamm-Horsfall-protein (clone CL 1032) from Cedarlane Laboratories Limited (Hornby,

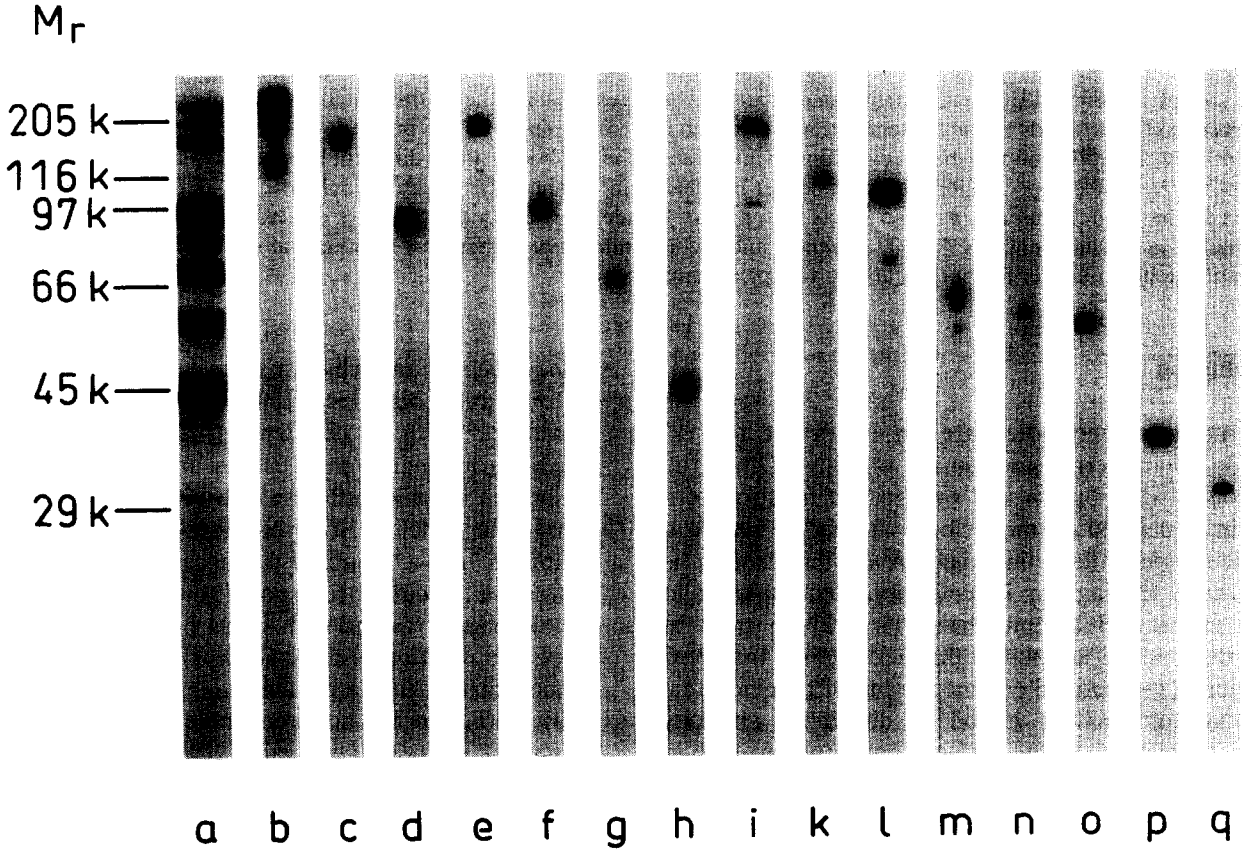

Fig. 1. Determination of apparent molecular weights of renal brush-border proteins in Western blots employing reference antibodies. Purified porcine renal brush-border membranes and molecular mass marker proteins were separated by SDS-PAGE and stained with silver (lane a) or electrically transferred to nitrocellulose. The nitrocellulose was either stained with Amido black (marker proteins indicated by their relative molecular weights) or tested for binding of reference antibodies (lanes b–q). The reference antibodies are the following proteins: aminopeptidase W (b), aminopeptidase N (c), endopeptidase 24.11 (d), myosin (e), villin (f), fimbrin (g), α-actin (h), clathrin-heavy chain (i), vinculin (k), α-actinin (l), cytokeratin (m), vimentin (n), α-tubulin (o), PI-protein (p) and PII-protein (q). Comparing the mobilities of the antigenic polypeptides with those of the marker proteins the following apparent molecular weights were determined for the reference proteins: Aminopeptidase W (240000, 120000), aminopeptidase N (160000), endopeptidase 24.11 (92000), myosin (200000), villin (96000), fimbrin (68000), α-actin (43000), clathrin-heavy chain (180000), vinculin (112000), α-actinin (100000), cytokeratins (64000, 59000, 53000), vimentin (57000), α-tubulin (55000), PI-protein (36000) and PII-protein (32000).

Canada). MAbs against endopeptidase-24.11 (clone GK 7C2 in Ref. 11), aminopeptidase N (clone F35 8C) and aminopeptidase W (clone GK 5C1 in Refs. 12 and 13) were a gift of Dr. A.J. Kenny.

Preparation of brush-border membrane vesicles. Porcine kidneys were obtained from the local slaughterhouse and human kidney material from normal regions of nephrectomized hypernephroma kidneys. Cortical tissue was dissected and brush-border membrane vesicles were prepared as described by Booth and Kenny [14] with the variation that 0.25 mM phenylmethylsulfonyl fluoride and 20 U/ml Trasylol were added.

Protein determination. Protein was determined according to Lowry [15] employing bovine serum albumin as protein standard. Before the measurement the samples were treated with 10% (v/v) trichloroacetic acid, spun down and resuspended in 1 M NaOH containing 1% (w/v) SDS.

Production of monoclonal antibodies against brush-border membrane proteins. Balb/c mice were immunized with a protein fraction which was isolated from porcine brush-border membranes (see PI in Ref. 16). The immunization was performed with combined intraperitoneal and subcutaneous injections of 100 μg of protein on day 1 (with complete Freund's adjuvant) and day 28 (with incomplete Freund's adjuvant). On day 38 the antisera were tested for their reaction with porcine brush-border membranes in Western blots. One mouse which showed strong reactions with several brush-border membrane polypeptides received a third intraperitoneal injection of protein (100 μg without adjuvant) on day 56. Spleen cells were fused on day 59 with mouse myeloma cells of the nonsecreting mouse myeloma cell line X63-Ag 8.6.5.3 [17]. The hybridoma supernatants were screened for binding to brush-border membranes by radioimmunoassay [3] and by light-microscopic immunohistochemistry. The positives were analyzed for binding to specific brush-border membrane polypeptides in Western blots as described earlier [4]. The monoclonal hybridoma cell lines described below were obtained by twice single-cell cloning employing limiting dilution. Their immunoglobulin subclasses were determined as described earlier [2]. The cross-reactivity of the monoclonal antibodies with man was determined by radioimmunoassays with purified human renal brush-border membrane vesicles [3] and by immunohistochemistry.

SDS-PAGE and Western blotting. Brush-border membrane polypeptides and marker proteins were solubilized in SDS and separated in 10% (w/v) SDS polyacrylamide gels which were run according to Laemmli as described earlier [4,18]. The separated proteins were blotted to nitrocellulose, stained with Amido black or reacted with antibodies [4]. For the immunoreactions the blots were primarily incubated with either murine monoclonal IgG- or IgM-antibodies or with polyclonal antisera from rabbit. The reaction of

TABLE I

Characteristics of monoclonal antibodies directed against porcine renal proximal tubules

The apparent molecular weights of antigenic proteins in porcine renal brush-border membrane vesicles were determined in Western blots (N4A4, L3C2, R1A2, N1D3, F1B4, P2D4, P1B6, N10C6) or by immunabsorption (N7A5, L4D6, I2D2, N7C2). The antigen localization was analysed on acetone treated cryosections of porcine kidney employing alkaline phosphatase-coupled rabbit anti-mouse-immunoglobulin antisera or APAAP and demonstration of alkaline phosphatase activity. Cross-reactivity of the antibodies with human kidney was determined by light-microscopic immunohistochemistry and radioimmunoassays with purified brush-border membrane vesicles of human kidney cortex. Abbreviations (for nephron segments, see Ref. 35): GL, glomerulums; PT, proximal tubule; IT, intermediate tubule; DT, distal tubule; DCT, distal convoluted tubule; CCD, cortical collecting duct; INT, interstitium; LM, luminal membrane; BLM, basolateral membrane; CP, cytoplasm; n.d., not detected.

Monoclonal antibody	M_r of antigen ($\times 10^{-3}$)	Antigen localization in the nephron	Subcellular antigen localization	Cross reaction with human kidney
N4A4	400	PT (S1-S3)	LM	yes
N7A5	900, 185	PT (S1-S3)	LM	no
L4D6	440, 50	PT (S1-S3)	LM	no
L3C2	96	PT (S1-S3)	LM	yes
R1A2	180	PT (S3)	LM	yes
N1D3	400	PT (S3), DCT	LM	yes
F1B4	45	PT (S1-S3)	LM, BLM	no
I2D2	130	PT (S1-S3), INT	LM, INT	yes
P2D4	220	PT (S1-S3), IT, DT	BLM	yes
P1B6	220	PT (S1-S3), IT, DT	BLM	yes
N10C6	57	GL [a], PT (S1-S3), IT, DT, CCD	LM [c], CP [d]	yes
N7C2	90	GL [b], PT (S1-S3), IT, DT, CCD	LM, INT	yes

[a] Staining of mesangial cells.
[b] Staining of parietal sheet of Bowman's capsule.
[c] Staining of terminal web region.
[d] In CC cells.

the mAbs was visualized with anti-mouse antibodies from rabbit followed by ^{125}I-labelled protein A, and the reaction of the polyclonal rabbit-antibodies only with ^{125}I-labelled protein A. The incubation of the Western blots with antibodies and ^{125}I-labelled protein A, the washing steps and the autoradiography were performed as described before [4]. The apparent molecular weights of the separated proteins were calculated by determination of their relative mobilities in comparison to those of stained marker proteins.

Immunoabsorption. For immunoabsorption the primary antibodies were biotinylated, incubated with solubilized brush-border membranes and the antigen-antibody complexes were purified by streptavidin affinity chromatography [19,20]. The mAbs N7A5, L4D6, I2D2 and N7C2 isolated from hybridoma supernatants by affinity chromatography on protein G-Sepharose, were biotinylated by incubation (2 h (pH 8.5), 0°C) with a five-fold molar excess of NHS-LC-Biotin and the unreacted NHS-LC-biotin was removed by 16 h (4°C) dialysis against excess of phosphate buffered saline (PBS). Brush-border membrane vesicles containing 100 μg of protein were labelled with 1 mCi ^{125}I by employing immobilized lactoperoxidase as described by the manufacturer, the unbound ^{125}I was removed by gel chromatography on Sepharose G-25 and the iodinated vesicles were solubilized. Thereafter the vesicles were incubated for 30 min (37°C) with 1% (w/v) NP-40 and separated from non-solubilized material by centrifugation at 37000 $\times g$ (30 min, 22°C). 20 μg of the biotinylated antibodies were immobilized on 20 μl of streptavidin beads and incubated (16 h at 4°C) with supernatants of the solubilized ^{125}I-labelled (10^6 cpm) brush-border vesicles. After three washings with 1% (w/v) NP-40 in PBS the beads were boiled 3 min in sample buffer (20 μl) of the Laemmli electrophoresis system [18] and the eluted proteins were applied to SDS-PAGE. After electrophoresis the gels were dried and exposed for 10 days (−20°C) to X-Omat S films.

Comparison of antigenic proteins from the proximal tubule in Western blots. To investigate whether the monoclonal antibodies described below are directed against brush-border membrane polypeptides which have been characterized earlier, some antibodies against brush-border proteins were obtained and the reaction of these reference antibodies in Western blots was compared with the reaction of the new mAbs. Fig. 1 shows a Western blot in which the apparent molecular weights of the antigenic polypeptides of the reference antibodies were determined.

Immunohistochemistry. For light-microscopic investigations immunoreactions were performed on 6-μm thick cryosections which were prepared and blocked as described earlier [3]. For reaction with the mAbs the sections were incubated (30 min, 22°C) with hybridoma supernatants which were diluted 10–100-fold with PBS. Binding of IgM-antibodies was visualized by incubation with alkaline phosphatase-labelled anti-mouse-IgM antiserum from rabbit (diluted 1 : 100 with PBS), whereas binding of IgG-antibodies was visualized by incubation with APAAP-complex as described by Dako Diagnostik (Hamburg, Germany). The alkaline phosphatase activity was analyzed with naphthol as biphosphate and newafuchsin, and the sections were counterstained with hematoxilin.

Electron microscopic immunostaining with F1B4 (Fig. 7) employing preembedding immunostaining and visualization with peroxidase-labelled anti-mouse-IgG serum from sheep was performed as described earlier [3]. The procedure for the immunostaining of plastic-embedded ultrathin sections employing protein A-gold (see Fig. 6) has been described in Ref. 21. No immunostaining was detected in control experiments, in which the primary antibodies were replaced either by hybridoma cell lines producing non-reactive IgG or IgM, or by different concentrations of mouse-myeloma-IgG and -IgM.

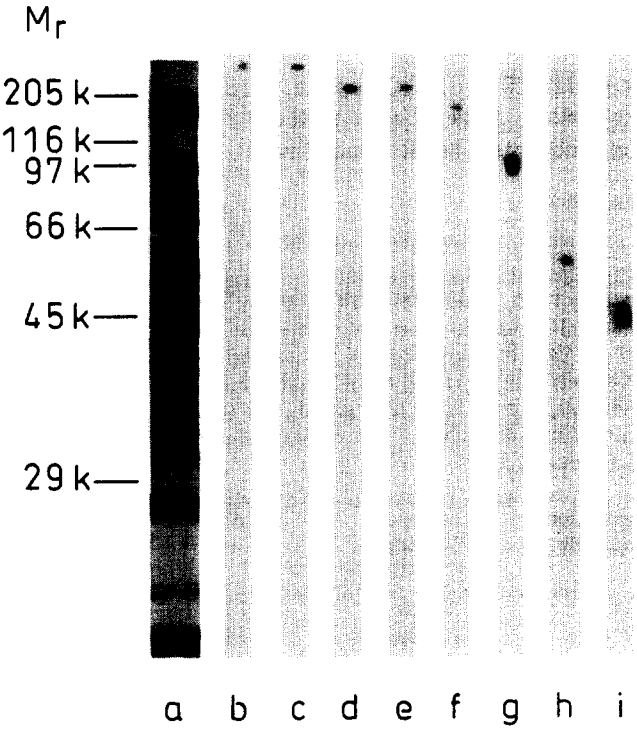

Fig. 2. Identification of antigenic polypeptides of monoclonal antibodies against renal proximal tubules and determination of their apparent molecular weights in Western blots. Porcine renal brush-border membrane vesicles and molecular mass marker proteins were solubilized in SDS, separated by SDS-PAGE and either stained with silver (lane a) or electrically transferred to nitrocellulose. The nitrocellulose was cut into strips which were stained with Amido black (marker proteins indicated by their relative molecular weights) or immunostained with mAbs (b–i). The tested mAbs were: N1D3 (b), N4A4 (c), P2D4 (d), P1B6 (e), R1A2 (f), L3C2 (g), N10C6 (h), F1B4 (i).

Results

Antibody production

Eight mice were immunized with proteins from porcine brush-border membranes (see PI in Ref. 16) and the antisera were tested in Western blots for reaction with brush-border membrane polypeptides. One mouse which showed strong reactions with several brush-border membrane proteins was used for fusion. About 1000 hybridoma supernatants were screened for their binding to brush-border membrane proteins by a radioimmunoassay. The 200 positives were tested for their reaction in Western blots of PI and in tissue sections of pig kidney. 12 hybridoma cell lines were selected, single-cell cloned twice and further characterized. For these antibodies the following antibody classes were determined: N4A4 (IgG_1), N7A5 (IgG_1), L4D6 (IgG_1), L3C2 (IgM), R1A2 (IgM), N1D3 (IgM), F1B4 (IgG_1), I2D2 (IgG_{2a}), P2D4 (IgM), P1B6 (IgM), N10C6 (IgM) and N7C2 (IgG_1).

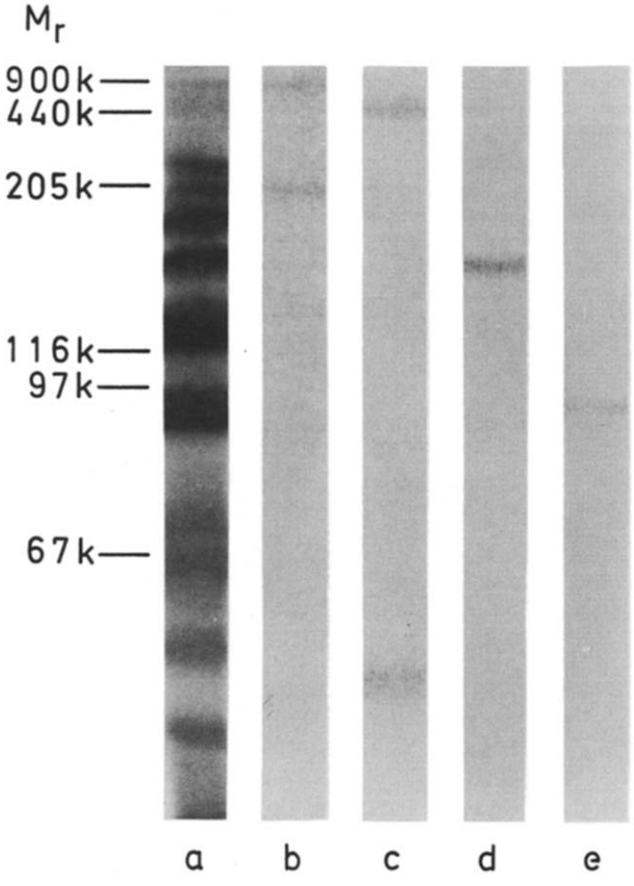

Fig. 3. Immunoabsorption experiments to identify antigenic polypeptides of monoclonal antibodies which do not react in Western blots. The mAbs N7A5, L4D6, I2D2, N7C2 were isolated by affinity chromatography on protein G-Sepharose, biotinylated and bound to streptavidin beads. The antibody-containing beads were incubated with ^{125}I-labelled and solubilized brush-border membrane proteins. After washing, the bound membrane proteins were removed and separated by SDS-PAGE in which marker proteins (indicated by their relative molecular weights) and non-fractionated ^{125}I-labelled brush-border membrane proteins (a) were run in parallel. The marker proteins were stained with Coomassie brilliant blue and the ^{125}I-labelled polypeptides were visualized by autoradiography. The polypeptides which were immunoabsorbed by N7A5 (b), L4D6 (c), I2D2 (d) and N7C2 (e) are indicated.

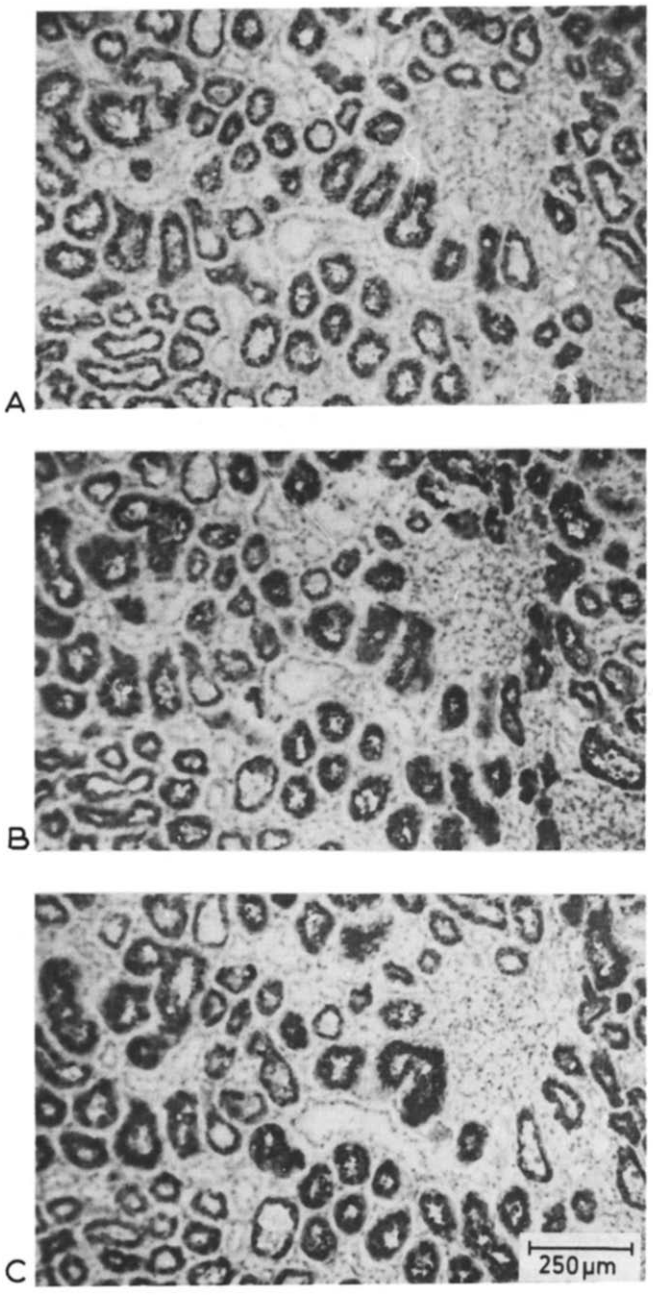

Fig. 4. Light-microscopic immunostaining of porcine kidney cortex with monoclonal antibodies which are kidney-specific and react only with proximal tubules. Serial cryosections through the inner renal cortex were fixed with acetone and incubated with the monoclonal antibodies L4D6 (A), N7A5 (B) and N4A4 (C). The immunoreactions were visualized by incubation with APAAP-complex and demonstration of the alkaline phosphatase activity, and the tissue was counterstained with hematoxilin.

Apparent molecular weights and distinction of antigenic polypeptides

For the monoclonal antibodies the apparent molecular weights of the antigenic polypeptides in the porcine brush-border membrane were determined in Western blots and by immunoabsorption (Table I, Figs. 2 and 3). To investigate whether the antigenic polypeptides are identical to previously described proteins parallel lanes of Western blots were stained with our monoclonal antibodies and with control antibodies (see Fig. 1). The antigenic polypeptides of P2D4 and P1B6 and aminopeptidase W (M_r 240000) could not be distinguished. However, they could be distinguished from myosin (M_r 200000). For the antigens of R1A2 and clathrin (M_r 180000) no difference in mobility was observed, however, since the antigen of R1A2 but not clathrin was solubilized from the brush border membranes after 30 min incubation (22°C) with 5% (v/v) Triton X-100 (data not shown), the antigen of R1A2 is different from clathrin. On the basis of different apparent molecular weights the antigen of L3C2 (M_r 96000) could be distinguished from α-actinin (M_r 100000) and from endopeptidase 24.11 (M_r 92000) and the antigen of L3C2 could be distinguished from villin by a different solubility in Triton X-100 [1] (data not shown). An identical apparent molecular weight was determined for vimentin and the antigen of N10C6 (M_r 57000) [2] which was different from a cytokeratin-component (Fig. 1, lane m, M_r 59000) and from α-tubulin (Fig. 1, lane o, M_r 55000). The apparent molecular weight of the F1B4-antigen (M_r 45000) was larger than that of α-actin (Fig. 1, lane h, M_r 43000).

Immunohistological localization of antigenic polypeptides in kidney

Light-microscopic immunohistochemical investigation showed that the monoclonal antibodies described in this paper bind to proximal tubular cells of pig kidney cortex (Table I). Six antibodies (N4A4, N7A5, L4D6, L3C2, R1A2 and F1B4) react exclusively with proximal tubular cells (see, e.g., Fig. 4) and the binding of one antibody (R1A2) was restricted to the brush border of the S3-segment in pig (Fig. 5). Interestingly, the selective staining of the S3-segment by R1A2 was species-specific, since R1A2 reacted with S1-, S2- and S3-segments in man (data not shown). With one exception (F1B4), the antibodies specific for the proximal tubule bind exclusively to the brush-border, as revealed from light-microscopic immunostaining (see, e.g., Figs. 4 and 5). For two antibodies (N4A4 and F1B4) in which electron-microscopic immunostaining was successful, the light-microscopic localization to the luminal (N4A4) and luminal plus basal membrane (F1B4)

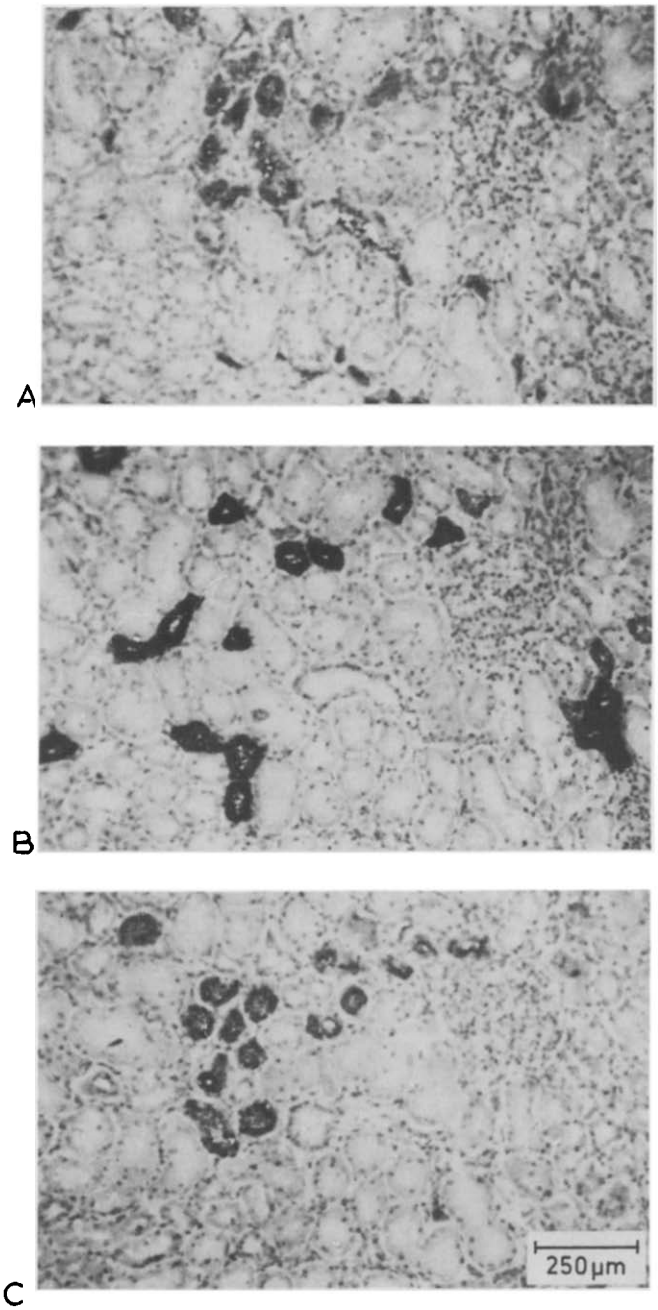

Fig. 5. Staining of the S3-segment of porcine proximal tubule by the monoclonal antibodies N1D3 (A) and R1A2 (C). Serial cryosections through the innermost cortex following those in Fig. 4 are shown. The sections were fixed, immunostained and counterstained as in Fig. 4. The binding of N1D3 and R1A2 can be attributed to S3-segments of proximal tubules by identifying the S3-segments through their neighbouring position to the thick ascending limbs of Henle's loops [33,34] which are identified by their reaction with the monoclonal antibody (CL 1032) directed against Tamm-Horsfall-protein (B).

[1] The antigens of L3C2 and villin can be also distinguished by different tissue distribution (compare Table II of this paper and Table I in Ref. 31).

[2] Since vimentin is restricted to mesenchymal cells [32] and N10C6 reacted with mesenchymal and epithelial cells (Table II), the N10C6-antigen and vimentin are not identical.

was confirmed (Figs. 6 and 7). Binding of N4A4 to the brush-border membrane (Fig. 6) is restricted to the basal parts of the microvilli and to apical endocytotic vesicles whereas F1B4 showed a relatively homogeneous binding to luminal and basolateral membranes of proximal tubules (Fig. 7).

Three of the monoclonal antibodies which are not specific for the proximal tubule exhibit interesting properties. Thus, with N1D3 staining of the luminal membrane in the S3-segment of the proximal tubule and in convoluted distal tubules was observed (Fig. 5). In this case, the specific staining of the S3-segment was also observed in man. Two antibodies (P1B6, P2D4) which are supposed to be directed against an identical antigenic polypeptide labelled the basolateral membrane of the proximal, intermediate and distal tubules [3].

Tissue distribution of the antigenic polypeptides

To further define the prospective use of the generated monoclonal antibodies, the tissue distribution of their antigenic polypeptides was investigated in pig, and the cross-reactivity of the antibodies with man was tested. Considering the antibodies which were specific for the luminal membrane of proximal tubules in kidney (N4A4, N7A5, L4D6, L3C2 and R1A2), it was found that three of them (N4A4, N7A5 and L4D6) were kidney-specific, whereas the other two (L3C2, R1A2) also reacted with epithelial cells in several extrarenal porcine tissues (Table II). Cross-reactivity with man was observed for N4A4, L3C2 and R1A2 but not for N7A5 and L4D6. In the case of R1A2 but not of N4A4 and L3C2, the apparent tissue distribution of the antigen was species-dependent. Thus at variance to pig (Table II), R1A2 did not react with urothelium, prostate, testis and lung in man. Antibody F1B4, which reacts with luminal and basolateral membranes of porcine renal proximal tubules, does not cross-react with man and binds to epithelial cells of several other porcine tissues. A selective species-specific reactivity with epithelial cells was also observed for antibody N1D3 which reacts in porcine and human kidney with the S3-segment of the proximal tubule and with distal convoluted tubules. In pig N1D3 cross-reacts with epithelial cells in breast and testis (Table II), whereas in man cross-reaction with epithelial cells in breast and in the lung was observed. The other antibodies without specificity for the proximal tubule in kidney (I2D2, P2D4, P1B6, N10C6 and N7C2) reacted with epithelial and non-epithelial cells. Two of them (I2D2 and N7C2) bound to interstitial components (Table I).

Fig. 6. Ultrastructural localization of the antigenic polypeptide of mAb N4A4 in the proximal tubule. Ultrathin plastic-embedded sections were etched and reacted with murine mAb N4A4 which was detected with the help of secondary antibodies from rabbit and gold-labelled protein A. The immunreaction was confined to the intervillar region and subapical vesicles at the luminal cell side. The same result was obtained when preembedding immunostaining was employed (see Fig. 1c in Ref. 3).

Discussion

11 different monoclonal antibodies are described which bind to renal proximal tubules. Six antibodies are selective for the porcine proximal tubule and react either with the entire tubule (N4A4, N7A5, L4D6, L3C2 and F1B4) or only with the S3-segment (R1A2). The antibodies bind to the brush-border (N4A4, N7A5, L4D6, L3C2 and R1A2) or to the luminal and basolateral cell side (F1B4). Three of them (N4A4, N7A5 and L4D6) exhibit an absolute specificity for porcine renal tissue. These antibodies are directed against at

[3] Since the IgM-antibodies P1B6 and P2D4 are directed against a polypeptide with the same apparent molecular weight and show an identical species specificity and immunohistochemical staining in kidney cortex and other tissues, they are presumed to be directed against the same antigen. For technical reasons it cannot be excluded that both antibodies are derivatives of the same clone. Since the antigen of P1B6 and P2D4 is localized in the basolateral membrane of renal proximal tubules it is not identical with aminopeptidase W which is localized in the brush-border membrane [12].

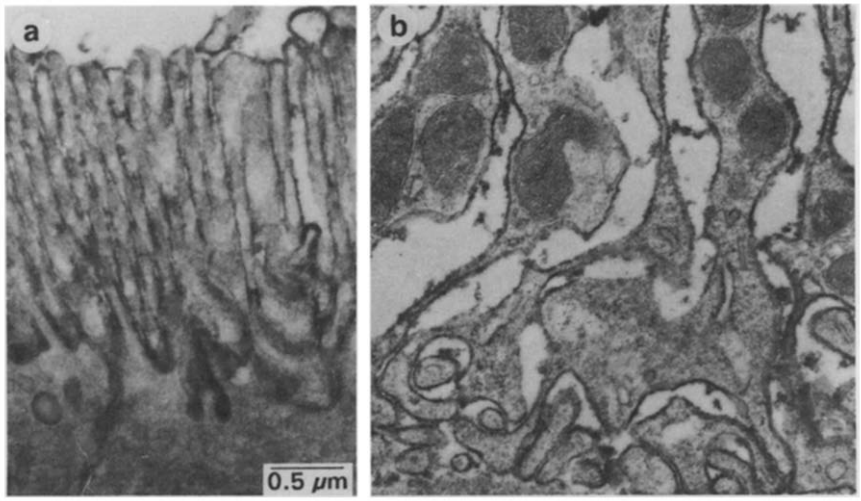

Fig. 7. Ultrastructural localization of the antigenic polypeptide of F1B4 in the proximal tubule. 100-μm thick sections of fixed porcine kidney cortex were incubated with mAb F1B4, reacted with anti-mouse-IgG and peroxidase activity was revealed. Then the sections were fixed for electron microscopy, embedded in plastic and ultrathin sections were prepared. F1B4 binds over the entire length of the brush-border (a) and basolateral membrane (b).

TABLE II

Antigen distribution of monoclonal antibodies against renal proximal tubules in extrarenal tissues of pig

The antigen distribution in extrarenal tissues was analysed by light-microscopic immunohistochemistry on acetone treated cryosections of various normal porcine tissues which was performed as in Fig. 4.

Normal tissues	Monoclonal antibody											
	N4A4	N7A5	L4D6	L3C2	R1A2	N1D3	F1B4	P2D4	P1B6	N10C6	I2D2	N7C2
Epithelial cells of:												
Urothelium	–	–	–	+	+	–	+	+	+	+	–	–
Prostate	–	–	–	–	+	–	–	+	+	+	+	–
Testis	–	–	–	–	+	+	+	+ [a]	+ [a]	+	–	–
Breast	–	–	–	–	+	+	–	+	+	+	–	+
Uterus	–	–	–	–	–	–	–	+	+	+	–	–
Placenta	–	–	–	+	+	–	–	–	–	+	–	–
Lung	–	–	–	+	+	–	–	+	+	+	+	–
Esophagus	–	–	–	–	–	–	+	–	–	+	+	–
Small intestine	–	–	–	+	–	–	+	+ [a]	+ [a]	+	+	–
Colon	–	–	–	+	–	–	+	+	+	+	+	–
Pancreas	–	–	–	–	–	–	+	+	+	+	+	–
Liver	–	–	–	–	–	–	+	–	–	+	+	–
Skin	–	–	–	–	–	–	–	–	–	–	–	–
Thyroid	–	–	–	–	–	–	–	–	–	+	–	–
Parotis	–	–	–	+	+	–	–	+	+	+	+	–
Non-epithelial cells of:												
Spinal cord	–	–	–	–	–	–	–	–	–	–	–	–
Spleen	–	–	–	–	–	–	+ [b]	–	–	+ [b]	–	–
Heart muscle	–	–	–	–	–	–	–	–	–	+	–	–
Smooth muscle	–	–	–	–	–	–	–	–	–	–	–	–
Striated muscle	–	–	–	–	–	–	–	–	–	+	–	–
Endothelium	–	–	–	–	–	–	–	+	+	+	+	–
Extracellular antigens:												
Interstit. matrix	–	–	–	–	–	–	–	–	–	–	+	+

[a] Basal epithelial layer.
[b] Sinusoids.

least two different antigenic proteins. Thus, for N4A4 and L4D6 antigenic polypeptides with similar apparent molecular weights (400000 and 440000) [4] were estimated whereas the antigenic polypeptides of N7A5 (900000 and 185000) are different [5]. For the three kidney-specific antibodies an identical light-microscopic immunohistological staining pattern was observed in porcine kidney sections (Fig. 4). One of these antibodies (N4A4) cross-reacts with man where it shows an identical immunostaining and kidney specificity as in pig (data not shown). The subcellular antigen localization in pig was determined for N4A4 which bound to the intermicrovillar domain in the brush-border and to subapical vesicles of proximal tubular cells. This subcellular localization in the proximal tubule is similar to that described for the antigen of Heyman nephritis gp330 [22–24]. However, the N4A4-antigen is not identical with gp330, since at variance to antibodies directed against gp330 [22,25] N4A4 does not label the epididymis (data not shown) and pneumocytes type II (Table II). Kidney-specific antigens from the proximal tubule which are neither present in intestinal epithelial cells like, e.g., villin [26] nor in any other tissue have not been described before. The only kidney-specific protein known so far is Tamm-Horsfall protein which is synthesized in the thick ascending limb of Henle's loop [27]. The immunological targeting of kidney-specific antigens may become important for the non-invasive detection and attack of kidney-derived tumors. Therefore, the above described antigens may be useful, since it was shown that antibody N4A4 crossreacts with human kidney and reacts with human nephroblastomas [28] and human renal cell carcinomas (data not shown).

The S3-segment of proximal tubules is a preferred target for toxic effects of chemical substances and drugs [29] which are supposed to induce the release of proteins from this segment into the urine [3]. When antibodies against S3-segment-specific polypeptides are available the urinary diagnosis of early renal dysfunction may become possible. Recently, such an antibody has been described [30] and is now tested in clinical settings. In our paper, a mAb (R1A2) against another S3-type-specific polypeptide has been prepared. However, since the S3-specific expression of this antigen was species-dependent being only observed in pig but not in man, the clinical application of this mAb is excluded. The antigen of another mAb (N1D3) is expressed in the S3-segment of the porcine and human proximal tubule but binds also to the distal convoluted tubule. In this case the nephron segment-specificity of N1D3 is identical in pig and man whereas the tissue-specificity is different. Thus, for the N1D3-antigen extrarenal expression in pig was observed in testis and breast, whereas in man extrarenal expression was observed in breast and lung.

In discussing specific expression of antigenic polypeptides on the basis of antibody reactions it must be stated that one cannot distinguish whether a certain polypeptide is absent or may exist as an isoform when no reaction with the mAb is observed. To differentiate between these possibilities the presented antibodies may be used to help in cloning and sequence determination of their antigenic polypeptides. Thereafter, hybridization experiments and the isolation of homologous clones from different tissues may be performed to better understand the molecular basis of the specific expression of renal membrane proteins.

[4] The small difference in apparent molecular weights may not be significant, since it was determined in separate experiments. The finding that L4D6 also reacts with a smaller protein (M_r 50000) may be due to cross-reactivity of L4D6 with an unrelated protein or to the reaction with a proteolytic splitting product of the M_r 440000 antigen. It does not exclude the binding of N4A4 and L4D6 to the same polypeptide.

[5] Since the immunohistochemical staining pattern of N1D3 was different from that of N4A4 and L4D6 (Tables I and II), N1D3 is probably directed against a different M_r 400000 polypeptide than N4A4 and L4D6.

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References

1 Koepsell, H. and Seibicke, S. (1990) Methods In Enzymol. 191, 583–605.
2 Koepsell, H., Korn, K., Raszeja-Specht, A., Bernotat-Danielowski, S. and Ollig, D. (1988) J. Biol. Chem. 263, 18419–18429.
3 Birk, H.-W., Piberhofer, S., Schütterle, G., Haase, W., Kötting, J. and Koepsell, H. (1991) Kidney Int. 40, 823–837.
4 Birk, H.-W. and Koepsell, H. (1987) Anal. Biochem. 164, 12–22.
5 Drenckhahn, D. and Dermietzel, R. (1988) J. Cell Biol. 107, 1037–1048.
6 Drenckhahn, D. and Franz, H. (1986) J. Cell Biol. 102, 1843–1852.
7 Gerke, V. and Weber, K. (1984) EMBO J. 3, 227–233.
8 Blose, S.H., Meltzer, D.I. and Feramisco, J.R. (1984) J. Cell Biol. 98, 847–858.
9 Bruder, G. and Wiedenmann, B. (1986) Exp. Cell Res. 164, 449–462.
10 Osborn, M., Debus, E. and Weber, K. (1984) Eur. J. Cell Biol. 34, 137–143.
11 Gee, N.S., Matsas, R. and Kenny, A.J. (1983) Biochem. J. 214, 377–386.
12 Gee, N.S. and Kenny, A.J. (1987) Biochem. J. 246, 97–102.
13 Gee, N.S. and Kenny, A.J. (1985) Biochem. J. 230, 753–764.
14 Booth, A.G. and Kenny, A.J. (1974) Biochem. J. 142, 575–581.
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.

16 Koepsell, H., Menuhr, H., Ducis, I. and Wissmüller, T.F. (1983) J. Biol. Chem. 258, 1888–1894.
17 Köhler, G. and Milstein, C. (1975) Nature 256, 495–497.
18 Laemmli, U.K. (1970) Nature 227, 680–685.
19 Updyke, T.V. and Nicolson, G.L. (1984) J. Immunol. Methods 73, 83–95.
20 Gretch, D.R., Suter, M. and Stinski, M.F. (1987) Anal. Biochem. 163, 270–277.
21 Haase, W. and Koepsell, H. (1989) Eur. J. Cell Biol. 48, 360–374.
22 Kerjaschki, D., Noronha-Blob, L., Sacktor, B. and Farquhar, M.G. (1984) J. Cell Biol. 98, 1505–1513.
23 Rodman, J.S., Mooseker, M. and Farquhar, M.G. (1986) Eur. J. Cell Biol. 42, 319–327.
24 Chatelet, F., Brianti, E., Ronco, P., Roland, J. and Verroust, P. (1986) Am. J. Pathol. 122, 500–511.
25 Chatelet, F., Brianti, E., Ronco, P., Roland, J. and Verroust, P. (1986) Am. J. Pathol. 122, 512–519.
26 Bretscher, A., Osborn, M., Wehland, J. and Weber, K. (1981) Exp. Cell Res. 135, 213–219.
27 Ronco, P., Brunisholz, M., Geniteau-Legendre, M., Chatelet, F., Verroust, P. and Richet, G. (1987) Adv. Nephrol. 16, 231–250.
28 Altmannsberger, M., Birk, H.-W., Wagner, F., Fitz, H. and Osborn, M. (1988) Verh. Dtsch. Ges. Path. 72, 150–163.
29 Kreisberg, J.I. and Venkatachalam, M.A. (1988) in Morphologic factors in acute renal failure, pp. 45–65, Churchill Livingstone, New York.
30 Verpooten, G.F., Nouwen, E.J., Hoylaerts, M.F., Hendrix, P.G. and De Broe, M.E. (1989) Kidney Int. 36, 617–625.
31 Robine, S., Huet, C., Moll, R., Sahuquillo-Merino, C., Coudrier, E., Zweibaum, A. and Louvard, D. (1985) Proc. Natl. Acad. Sci. USA 82, 8488–8492.
32 Steinert, P.M., Jones, J.C.R. and Goldman, R.D. (1984) J. Cell Biol. 99, 22s-27s.
33 Kriz, W. and Koepsell, H. (1974) Z. Anat. Entwickl.-Gesch. 144, 137–163.
34 Kriz, W. (1967) Z. Zellforsch. 82, 495–535.
35 Kriz, W. and Bankir, L. (1988) Kidney Int. 33, 1–7.