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# Localization of Tamm–Horsfall glycoprotein in the human kidney using immuno-fluorescence and immuno-electron microscopical techniques

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## INTRODUCTION

During the last decade or so there has been a revival of interest in the urinary glycoprotein first described by Tamm & Horsfall (1950, 1952) in human urine and which has subsequently become known as Tamm–Horsfall glycoprotein (THP). After earlier controversy it is now generally agreed that this protein (which is a constituent of normal urine) is associated with and almost certainly produced by the cells of the thick ascending limb of Henle's loop and the distal convoluted tubule. Until quite recently, all observations (with the exception of those of Pape & Maxfield, 1964) who used an immuno-electron microscopical technique on guinea-pig kidneys with ferritin as a label) have been based on the use of immuno-fluorescence microscopy. Much of this work has been concentrated on the human kidney (McKenzie & McQueen, 1969; Pollak & Arbel, 1969; Schenk, Schwartz & Lewis, 1971; Wallace & Nairn, 1971) largely because of the possible involvement of THP in renal disease (Zager, Cotran & Hoyer, 1978; Resnick, Sisson & Vernier, 1978; Hoyer & Seiler, 1979).

Very recently, work has been published in which, by the use of immuno-electron microscopical techniques, THP was shown to be associated with the total plasma membrane systems of the cells of the thick ascending limb of Henle's loop in the rat (Hoyer, Sisson & Vernier, 1978, 1979) and of the thick ascending limb of Henle's loop and the distal convoluted tubule in the hamster (Foster *et al.* 1979; Sikri, Foster, Bloomfield & Marshall, 1979). Furthermore, these authors independently suggested that, in view of its precise localization, THP might, in some manner, be involved in the urine-diluting mechanism of the nephron.

This article describes an investigation into the localization of THP in the cells of the human nephron using immuno-fluorescence and immuno-electron microscopical techniques – the latter, it is believed for the first time.

## MATERIALS AND METHODS

Human THP was isolated by the method of Tamm & Horsfall (1952). Solid sodium chloride was added to the normal pooled urine until the concentration was brought to 0·58 M. On standing at 4 °C overnight, sedimentation of the white

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flocculent precipitate was obtained and was separated from the supernatant by centrifugation (1500 g, 4 °C). This was dissolved in water and re-precipitated twice with 0·58 M sodium chloride. Homogeneity of the glycoprotein was checked by disc gel electrophoresis and the purity was shown by the single band obtained.

Antibodies to human THP were raised in rabbits by the intramuscular injection of 1·0 ml of an emulsion containing equal volumes of the glycoprotein (1·0 mg/ml in water) and complete Freund's adjuvant. After giving two more such injections at fortnightly intervals, the blood was drawn from an ear vein. Pure immunoglobulin G (IgG) fractions were isolated from the serum by the method of Sober & Peterson (1958).

Small representative pieces from four (two male and two female) adult human kidneys were fixed in the operating theatre. Each of the male kidneys was removed on account of an intrarenal tumour; those from the females because of a tumour located in the ureter.

For immuno-fluorescence microscopy pieces of kidney were fixed in formalin–calcium chloride (FCC), embedded in paraffin wax and 5 μm sections were stained with fluorescein isothiocyanate by the indirect (sandwich) method. For immuno-electron microscopy, on the other hand, small pieces of tissue were fixed in the periodate–lysine–paraformaldehyde fixative of McLean & Nakane (1974). From these 30–40 μm thick sections were cut on a cryostat and stained by the perioxidase–antiperoxidase method. The techniques of tissue preparation and staining were similar to those used for the hamster kidney and have been described in detail elsewhere (Sikri *et al.* 1979).

In order to check for the specificity of the reaction, control tests were done in which anti-THP antibodies were either omitted altogether or replaced by normal rabbit serum. Paraffin sections were also stained with haematoxylin and eosin for routine examination.

The preparations for immuno-fluorescence microscopy were examined with a Gillet and Siebert conference microscope fitted with a Zeiss FL epifluorescence condenser and an HB0 50W mercury lamp. A Zeiss recommended FITC specific filter set with blue excitation at 450 nm was also used. Ultrathin sections cut from the peroxidase-stained material were mounted on uncoated 200-mesh copper grids and examined without further heavy metal staining with a Miles MR60 electron microscope at 60 kV and a Philips 300 at 40 kV.

## RESULTS

No gross pathological changes were observed in those portions of the kidneys chosen for this study.

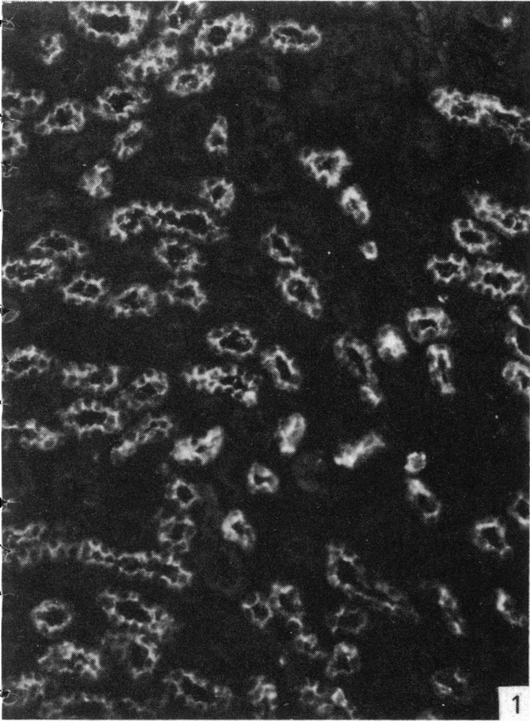
A generalized intracellular fluorescence, sometimes brighter on the luminal

Fig. 1. Localization of THP in the adult human kidney. Specific fluorescent staining in the cells of the thick ascending limb of Henle's loop and the distal convoluted tubule of the inner cortex. × 240.

Fig. 2. Outer medullary zone. Specific fluorescent staining indicating the presence of THP is seen in the cells of the thick ascending limb of Henle's loop. × 350.

Fig. 3. Outer cortical zone. Uniform distribution of immuno-fluorescence is seen in the cells of the distal convoluted tubule (*DCT*). Cells of the proximal convoluted tubule (*PCT*) are unstained. × 600.

Fig. 4. Inner cortical zone. Specific fluorescent staining is present in the cells of the distal convoluted tubule (*DCT*). Cells of the macula densa (*MD*) along with those of the proximal convoluted tubule (*PCT*) and glomerulus (*GL*) are negative. × 750.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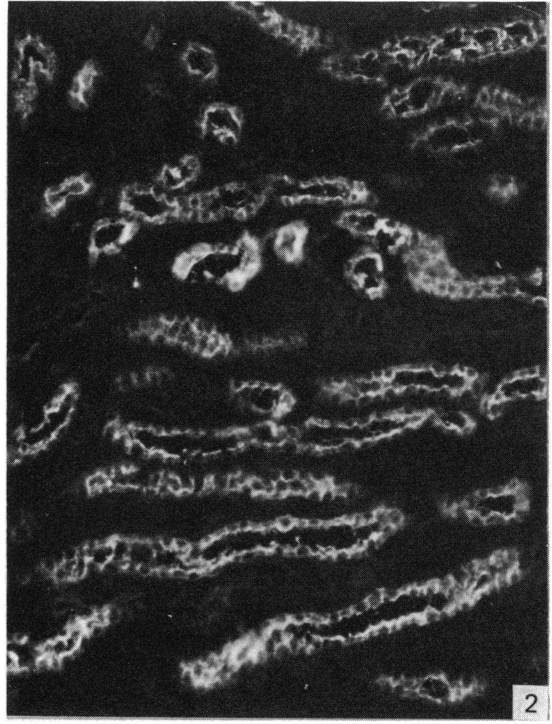
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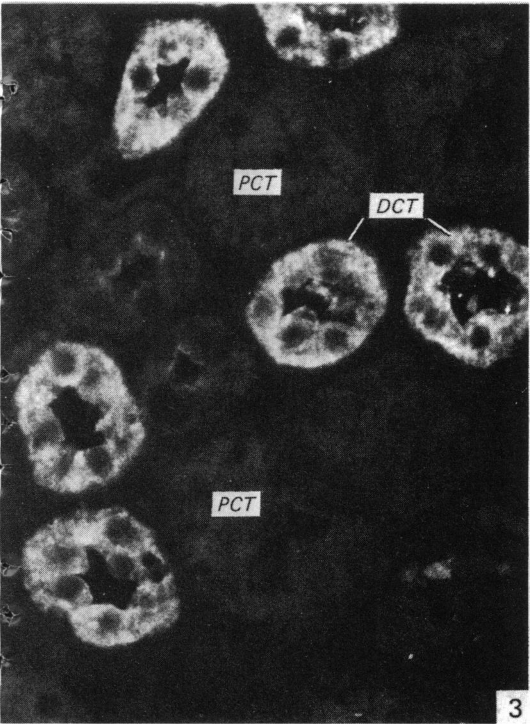
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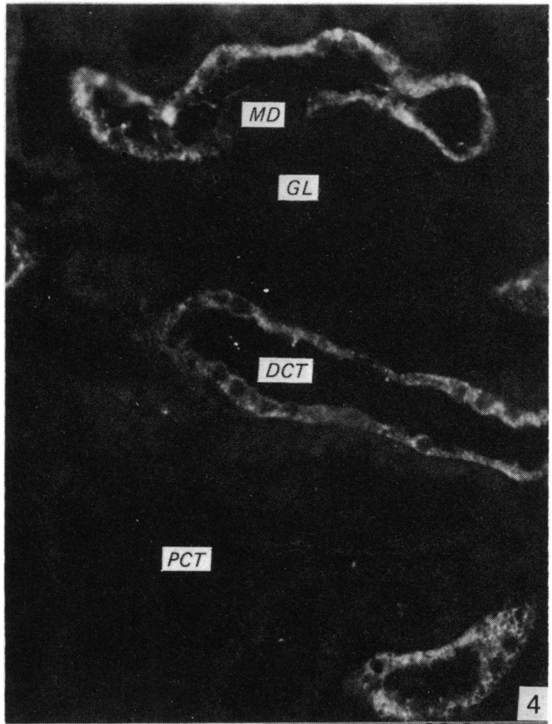
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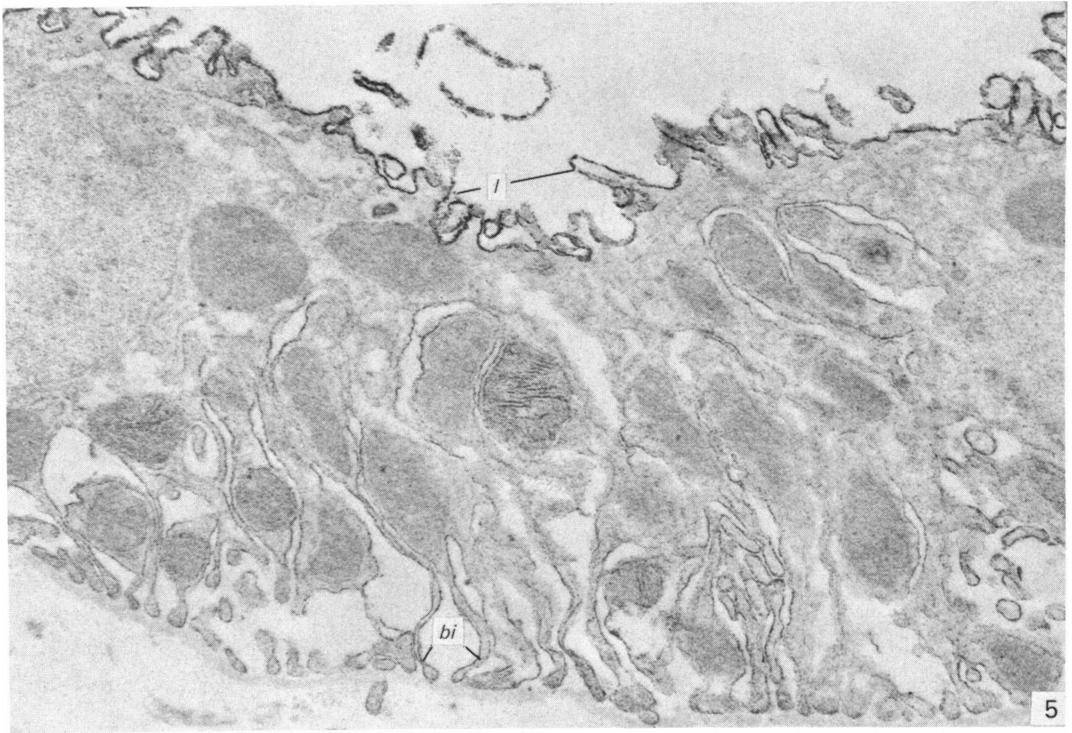


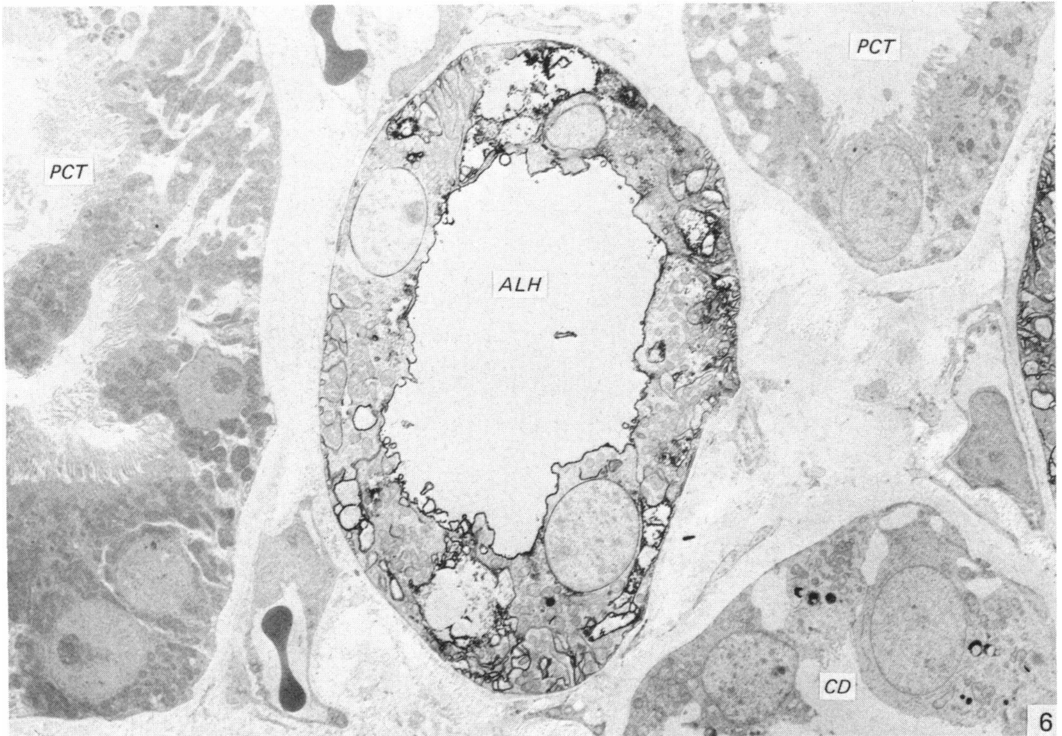


Fig. 5. Electron micrograph of part of a distal convoluted tubule cell. Immunoperoxidase reaction is present on the luminal surface (*l*) and basal plasma membrane, including its invaginations (*bi*). ×20000.

Fig. 6. Low power electron micrograph of inner cortex showing the presence of THP specific peroxidase reaction in the plasma membrane systems of the cells of the thick ascending limb of Henle's loop (*ALH*) and its absence from those of the proximal convoluted tubules (*PCT*) and collecting duct (*CD*). ×2000.

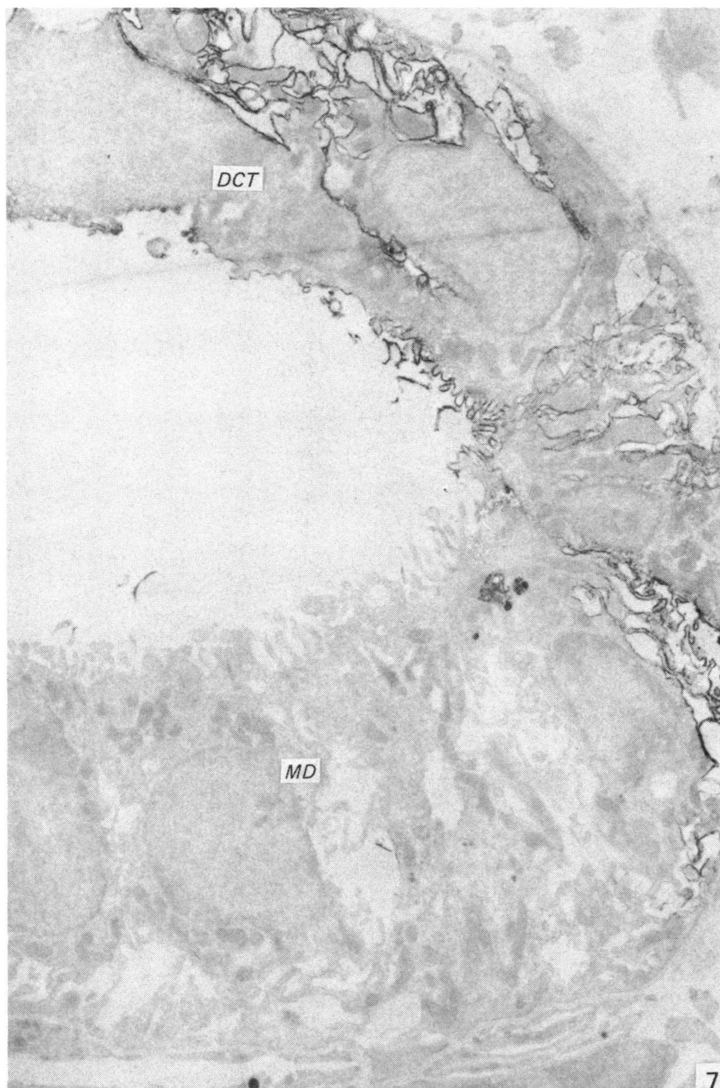


Fig. 7. Electron micrograph showing a part of the macula densa (*MD*). Note that its cells are completely devoid of the peroxidase reaction which is present on the membranes of the cells of the distal convoluted tubule (*DCT*). × 15000.

borders, was observed in the cells of the thick ascending limb of Henle's loop and the distal convoluted tubule (Figs. 1, 2, 3); the cells of the macula densa, however, were always negative (Fig. 4) as were those of the proximal convoluted tubules, the thin parts of Henle's loop, collecting ducts and glomeruli. Control preparations in which anti-THP antibody was omitted were always negative.

The results obtained by immuno-electron microscopy once again demonstrated that THP was localized in the cells of the thick ascending limb of Henle's loop and the distal convoluted tubule (Figs. 5, 6) but that those of the glomeruli, proximal convoluted tubule (Fig. 6), macula densa (Fig. 7) and collecting duct lacked any immuno-peroxidase reaction. The reaction product indicating the sites of THP

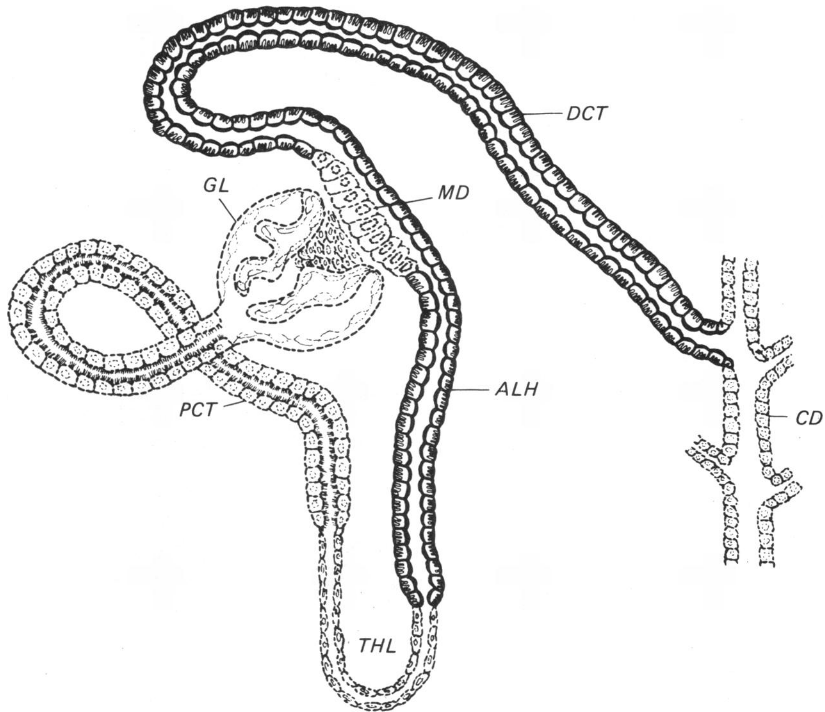


Fig. 8. Diagram of a single nephron, summarising the distribution of THP in the cells of the thick ascending limb of Henle's loop (*ALH*) and the distal convoluted tubule (*DCT*) and its absence in those of the proximal convoluted tubule (*PCT*), thin limb of Henle's loop (*THL*), glomerulus (*GL*) and collecting duct (*CD*). The presence of a THP-positive reaction in the thick ascending limb of Henle's loop and the distal convoluted tubule is indicated by dark bold lines.

was confined to the plasma membranes of the cells: the luminal borders of the cells of the thick ascending limb of Henle's loop and the distal convoluted tubule together with their lateral and basal plasma membranes and including the infoldings of the latter. The most massive and consistent reaction was observed on the luminal borders of the cells. In others parts of the membrane system the staining was sometimes capricious. This is probably related to the fact that the immunochemical section of the technique relies on the diffusion of THP antibody and other reagents of fairly high molecular weight into circa 30 $\mu$m sections of fixed tissue. This fact could clearly lead to some variability in the intensity of the reaction, particularly in those regions of cells more remote from a lumen.

It should be pointed out also that a compromise had to be made with regard to the primary fixative used since, although the periodate–lysine–paraformaldehyde fixative is excellent for the preservation of THP and the maintenance of its antigenicity, it is less good (but adequate) for the preservation of ultrastructural detail.

A diagrammatic representation of the distribution of this glycoprotein in a single nephron of the human kidney, as seen by immuno-electron microscopy is shown in Figure 8.

As in the case of immuno-fluorescence, control specimens were invariably negative.

## DISCUSSION

The results from immuno-fluorescence microscopy confirm those of others who have recently investigated the human kidney (see Introduction). The absence of fluorescence in the macula densa does not, however, appear to have been reported before.

The results obtained by immuno-electron microscopy indicate an identical distribution of THP in human and hamster kidneys (Sikri *et al.* 1979), that is to say the protein is associated with the plasma membranes of that part of the nephron where, in consequence of a low permeability to water and an active transport of Cl ions into the interstitium, a progressive dilution of urine takes place.

There is evidence that solutions of THP are highly viscous (Stevenson, Cleave & Kent, 1971) and therefore can trap water. If the protein behaves in the same manner when associated with the cell surface one can envisage a situation in which a barrier of relatively stationary water molecules, occurring within an ordered structure, is present on the surface of the cells of the thick ascending limb of Henle's loop and the distal convoluted tubule (Sikri *et al.* 1979). This layer might, therefore, act as a barrier to water molecules but not to the ions and small molecules of the tubular urine.

As in the hamster (Sikri & Foster, 1981), the cells of the macula densa lacked the glycoprotein. This fact may be of particular significance in the context of the hypothesis put forward above, since the absence of THP would allow direct assessment of the ionic strength of the tubular fluid and thus aid the monitoring function which the macula densa is thought by some to possess (Latta, 1973; Barajas, 1979).

The early work of Pape & Maxfield (1964), who were the first to apply an immuno-electron microscopical technique to this problem, reported THP in association with the cells of the proximal convoluted tubule in the guinea-pig kidney. This result runs counter to the findings in other species and in view of the fact that ferritin was used as a label (now considered to be less reliable) and that their methods of antibody preparation were cruder than those available now, their observations must be viewed with considerable doubt.

As a result of studies on the ultrastructural localization of THP in the rat kidney, Hoyer *et al.* (1978) in a preliminary report and at length in 1979 independently put forward a similar hypothesis based on almost comparable morphological findings. Sikri *et al.* (unpublished results) have also studied the rat kidney and although their results concur with those of Hoyer *et al.* (1979) in relation to the distribution of THP in the plasma membranes of the cells of the thick ascending limb of Henle's loop, there are certain differences in respect of the distal convoluted tubule, where, according to these latter authors, the protein is associated only with the periluminal plasma membrane. However, in a more recent paper Sisson & Vernier (1980) seem to suggest that THP is associated also with the basal plasma membranes and their infoldings as in the thick ascending limb of Henle's loop. This would bring into line the ultrastructural findings relating to the intrarenal localization of this protein in the three species studied so far, thereby strengthening the notion that THP has some role in the urine-diluting segment of the mammalian nephron.

SUMMARY

Small pieces of tissue obtained from apparently normal areas of four surgically removed adult human kidneys were used in the present study.

The results obtained by immuno-fluorescence and immuno-electron microscopical techniques show that Tamm–Horsfall glycoprotein (THP) is present in the thick ascending limbs of the loops of Henle and the distal convoluted tubules.

Within the cells concerned, the protein is associated with the luminal, lateral as well as basal, plasma membranes and their infoldings. The cells of the macula densa are completely negative as are those of proximal convoluted tubules, glomeruli and collecting ducts. The possible significance of these findings in relation to the process of urine dilution in the nephron is discussed.


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REFERENCES


BARAJAS, L. (1979). Anatomy of the juxtaglomerular apparatus. *American Journal of Physiology* **237**, 333–343.

FOSTER, C. L., MARSHALL, R. D., SIKRI, K. L., BLOOMFIELD, F. J. & ALEXANDER, D. P. (1979). The localization of Tamm–Horsfall glycoprotein in the nephrons of the Syrian hamster kidney as demonstrated by immunofluorescence and immuno-electron microscopical techniques. *Journal of Anatomy* **128**, 417–418.

HOYER, J. R. & SEILER, M. W. (1979). Pathophysiology of Tamm–Horsfall protein. *Kidney International* **16**, 279–289.

HOYER, J. R., SISSON, S. P. & VERNIER, R. L. (1978). Immunoelectron microscopical localization of Tamm–Horsfall protein (THP) in rat kidney. *Federation Proceedings* **37**, 837.

HOYER, J. R., SISSON, S. P. & VERNIER, R. L. (1979). Tamm–Horsfall glycoprotein – Ultrastructural immunoperoxidase localization in the rat kidney. *Laboratory Investigation* **41**, 168–173.

LATTA, H. (1973). Ultrastructure of the glomerulus and juxtaglomerular apparatus. In *Handbook of Physiology*, Ch. 1, Sect. 8 (ed. J. Orloff & R. W. Berliner), pp. 1–29. Washington: American Physiological Society.

MCLEAN, I. W. & NAKANE, P. K. (1974). Periodate-lysine-paraformaldehyde fixative – A new fixative for immunoelectron microscopy. *Journal of Histochemistry and Cytochemistry* **22**, 1077–1083.

MCKENZIE, J. K. & MCQUEEN, E. G. (1969). Immuno-fluorescence localization of Tamm–Horsfall mucoprotein in human kidney. *Journal of Clinical Pathology* **22**, 334–339.

PAPE, L. & MAXFIELD, M. (1964). Localization of cellular site of the Tamm–Horsfall urino-glycoprotein. In *Proceedings of the 3rd International Conference on Research on Pathogenesis of Cystic Fibrosis of the Pancreas (Mucoviscidosis)* (ed. P. A. Di Sant' Agnese), pp. 260–273. Lancaster, PA: Wickersham Printing Co.

POLLAK, V. E. & ARBEL, C. (1969). The distribution of Tamm–Horsfall mucoprotein (Uromucoid) in the human kidney. *Nephron* **6**, 667–672.

RESNICK, J. S., SISSON, S. & VERNIER, R. L. (1978). Tamm–Horsfall glycoprotein – Abnormal localization in renal disease. *Laboratory Investigation* **38**, 550–555.

SCHENK, E. A., SCHWARTZ, R. H. & LEWIS, R. A. (1971). Tamm–Horsfall mucoprotein. Localization in the kidney. *Laboratory Investigation* **25**, 92–95.

SIKRI, K. L., FOSTER, C. L., BLOOMFIELD, F. J. & MARSHALL, R. D. (1979). Localization by immunofluorescence and by light and electron-microscopical immunoperoxidase techniques of Tamm–Horsfall glycoprotein in adult hamster kidney. *Biochemical Journal* **181**, 526–531.

SIKRI, K. L. & FOSTER, C. L. (1981). Light and electron microscopical observations on the macula densa of the Syrian hamster kidney. *Journal of Anatomy* **132**, 57–69.

SISSON, S. & VERNIER, R. L. (1980). Methods for immunoelectron microscopy: Localization of antigens in rat kidney. *Journal of Histochemistry and Cytochemistry* **28**, 441–452.

SOBER, H. A. & PETERSON, E. A. (1958). Protein chromatography on ion exchange cellulose. *Federation Proceedings* **17**, 1116–1127.

STEVENSON, F. K., CLEAVE, A. J. & KENT, P. W. (1971). The effect of ions on the viscometric and ultracentrifugal behavior of Tamm–Horsfall glycoprotein. *Biochimica et biophysica acta* **236**, 59–65.

TAMM, I. & HORSFALL, F. L. JR. (1950). Characterization and separation of an inhibitor of viral haemagglutination found in urine. *Proceedings of the Society for Experimental Biology and Medicine* **74**, 108–114.

TAMM, I. & HORSFALL, F. L. JR. (1952). A mucoprotein derived from human urine which reacts with influenza, mumps and Newcastle disease viruses. *Journal of Experimental Medicine* **95**, 71–97.

WALLACE, A. C. & NAIRN, R. C. (1971). Tamm–Horsfall protein in kidneys of human embryos and foreign species. *Pathology* **3**, 303–310.

ZAGER, R. A., COTRAN, R. S. & HOYER, J. R. (1978). Pathological localization of Tamm–Horsfall protein in interstitial deposit in renal disease. *Laboratory Investigation* **38**, 52–57.