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Reconsideration of the development of the distal tubule of the human kidney

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ABSTRACT

The human kidney develops from 2 embryonic tissues, the ureteric bud and the metanephric blastema. The site in the adult renal distal tubule corresponding to the junction between these tissues has never been established unequivocally and is usually said to be the union between the collecting duct and the connecting piece, based on microdissection evidence. We have examined kidneys from 21 human fetuses of various ages using an immunohistological method for substances related to the ABO blood group system, various cytokeratins including those detected by the monoclonal antibody PKK2, and Tamm–Horsfall protein. The ureteric bud and connecting piece expressed the type 1 precursor chain of ABO antigens mostly early in gestation, the H antigen of the ABO system mostly later in gestation, and cytokeratins detected by PKK2. The induced nephrons after the S-shaped body stage expressed Tamm–Horsfall protein. In the adult renal tubule, distal from the macula densa, it was already known that there is a sharp junction between the segment expressing Tamm–Horsfall protein and the more distal segment that expresses the H antigen and cytokeratins detected by PKK2. The finding that the ureteric bud and connecting piece express the same antigens as this segment while the S-shaped body eventually expresses Tamm–Horsfall protein is consistent with the concept that (1) the connecting piece arises from the ureteric bud, not the S-shaped body, and (2) the junction of ureteric bud derivatives and metanephric blastema derivatives is on the distal side of the macula densa at the distal end of Tamm–Horsfall staining.

INTRODUCTION

There is no dispute that the human kidney develops by growth and branching of the ureteric bud with the induction of nephrons from the metanephric blastema. What has not previously been shown satisfactorily is the site in the adult kidney that corresponds to the junction between the branches of the ureteric bud and the induced nephrons. Oliver (1968) and Potter (1972), who both based their interpretations on microdissection, said that this junction was between the connecting piece and the collecting duct, and most accounts of renal development repeat this statement. The connecting piece or tubule is the term used by Oliver and Potter to refer to the segment that shows marked elongation soon after the junction of the ampulla of the ureteric bud and the early S-shaped body and eventually runs from near the glomerulus, distal to the macula densa, to the straight collecting duct as it passes radially through the cortex (Fig. 1).

The published evidence on which Oliver and Potter made their assertion can be interpreted in other ways. We have previously studied the adult human kidney using an immunohistological method and have shown that there are 2 fundamentally different parts in the tubule on the distal side of the macula densa, with a sharp junction between them. The proximal part contains Tamm–Horsfall protein, the main protein in normal urine. The distal part marks with the anticytokeratin antibody PKK2 and the lectin UEA-1, with a gap before the gradual onset of staining with other anticytokeratin antibodies (Howie & Johnson, 1992). To test whether these parts have any relation to the development of the kidney, we applied the immunohistological method to sections of human fetal kidneys, using the most informative reagents in our previous study which were antibodies to Tamm–

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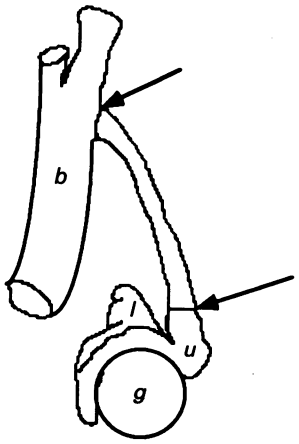


Fig. 1. Diagram of nephron development based on the illustrations of Potter (1972), showing a developing S-shaped body attached to a ureteric bud (b); g, glomerulus; l, loop of Henle; u, upper limb of the S-shaped body. The segment between the arrows is the connecting piece, stated by Oliver (1968) and Potter (1972) to be derived from the upper limb of the S-shaped body. Evidence in this paper suggests that it may be derived from the ureteric bud.

Horsfall protein and cytokeratins, and markers of antigens related to the ABO blood group system, as described by Fudenberg et al. (1978) and Gooi et al. (1983).

MATERIALS AND METHODS

Kidneys

Kidneys at various stages of gestation from 14 to 38 wk were taken at 21 necropsies following spontaneous abortion or stillbirth or neonatal death. Samples were fixed in 10% formol–saline and processed into paraffin wax. In 5 cases, small pieces were frozen in liquid nitrogen and stored at −70 °C.

Immunohistological methods

Frozen blocks were cut in a cryostat to give sections ~ 5 μm thick. These were dried in air for 4 h, fixed in acetone for 10 min, and then used in the first stage of the immunohistological method without further pretreatment. Paraffin blocks were cut to give sections ~ 5 μm thick that were dried on glass slides, dewaxed in xylene and industrial methylated spirit, and washed in water. Before reaction with monoclonal antibody AE1, sections were pretreated with trypsin by immersion for 15 min at 37 °C in a solution of 0.1% trypsin in phosphate-buffered saline, pH 7.4 (PBS). Some sections to be reacted with markers of ABO-related antigens were pretreated with neuraminidase, 1 U/ml in veronal acetate buffer, pH 5, for 2 h at room temperature, as neuraminidase can alter the apparent distribution of carbohydrate antigens obscured by sialic acid residues (Howie & Brown, 1985). Endogenous peroxidase activity in sections was blocked either by covering them with a solution of 0.5% hydrogen peroxide in methanol for 10 min, or by sequential immersion in 2.5% hydrogen peroxide in water for 5 min, 0.1 M periodic acid in water for 5 min, and 0.02% potassium borohydride in water for 2 min (Heyderman, 1979). Reagents used in the first stage of the immunohistological method were as follows, diluted if appropriate in PBS containing 1% ovalbumin: mouse monoclonal antibodies FC10.2, undiluted tissue culture supernatant (M. J. O'Hare, Institute of Cancer Research, Sutton, Surrey), AGF4.48, undiluted tissue culture supernatant (G. Brown, University of Birmingham), 111127, undiluted tissue culture supernatant (Seraclone), C14/1/46/10, 1:100 purified immunoglobulin (L. Durrant, University of Nottingham), PKK2, 1:100 purified immunoglobulin (Labsystems), and AE1, 1:100 purified immunoglobulin (ICN Immunobiologicals); sheep anti-Tamm–Horsfall protein, 1:1000 purified immunoglobulin (Binding Site); and lectin *Ulex europaeus* type 1 (UEA-1), 1:50 (Dako). Second-stage reagents, used as appropriate, were sheep antimouse immunoglobulins, peroxidase-conjugated, 1:100 (Binding Site); donkey antisheep immunoglobulins, peroxidase-conjugated, 1:100 (Binding Site); and rabbit anti-UEA-1, peroxidase-conjugated, 1:100 (Dako). Sections were covered with reagents and rocked at room temperature for 30 min in each stage. Between the stages and at the end, sections were washed for 15 min in a bath of PBS with 0.001% polyoxyethylene 10 oleyl ether detergent (Heyderman, 1979). Tetra-amino-biphenyl hydrochloride (diaminobenzidine), 0.1% in PBS, with 0.01% hydrogen peroxide, was used to detect peroxidase activity. Sections were counterstained with Mayer's haemalum.

Conditions found to be necessary for successful immunohistological staining are given in Table 1.

Controls

With each batch of fetal kidneys, a section of normal adult kidney known to react with the first-stage reagent was included as a positive control. For carbohydrate antigens such as the ABO blood group system, a negative control was use of the periodic acid method of blocking endogenous peroxidase, which destroyed the antigen (Manners, 1961). For the reagents whose appropriate ligand was available, negative controls were studied by use of the reagents absorbed with their ligands. Tamm–Horsfall protein

Table 1. *Conditions necessary for reagents used in immunohistological study of fetal kidneys*

Reagent	Type of section	Pretreatment	Peroxidase block
FC10.2	Paraffin	±neuraminidase	H_2O_2 in methanol
AGF4.48	Paraffin	±neuraminidase	H_2O_2 in methanol
111127	Paraffin	±neuraminidase	H_2O_2 in methanol
C14/1/46/10	Paraffin	±neuraminidase	H_2O_2 in methanol
PKK2	Frozen	None	None
AE1	Paraffin	Trypsin	H_2O_2 in methanol
Anti-Tamm–Horsfall protein	Paraffin	None	Periodic acid
Lectin UEA-1	Paraffin	±neuraminidase	H_2O_2 in methanol

was prepared from human urine (Tamm & Horsfall, 1952); 6 mg Tamm–Horsfall protein were coupled to 1 g cyanogen bromide-activated Sepharose following the manufacturer's instructions (Pharmacia). Anti-Tamm–Horsfall protein was passed through a column packed with Tamm–Horsfall protein–Sepharose and the eluted fractions used in the immunohistological method. To a solution of UEA-1 lectin at its working dilution, α-L-fucose was added to give a concentration of 0.2 M, and the mixture was used in the immunohistological method.

RESULTS

All the kidneys were normal and showed nephrons at various stages of development from the S-shaped body stage in the outer cortex to virtual maturity in the inner cortex. Connecting pieces were identified as those parts running from the ampullae of the ureteric bud to S-shaped bodies and those tubules joining the straight collecting ducts in the cortex at right angles (Oliver, 1968; Potter, 1972). Control adult kidneys had patterns of staining as previously reported (Howie & Johnson, 1992). Reagents adsorbed with appropriate ligands showed no staining of sections, and the periodic acid method abolished reactivity of ABO-related antigens.

Markers of blood-group-related antigens

Monoclonal antibody FC10.2 against the type 1 precursor chain of ABO antigens (Gooi et al. 1983) reacted with the ureteric bud from the medulla to the capsular surface in kidneys from 3 fetuses of under 26 wk, and 1 of 31 wk. The ampulla of the ureteric bud was strongly stained and its junction with the unstained S-shaped body was seen. The connecting piece was stained (Fig. 2). In 2 fetuses of 26 and 34 wk, the outer cortical ureteric bud, ampulla and connecting piece were the only parts of the kidney that stained, as reactivity was not seen in the medulla (Figs 3, 4). There was no staining in the cortex of adult kidneys. Neuraminidase had no effect on the pattern of staining with FC10.2.

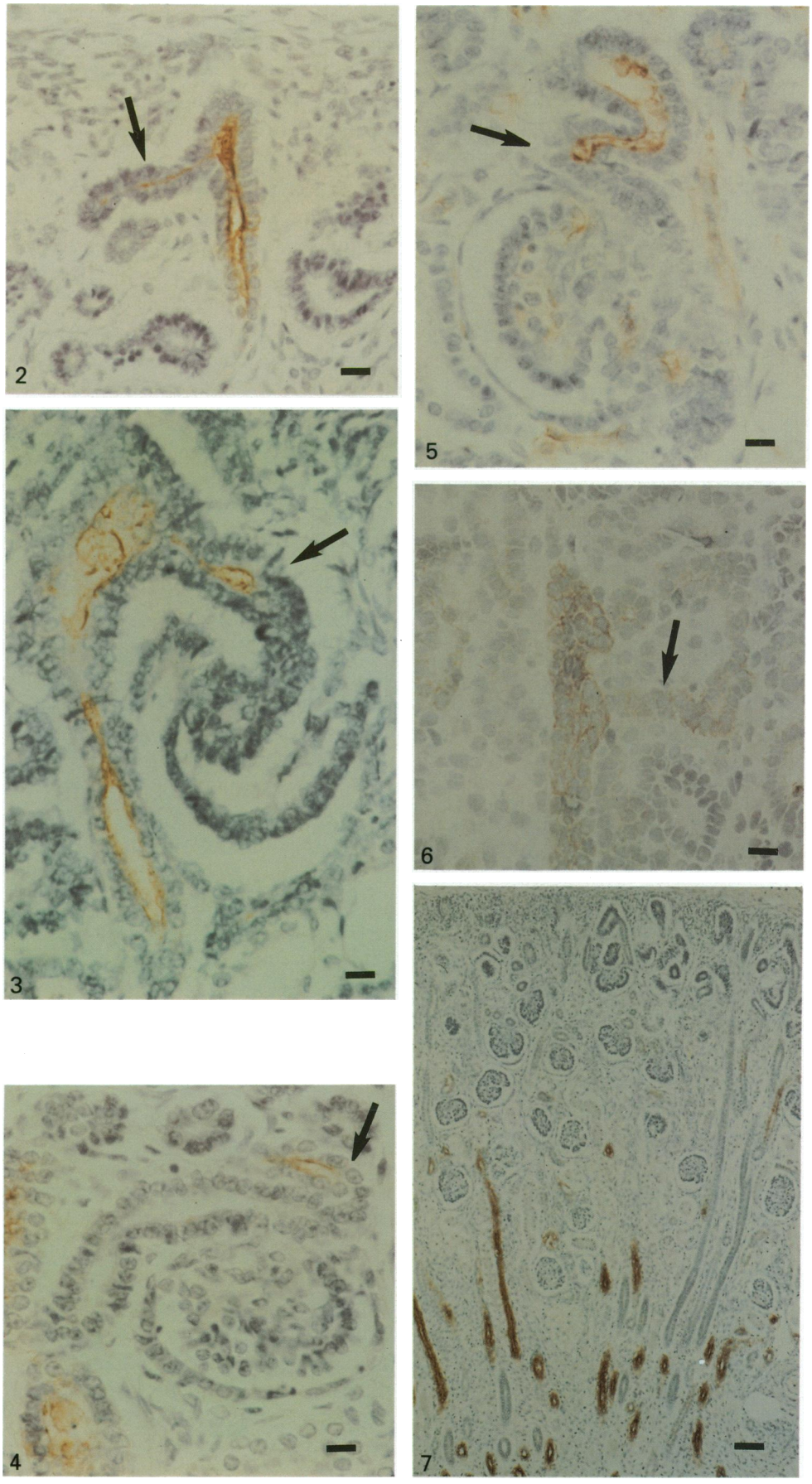
Lectin UEA-1, that recognises the H antigen of blood group O (Pereira et al. 1978), was similar to FC10.2 in that it marked the ureteric bud, but roughly complementary in that in 5 fetuses of 26 wk or less there was little staining with UEA-1 except in inner medullary collecting ducts, but in 5 fetuses, 3 under 26 wk and 2 over, UEA-1 stained the ureteric bud strongly, including the ampulla and connecting piece (Fig. 5). UEA-1 did not react with the S-shaped body but unlike FC10.2 stained endothelium. Neuraminidase had no effect on UEA-1 staining. Another 5 fetal kidneys showed no epithelial staining with UEA-1, presumably because they were from nonsecretors (Oriol et al. 1980; Cordon-Cardo et al. 1986).

Other markers of ABO-related antigens were not informative about the relation between the ureteric bud and S-shaped body. Monoclonal antibody 111127, against Lewis a antigen on type 1 chains, stained the ureteric bud in the inner medulla and interrupted patches on the cortical ureteric bud in 3 kidneys, with or without neuraminidase. Monoclonal antibody AGF4.48, against Lewis a antigen on type 2 chains, also known as Lewis x (Howie et al. 1984), reacted with thin limbs of the loop of Henle and parts of proximal tubules without neuraminidase, but after neuraminidase reacted with thin limbs and all proximal tubules, in all 4 kidneys examined, that is, irrespective of secretor status. Monoclonal antibody C14/1/46/10 against Lewis b antigen on type 2 chains, also known as Lewis y (Brown et al. 1983), was similar to AGF4.48, and also stained patchily the medullary collecting ducts in 2 kidneys examined.

Antibodies to cytokeratins

Monoclonal antibody PKK2, against cytokeratins 7, 16, 17 and 19, only reacted with frozen sections. All 5 fetal kidneys stained and all had strong reactivity throughout the ureteric bud and connecting piece but not in the early S-shaped body (Fig. 6). As the nephron matured, staining appeared in the parietal epithelium of Bowman's capsule, the straight proximal tubule and the thin limb of the loop of Henle.

Monoclonal antibody AE1, against cytokeratins 14, 15, 16 and 19, reacted with paraffin sections after trypsin pretreatment. The ureteric bud and connecting piece were stained at all stages of gestation in 9 kidneys. The early S-shaped body was unstained but



Figs 2–7. For legends see opposite.

as it developed, reactivity appeared in a distribution similar to that of PKK2.

Antibody to Tamm–Horsfall protein

No reactivity was seen in early S-shaped bodies but appeared in thick limbs as loops of Henle began to form, developing first around the macula densa region, where the tubule was attached to the base of the glomerulus (Fig. 7). All 16 fetal kidneys examined showed reactivity for Tamm–Horsfall protein.

DISCUSSION

These findings are consistent with the idea that the connecting piece develops from the ureteric bud and that the tubule distal from the junction shown in the adult kidney (Howie & Johnson, 1992) is derived from the ureteric bud, and proximal to the junction, from the metanephric blastema.

Most accounts of the development of the distal tubule are based on the views of Oliver (1968) and Potter (1972), both of whom used microdissection and therefore interpreted events from the external appearance of tubules. They stated that the connecting tubule is derived from the S-shaped body, not the ureteric bud. Oliver said that a distinct narrowing at their junction marked the point of union of the connecting tubule and the collecting duct, by which he meant the straight part of the collecting system in the cortex. Potter did not define the point of union of connecting piece and collecting duct, but her diagrams are similar to those of Oliver (Fig. 1). Bulger & Dobyan (1982) indicated that the views of Oliver and Potter are not necessarily correct: 'Embryological studies to determine whether the connecting tubule is derived from the metanephric blastema or the ureteric bud would also help in classifying this region.'

Several problems arise from the interpretation of Oliver and Potter. One is that the cells of the connecting tubule resemble the cells of the collecting duct structurally and functionally (Woodhall & Tisher, 1973; Bulger & Dobyan, 1982) and therefore, if they have different embryological origins, they must converge in structural and functional differentiation. For instance, both collecting duct and connecting piece contain intercalated cells (Oliver, 1968) which are thought to arise from principal cells of the collecting duct (Minuth et al. 1989). Another problem is that the S-shaped body, which in other respects differentiates from the glomerular end first (Huber, 1910), is postulated to show elongation of its upper limb and differentiation into the connecting piece before glomerular development begins. A distinct notch is seen between the connecting piece and the S-shaped body (Potter, 1972; her fig. 1. 95), which could be an external marker of the 2 embryological precursors. Further, the schemes of both Oliver and Potter postulate the union of several connecting pieces in arcades, although branching is a feature of the ureteric bud, not the S-shaped body (Huber, 1910).

A major problem is described by Huber (1910): 'It is also impossible to state with any degree of certainty whether the initial collecting tubule develops as an outgrowth from the collecting tubule or is differentiated with the other parts of the renal tubule from the renal vesicle, since soon after the fusion of the S-shaped renal tubule with the ampulla of the collecting tubule, all trace of place of fusion is lost.' The photographs of dissected tubules on which Oliver (1968; his plate 4, figs 8–15) and Potter (1972; her figs 1.100–1.103) base their ideas could be interpreted as showing the connecting piece arising from the ampulla of the ureteric bud just as feasibly as their interpretation. In addition, photographs of dissected tubules in infantile polycystic disease, called type 1 by Potter (1972; her figs 5.10, 5.11), show dilatation of tubules from just the distal side of the glomerulus throughout the collecting system, that is, including the connecting piece, consistent with a cystic disorder of all ureteric bud derivatives in our postulated scheme.

Our findings in fetal kidneys are consistent with the idea that the fundamental junction we have described in the adult kidney (Howie & Johnson, 1992) marks

Fig. 2. Section of outer cortex of fetal kidney at 24 wk of gestation, stained immunohistologically with antibody FC10.2. Ureteric bud and connecting piece (arrow) are stained. Bar, 13 μm.

Fig. 3. Section of outer cortex of fetal kidney at 26 wk of gestation, stained immunohistologically with antibody FC10.2. The junction of the connecting piece, which is stained, and the S-shaped body is seen (arrow). Bar, 10 μm.

Fig. 4. Section of midcortex of fetal kidney at 34 wk of gestation, stained immunohistologically with antibody FC10.2. The junction of connecting piece, which is stained, and developing tubule near the macula densa is seen (arrow). Bar, 10 μm.

Fig. 5. Section of midcortex of fetal kidney at 36 wk of gestation, stained immunohistologically with lectin UEA-1. The junction of connecting piece, which is stained, and developing tubule near the macula densa is seen (arrow). Endothelial cells are also stained. Bar, 10 μm.

Fig. 6. Frozen section of outer cortex of fetal kidney at 25 wk of gestation, stained immunohistologically with antibody PKK2. Ureteric bud and connecting piece (arrow) are stained. Bar, 16 μm.

Fig. 7. Section of fetal kidney at 21 wk of gestation, stained immunohistologically with antibody to Tamm–Horsfall protein. Thick limbs of loop of Henle are stained. Bar, 65 μm.

the site of the union of ureteric bud derivatives with induced metanephric blastema (Fig. 1). The ureteric bud expresses antigens related to the ABO blood group system and various cytokeratins, particularly those recognised by monoclonal antibody PKK2. The antigen recognised by monoclonal antibody FC10.2 is expressed early in embryonic endodermal cells and is apparently lost in development as it is converted into ABO blood group or other determinants (Williams et al. 1982; Gooi et al. 1983), which is consistent with our findings. The ureteric bud is usually said to arise from the mesonephric duct near the junction with the cloaca (Potter, 1972), but the reactivity with FC10.2, an endodermal marker, suggests the possibility that the bud arises from the cloaca itself. Antibodies to cytokeratins are not specific markers of the ureteric bud since they also detect parts of the developing S-shaped body, but the findings with monoclonal antibody PKK2 in particular are consistent with our idea. Some cytokeratins may be lost from ureteric bud derivatives near the junction, leaving a gap before the gradual onset of staining (Howie & Johnson, 1992).

The immunohistological characteristics of the ureteric bud are shared with the more distal part of the tubule after the macula densa (Howie & Johnson, 1992). If this part is not derived from the ureteric bud, then it is necessary to postulate that the bud induces next to it a segment of tubule that is similar to the bud itself in immunohistological characteristics, followed by a segment that is abruptly different, namely, that part expressing Tamm–Horsfall protein. There is in vitro evidence that the S-shaped body gives rise to the tubular segment that expresses Tamm–Horsfall protein (Ekblom et al. 1981), which therefore seems unequivocally derived from the metanephric blastema.

This idea that the distal end of the Tamm–Horsfall staining marks the junction of ureteric bud and metanephric blastema receives support from phylogeny. Tubules in the mesonephric kidney, as in vertebrates such as amphibians and early mammalian embryos, develop through the S-shaped body stage and each tubule attaches directly to the nephric duct (Potter, 1972; Hildebrand, 1974). Tamm–Horsfall protein is seen in the distal tubules and nephric duct of the frog (Howie et al. 1991). Tamm–Horsfall protein therefore seems to mark the most distal part of the mesonephric tubule. The metanephric kidney differs from the mesonephric kidney in having the ureteric bud in development and collecting tubules when mature, but the metanephric blastema and its S-shaped bodies seem to resemble the equivalent mesonephric structures. Thus the junction we postulate lies between the mesonephric-like metanephric blastema derivatives and the metanephric-specific ureteric bud derivatives.

We suggest that a widely accepted view of the development of the distal tubule is open to doubt and that it is possible that the parts of the adult tubule derived from the ureteric bud begin at the distal end of the tubular staining for Tamm–Horsfall protein. This site is closer to the glomerulus than usually considered but is consistent with our immunohistological findings and indeed with the findings of Oliver and Potter.

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