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Epithelial inclusions and Tamm-Horsfall protein in paranephric lymph nodes

A light microscopy and immunocytochemical study*

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Summary. An unusual histological pattern made up of tubular structures and clusters of apparently epithelial cells floating within sinusoidal deposits of Tamm-Horsfall (TH) protein was observed in several lymph nodes removed with renal tumours (3 Wilms' tumours, 1 cystic nephroma) and pathological but non neoplastic kidneys (2 cases of reflux nephropathy). Masses of TH protein, often containing desquamated tubular epithelial cells, were also found in tubules, interstitium and perivascular lymphatic vessels of the resected kidneys, but never in the tumour tissue. Lymph nodes draining renal tumours, although moderately enlarged because of reactive hyperplasia and TH protein deposits, did not contain metastases. Our findings suggest that these inclusions originate from renal tubular epithelium and are transported to paranephric lymph nodes along with TH protein.

Key words: Lymph nodes – Glandular inclusions – Glycoproteins – Immunochemistry

Tamm-Horsfall (TH) protein is a high molecular weight glycoprotein synthesized in the kidney by the cells of the ascending limb of the loop of Henle and distal convoluted tubules (Schenk et al. 1971; Hoyer et al. 1974). This substance was originally described by Tamm and Horsfall (1950) as an active urinary inhibitor of myxovirus agglutination reactions, but its physiological role is probably related to electrolyte and water transport (Sikri et al. 1979).

Intratubular and interstitial renal deposits of TH protein have been described by Vernier and Resnick (1976) in medullary cystic disease, and subsequently by Zager et al. (1978) and Resnick et al. (1978) in a variety of other pathological renal conditions such as chronic pyelonephritis, hydrone-

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phrosis and chronic interstitial nephritis of uncertain origin. The above mentioned authors have identified this protein in the renal deposits using immunofluorescence and immunochemistry; nevertheless, in routine histology, TH protein deposits are easily recognized as a waxy pink amorphous material positively stained with periodic acid-Schiff after diastase digestion.

Yunis and Jaffe (1981) identified TH protein deposits with immunofluorescence in peripheral and intermediate sinuses of paranephric lymph nodes from 4 cases of pediatric renal tumours (3 Wilms' tumors, 1 mesoblastic nephroma), as well as in the residual compressed kidney; no deposits were present in the Wilms' tumours or in the mesoblastic nephroma. The lymph node deposits were considered to have been transported from renal interstitial deposits of the protein, since similar material was also found in perivascular lymphatics within the kidneys.

Our report concerns cellular clusters and tubular inclusions observed within TH protein deposits in paranephric lymph nodes of children who underwent nephrectomy for both neoplastic and obstructive conditions. The results of a light microscopy and immunocytochemical study of these inclusions, and suggestions about their origin, are discussed.

Materials and methods

We studied formalin fixed paraffin-embedded histological material from 28 nephrectomies performed in children between 1975 and 1984 in which renal tissue showed TH protein deposits in tubules as well as in the interstitium and perivascular lymphatic channels. Nephrectomies were performed because of Wilms' tumour in 20 cases, cystic nephroma in 1 case, severe obstructive and reflux nephropathy in 7 cases. Regional lymph nodes were available only in 23 of these cases, and minimal to large deposits of TH protein were found in some nodes (1 to 4) in 19 cases. Criteria used to identify TH protein by light microscopy both in renal tissue and paranephric lymph nodes were (Zager et al. 1978; Resnick et al. 1978): the presence of pale pink amorphous material in routine haematoxylin-eosin stained sections; strong positivity and fibrillary appearance of the material with periodic acid-Schiff stain before and after diastase; negative Congo red staining.

Lymph nodes showing inclusions within TH protein deposits were removed in 3 cases with usual Wilms' tumour (1 positive peri-aortic node in each case), in 1 case with a cystic nephroma (3 positive peri-aortic nodes) and in 2 cases with hydronephrotic organs due to vescico-ureteral reflux (1 positive hilar node in each case). Multiple tissue sections from each node were available for the following procedures: haematoxylin and eosin stain, periodic acid-Schiff stain before and after diastase, Congo red stain for examination under polarized light and Weigert-van Gieson stain for elastin and connective tissue. The same tests were also made on the corresponding renal tissues, and, for neoplastic cases, on the tumour tissue.

Immunocytochemistry. Formalin fixed, paraffin-embedded tissue sections from both kidneys and regional lymph nodes were studied with polyclonal antisera to keratin proteins and Factor VIII-Related Antigen, and a monoclonal anti-Epithelial Membrane Antigen antibody. While the former were revealed by a slightly modified PAP-technique, the latter was evidenced by the ABC method. The technical details of these methods have been described elsewhere (Sternberger 1979; Taylor 1979; Pileri et al. 1980; Hsu et al. 1981). Rabbit anti-human keratin (DAKO A575) and rabbit anti-(human Factor VIII-Related Antigen) (DAKO A082) antisera were diluted with TBS 1:250 and 1:650 respectively. Mouse monoclonal anti-EMA was diluted 1:1 with TBS. TH protein was revealed by the ABC method using rabbit anti-(human TH protein) antiserum purified by affinity chromatography (Abbondanza et al. 1980) and diluted

1:1500 with TBS. The specificity of the immunostaining was confirmed by replacement of the primary antiserum with nonimmune serum or preabsorption of the primary antibodies with excess antigen. Specific staining was totally eliminated by both control procedures.

Results

The lymph nodes were all moderately enlarged because of reactive hyperplasia. The TH protein deposits varied from small subcapsular lumps to large masses occupying the peripheral and intermediate sinuses (Fig. 1). Less frequently, small deposits were seen dispersed within cortical lymphatic tissue and germinal centers. The larger sinusoidal deposits masses of TH protein manifested partial collagenous or hyaline transformation. Lymph nodes removed with neoplastic kidneys did not contain metastases.

The inclusions within TH protein deposits in lymph nodes were primarily tubular structures (Figs. 1, 2, 3) although small clusters or isolated elements of apparent epithelial cells were also present in some fields. These inclusions were often found lying in collagenized deposits of TH protein, especially at the periphery, where they appeared in close continuity with either lymphatic tissue or capsular wall. In a single case some tubules were observed only within young collagenous tissue obstructing the sinus, whereas a small contiguous deposit of protein did not show any cellular inclusion. The tubules were variable in size, ranging from small acini or parallel rows of cuboidal cells with absent or barely visible lumens, to cystic structures lined by flattened cells (Fig. 3). The tubules were invested by a distinct basement

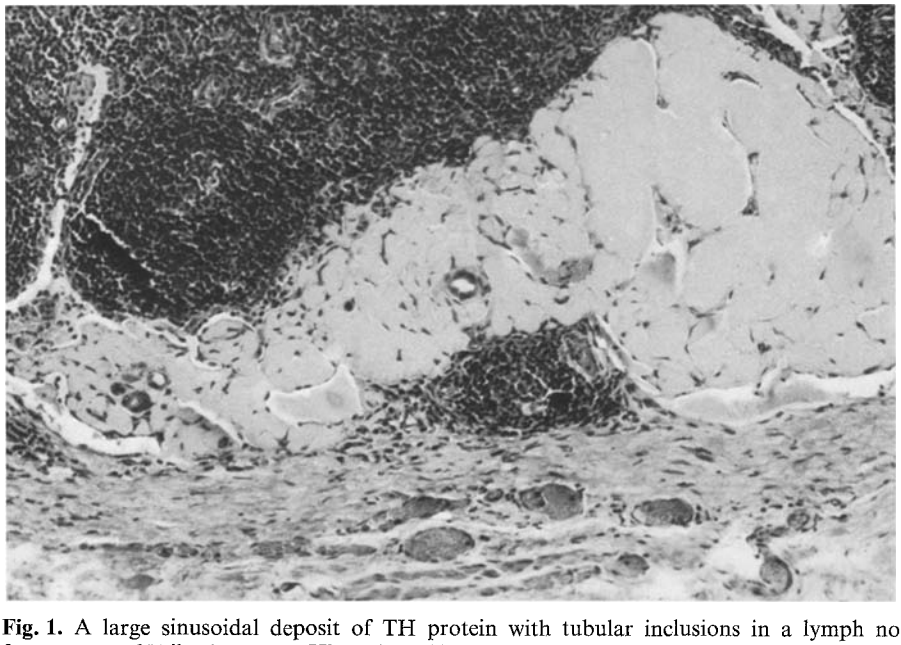

Fig. 1. A large sinusoidal deposit of TH protein with tubular inclusions in a lymph node from a case of Wilms' tumour. HE-stain ×125

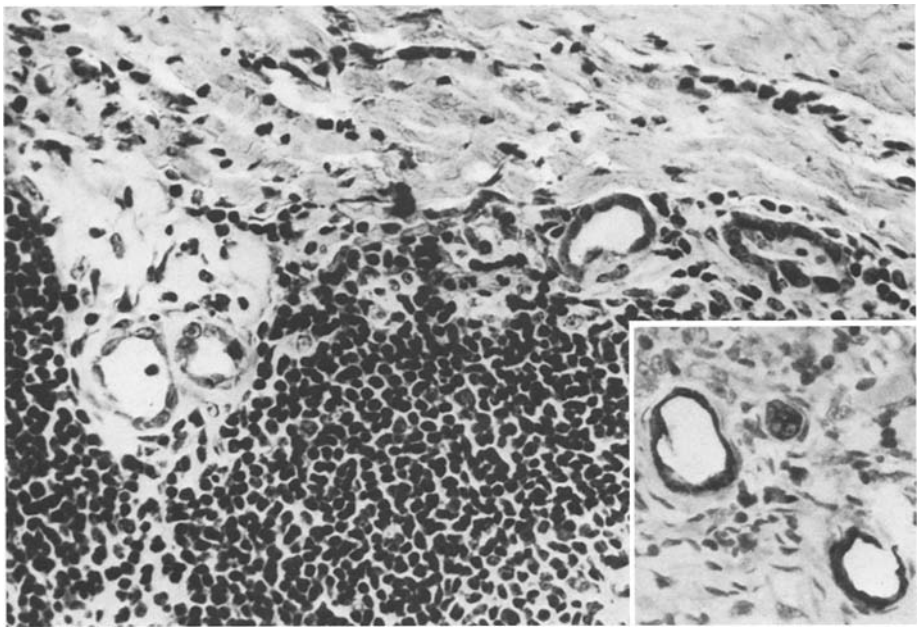

Fig. 2. A hilar lymph node from a case of reflux nephropathy. The inclusions are formed by a simple layer of cuboidal cells with bland nuclei. HE-stain ×250. Inset: Positive PAP-stain for keratin in the inclusions. ×250

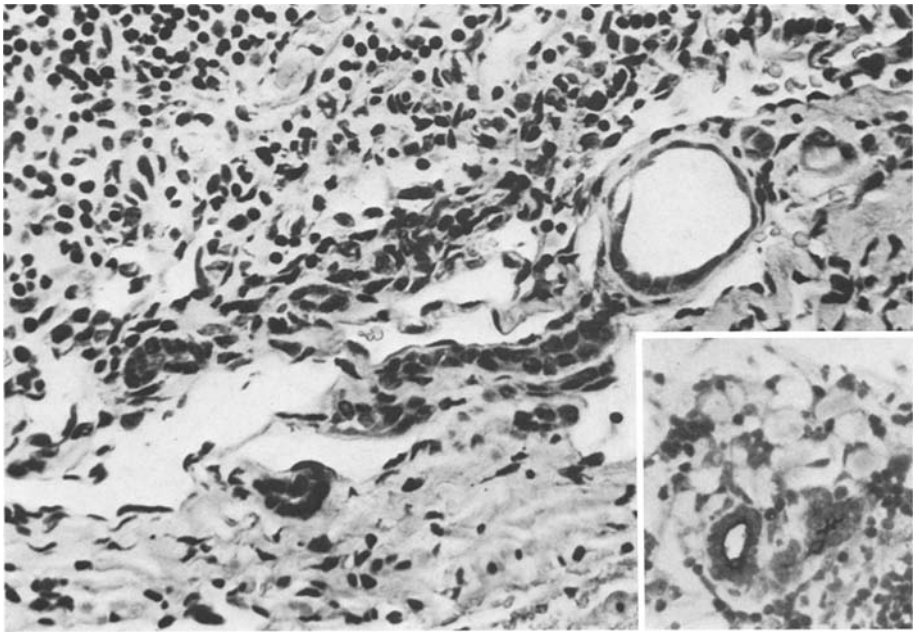

Fig. 3. Cellular clusters, thin tubules and a cystic structure in a lymph node from the case of cystic nephroma. HE-stain ×250. Inset: Positive immunochemical staining for EMA. ABC method ×250

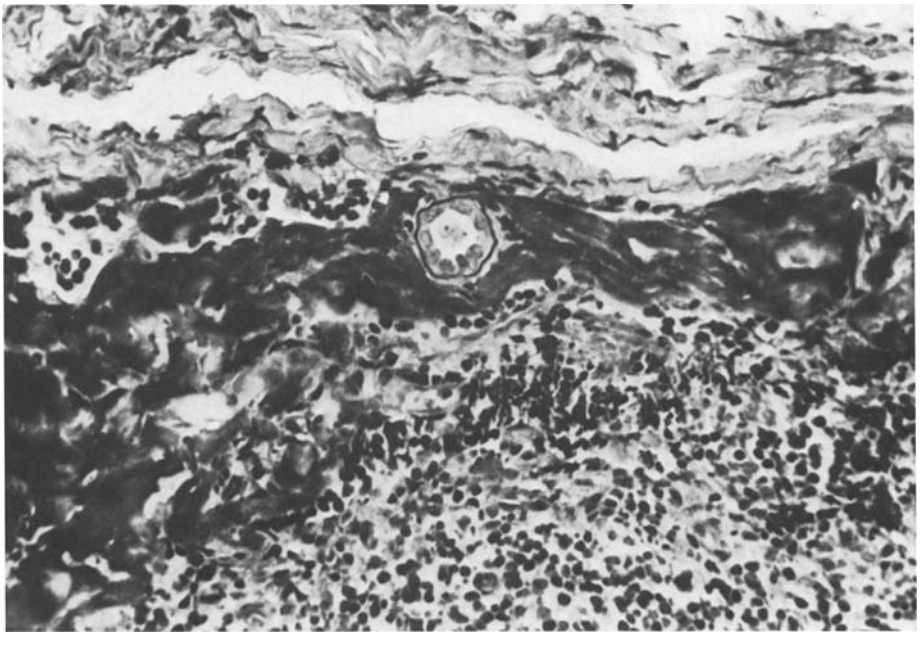

Fig. 4. Strongly positive PAS-diastase reaction of TH protein in a lymph node deposit. The tubular inclusion shows a distinct basement membrane. ×250

membrane that was best appreciated in PAS-stained sections, especially when the sorrounding protein had undergone fibrous or hyaline transformation (Fig. 4). Tubular lumens appeared optically empty in most cases but a few contained cellular debris or a small amount of TH protein. In sporadic fields, dense granular calcifications obscured the lumen and the cells of the tubules. Nuclei of the cells appeared round to oval in shape, with finely dispersed chromatin. No mitoses were seen. The cytoplasm was slightly eosiniphilic or amphophilic, and sometimes showed small vacuoles.

The epithelial nature of these inclusions was confirmed by immunocytochemistry as in all cases the cellular clusters and tubular structures stained positively for both keratin and EMA (Insets of Figs. 2 and 3). The staining pattern was diffuse cytoplasmic for keratin and distinctly membranous for EMA. None of the inclusions stained positively for Factor VIII-Related Antigen, whereas the endothelial linings of lymph nodal vessels were positive.

TH protein stained positively with rabbit anti-(human TH protein) antiserum in both renal and lymph node deposits; similary stained material was also present in renal as well as perinodal lymphatic vessels (Figs. 5 and 6). In neoplastic kidneys TH protein was restricted to the compressed residual parenchima and was never seen in the tumour tissue. In all cases renal tissue showed interstitial deposits of TH protein that appeared to originate from disrupted tubules. Small clusters or thin rows of desquamated

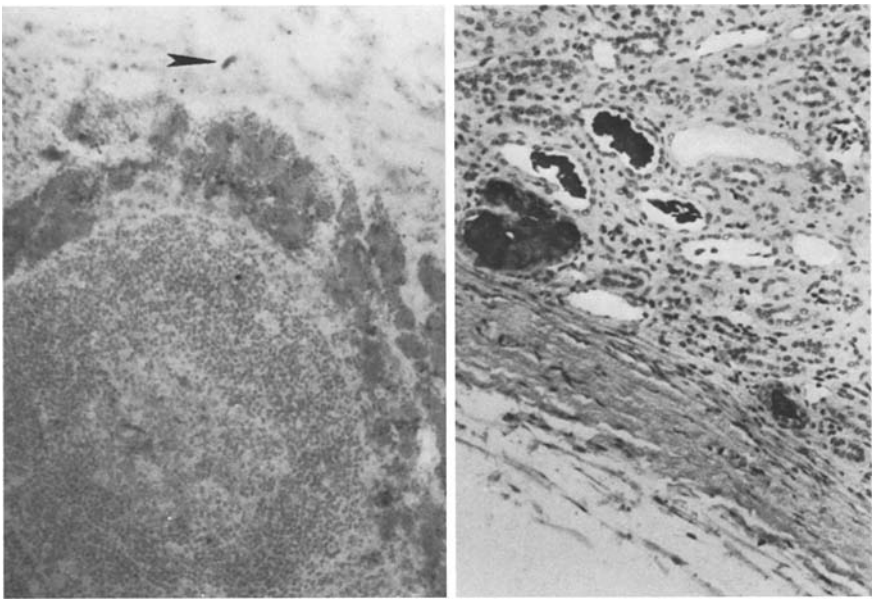

Fig. 5. Immunochemically-stained TH protein deposits in a lymph node (left) and kidney (right). A small lymphatic vessels in the perinodal tissue is filled with the protein (arrow). ABC method ×125

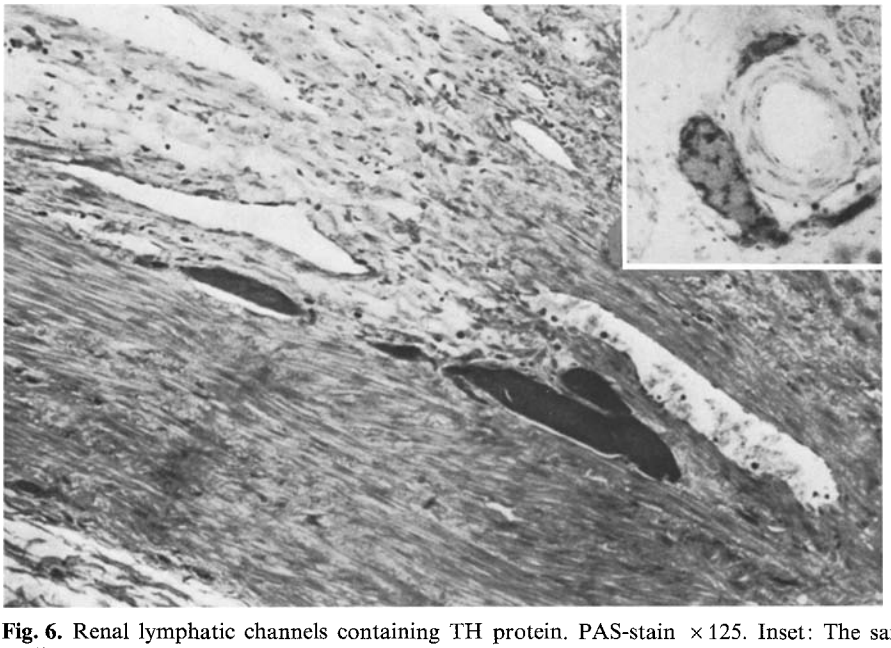

Fig. 6. Renal lymphatic channels containing TH protein. PAS-stain ×125. Inset: The same findings immunochemically stained. ABC method ×125

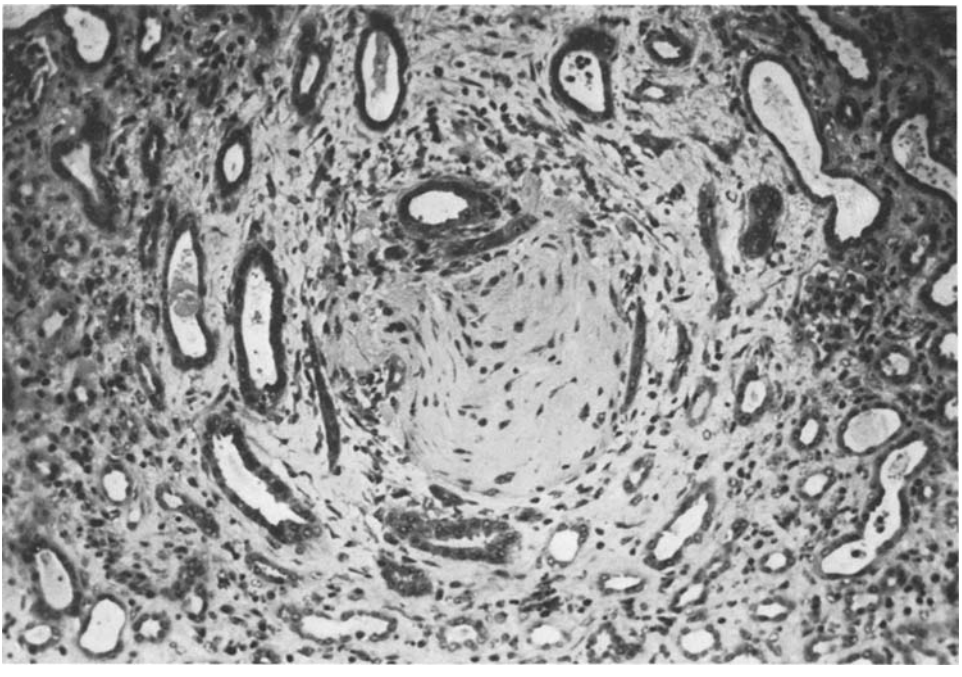

Fig. 7. Renal interstitial deposit of TH protein containing desquamated epithelial cells. HE-stain ×250

tubular epithelium were often seen within intratubular as well as interstitial deposits (Fig. 7).

Discussion

Benign glandular inclusions in lymph nodes are a well known finding. They occur in pelvic and abdominal para-aortic nodes of women (Karp and Czernobilsky 1969) where they have been considered to represent either "benign metastases" i.e. mullerian metaplasia of coelomic epithelium, or congenital rests. Salivary gland tissue is commonly observed in high cervical nodes (Brown et al. 1953) and thyroid follicles may be detected in the peripheral sinuses of midcervical lymph nodes in the absence of thyroid carcinoma (Meyer and Steinberg 1969).

Our report concerns cellular clusters and tubular inclusions observed within TH protein deposits in paranephric lymph nodes that were removed in 6 cases of nephrectomy for Wilms' tumour (3 cases), cystic nephroma (1 case) and reflux nephropathy (1 case). To our knowledge, no detailed description of this unusual association has been previously reported.

Yunis and Jaffe (1981), in their paper on TH protein in lymph nodes, briefly mentioned inclusions of epithelial cells which they observed in protein deposits of one node, suggesting these were desquamated tubular cells of the kidney; however, no reliable proof of their epithelial nature was given.

In our cases, inclusions appeared immunocytochemically positive for keratin and EMA, while Factor VIII-Related Antigen was negative. This substantiated their epithelial nature. On the other hand, the same findings in lymph nodes from the cases of reflux nephropathy tend to exclude the possibility of metastases.

We considered two possibilities for these histopathological findings: Lymph node inclusions and TH protein deposits may be coincidental and unrelated, or the two processes may be related by a common pathogenesis. It has been suggested that lymph node inclusions are related to TH protein deposits in lymph nodes and that both probably originated in the kidney (Dehner 1983) and we agree with this view. In all of our cases, TH protein deposits in lymph nodes were associated with conspicuous pools of TH protein in tubules and interstitium of the resected kidneys. Casts of the same material were also observed in renal lymphatic vessels. In lymph nodes and kidneys TH protein was immunocytochemically identified using a rabbit anti-TH protein antiserum, whereas no specific staining was observed in the Wilms' tumors or in the cystic nephroma. Hence, we believe that the TH protein in lymph node sinuses is transported to these sites through lymphatic vessels from renal pools of the same material.

An interesting feature suggesting that the epithelial inclusions in lymph nodes share a common origin with TH protein was the frequent detection of small epithelial clusters or isolated cells within the renal pools of the protein. These cells appeared to be desquamated tubular epithelium and closely resembled the cells forming the lymph node inclusions. Although we did not see any well formed tubular structure within TH protein in the kidney, it is conceivable that small epithelial clusters of desquamated tubular epithelium transported to paranephric lymph nodes with TH protein may have undergone tubular rearrangement.

Some inclusions were seen invested by a distinct basement membrane, especially at the periphery of TH protein deposits where fibrous or hyaline transformation had occurred. This finding is consistent with current knowledge concerning synthesis of basal lamina by epithelium when interacting with stroma (Pierce et al. 1963, 1964; Bernfield and Banerjee 1978; Kefalides et al. 1979). Therefore, it seems likely that the epithelial inclusions we have observed within TH protein deposits in paranephric lymph nodes constitute desquamated tubular epithelium transported to lymph nodes along with TH protein abnormally deposited in the kidney. Whereas in inflammatory and obstructive conditions of the kidney these lymph node inclusions are correctly evaluated as benign, it is important that they not be interpreted as metastases in cases associated with renal malignancies.

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