Immunohistochemical Localization of Renin in Renal Tumors

TATSUO TOMITA, MD, ALAN POISNER, MD, and TADASHI INAGAMI, PhD

From the Departments of Pathology and Pharmacology, University of Kansas Medical Center, Kansas City, Kansas, and Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee

Immunoperoxidase staining for renin was performed with renal tumors, including juxtaglomerular (JG) tumor, Wilms' tumors, renal adenocarcinomas, renal oncocytomas, and cortical adenomas. Compared with the JG apparatus adjacent to the glomerulus, JG tumor cells were less darkly but diffusely stained for renin. One of five Wilms' tumors revealed more numerous renin-containing tumor cells than the adjacent renal cortex, whereas three of ten renal adenocarcinomas and two of three renal oncocytomas revealed only focally renin-positive tumor cell cytoplasms. None of six cortical adenomas were positive for renin. With available fresh tumor tissue, renin activity was studied by measuring newly formed angiotensin I by radioimmunoassay. JG tumor contained markedly elevated renin activity, whereas one Wilms' tumor and two renal adenocarcinomas contained no more than 2% renin activity of the renal cortex, more than 50% of which was inactive renin. These findings suggest that the JG tumor elaborates enormous amounts of active renin, whereas other renal tumors produce lesser amounts of renin, more than half of which is inactive renin. (Am J Pathol 1987, 126:73–80)

RENAL TUMORS presenting hypertension and hyperreninemia are practically curable,[1,2] among which juxtaglomerular (JG) tumors and Wilms' tumors are the most frequently encountered renin-secreting tumors.[1-3] A syndrome of severe hypertension, hyperreninemia, and secondary aldosteronism in young patients is characteristic of JG tumor.[1,3] Wilms' tumor occurring in young children may elaborate renin and present hypertension.[1,4-6] Adenocarcinoma of the kidney accounts for about 90% of all malignant tumors of renal parenchyma and occurs most frequently in patients over 60 years of age.[7] Hypertension is estimated to occur in about 25% of the patients with renal adenocarcinoma; and in a few of these patients, hypertension is secondary to hyperreninemia.[7,8]

This study was attempted to localize renin immunoreactivity by immunohistochemical techniques in renal tumors, which include JG tumor, Wilms' tumor, renal adenocarcinoma, renal oncocytoma,[9] and cortical adenoma.

Materials and Methods

Tumor tissue was collected at surgery or autopsy and fixed with Bouin's fluid or buffered 10% formalin. Deparaffinized sections were processed by the indirect immunoperoxidase technique[10] with the use of rabbit anti-human renin and peroxidase-conjugated goat anti-rabbit gamma globulin (Bio Sys, Compiegne, France). The specificity of the renin antibody was previously reported,[11] and this antiserum recognizes both active and inactive renin. The tumors studied consist of one JG tumor, five Wilms' tumors, ten renal adenocarcinomas, three renal oncocytomas, and six renal cortical adenomas. Fresh tissue from

Supported in part by the American Heart Association, Kansas Affiliate, and USPHS Grant HL35323.

Accepted for publication August 8, 1986.

Address reprint requests to Tatsuo Tomita, MD, Department of Pathology, University of Kansas Medical Center, 39th and Rainbow Blvd., Kansas City, KS 66103.

one JG tumor, one Wilms' tumor, and two renal adenocarcinomas was also collected for studying renin activity. Tissues were extracted with 10 mM PO_4 buffer (pH 7.0) containing 10 mM EDTA. Extracts were assayed for renin and inactive renin by our previously described method.[12] Briefly, this consists of exposing the extract to buffer or trypsin solution (2 μg/ml) for 30 minutes at room temperature, incubation with sheep substrate to generate Angiotensin I, and radioimmunoassay of Angiotensin I.[12] The results are expressed as milliGoldblatt units per gram tissue with the use of an international reference standard for renin.

Results

Immunohistochemical Findings

The number of patients, their age, sex, size of tumors, and the result of immunohistochemical staining are given in Table 1.

JG Tumor

The tumor is a well-circumscribed subcapsular mass, measuring 2 cm in diameter (Table 1). Histologically, the tumor is surrounded by the pseudocapsule with pushing margin (Figure 1) and consists of solid sheets or cords of closely packed tumor cells containing uniform nuclei and plump cytoplasm larger than that of the adjacent renal tubules. The cytoplasm is polygonal and basophilic by hematoxylin and eosin (H&E) staining (Figure 1). By immunohistochemical staining, the adjacent renal cortex revealed darkly stained renin-containing cells in the JG apparatus (Figures 2 and 3), whereas the tumor cells were less dark, but diffusely mild to moderately stained (Figure 2).

Wilms' Tumors

Among the five cases, one case reveals scattered, strongly renin-positive cells in predominantly glandular tumor cells, which appear spindled in shape and are located along the longer axis of the cytoplasm (Figure 4).

Renal Adenocarcinomas

Among ten cases studied, three cases show sparsely scattered renin-positive tumor cells. Positive staining is round and focally occupies the cytoplasm.

Oncocytic Adenomas

Two of three cases reveal focally round renin-positive cytoplasm (Figure 5), similar to that of renin-positive renal adenocarcinomas.

Table 1—Summary of Cases

Case	Age	Sex	Tumor: Site (R or L)	Tumor: Size (cm)	Renin staining*
A. JG cell tumor					
1	14	F	R	2.0 × 2.0	++++
B. Wilms' tumors					
1	1	M	R	15 × 15 × 10	+++
2	2	F	R	7.5 × 7.5 × 7	−
3	7	F	R	19 × 10 × 7	−
4	8	F	L	5.6 × 4 × 4	−
5	10	M	L	4 × 4 × 3	−
C. Renal adenocarcinomas					
1	21	F	R	1.5 × 1.0 × 1.0	−†
2	28	F	R	11 × 8 × 7	−
3	39	M	R	13 × 10 × 8	+
4	61	F	L	9 × 8 × 8	+
5	61	F	L	7.5 × 7.5 × 5	−
6	63	M	R	7 × 5 × 3	+‡
7	65	M	R	4.5 × 4 × 3	−
8	71	M	R	4 × 3 × 2	−
9	72	M	L	5.5 × 5 × 4	−§
10	73	F	R	7.5 × 6 × 4.5	−
D. Renal oncocytomas					
1	65	M	R&L	1.2 × 0.8 × 0.8 1.5 × 1.0 × 1.0	+‖
2	72	M	L	0.7 × 0.4 × 3.0 0.4 × 0.4 × 0.3	−‖
3	76	M	R	1.5 × 1.0 × 1.0	+
E. Cortical adenomas					
1	53	M	L	1.2 × 0.9 × 0.8	−‡
2	64	M	R&L	1.3 × 1.2 × 1.0 1.0 × 0.8 × 0.7	−‖
3	65	M	R	0.7 × 0.5 × 0.3 0.6 × 0.5 × 0.5	−‖
4	65	M	R&L	1.5 × 1.2 × 1.0 1.4 × 1.0 × 0.8	−‖
5	72	M	R	0.5 × 0.4 0.3 × 0.3 0.4 × 0.3	−§‖
6	75	M	L	0.5 × 0.4	−

*Grading system: ++++, all tumor cells positively stained. +++, more, ++, the same number; or +, less tumor cells positive, compared with adjacent normal renal cortex.

†The patient with tuberous sclerosis also had an angiomyolipoma in the left kidney.

‡The patients had been on hemodialysis for more than 10 years.

§The same patient had both renal adenocarcinoma and cortical adenoma.

‖Multiple adenomas.

Cortical Adenomas

All of six cases are composed of either papillary or trabecular basophilic tumor cells and are negative for renin staining (Figure 6).

Renin Activity of Tumor Tissue

The renin activity of the tissue extract is given in Table 2. The two tissue samples of normal renal cortex revealed about 2700 mU renin/g tissue. The JG tumor contained about 100 times renin activity of normal renal cortex (Table 2), which was very similar to the value reported by Galen et al with another JG

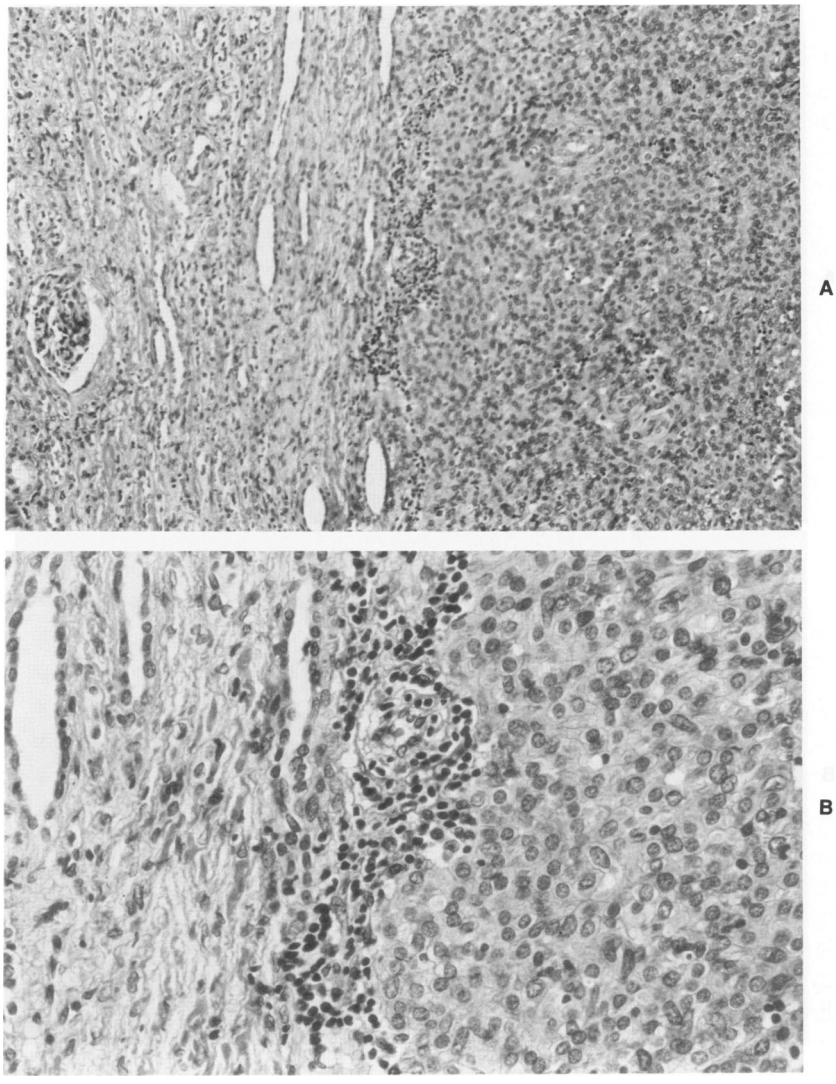

Figure 1—JG tumor. The tumor is surrounded by the pseudocapsule with pushing margin, containing lymphocytic infiltrates. It consists of uniform cells with larger nuclei and more abundant cytoplasm than those of adjacent renal tubules. (H&E, **A**, ×125; **B**, ×625)

tumor.[13] The active and total renin levels were the same for the normal renal cortex and the JG tumor.

The renin level of Case 1, Wilms' tumor, was about 2% that of the normal cortex; about half was active and half was inactive renin (Table 2). Case 3, renal adenocarcinoma, was studied with two tumor samples from the same tumor, which showed the same renin levels as the Wilms' tumor with one sample and about 15% of active renin and 85% inactive renin with the second sample (Table 2). Case 5, renal adenocarcinoma, revealed a total renin level less than 1% of normal cortex, and about 60% of this 1% was inactive renin. Thus, both Wilms' tumor and renal adenocarcinoma contained up to 2% of renin activity of the normal renal cortex, and more than half of this renin was inactive (Table 2).

Discussion

Renin immunoreactivity was detected by immunohistochemistry in the tumor cells of JG cell tumor, Wilms' tumor, renal oncocytoma, and renal adenocarcinoma: the latter two were only focally positive in tumor cell cytoplasms. Only the JG tumor presented clinical symptoms characteristic for hyperreninemia as reported previously.[14]

In the human kidney, renin-containing cells are detected in early metanephros (5-week-old fetus), and most of them are located in the wall of the renal artery branches, particularly in the prospective vascular pole of the glomeruli.[15] Renin-containing cells can be found in the poorly differentiated peripheral cortical blastema, in nearby arterioles of pocketlike S-shaped tubules as well.[15]

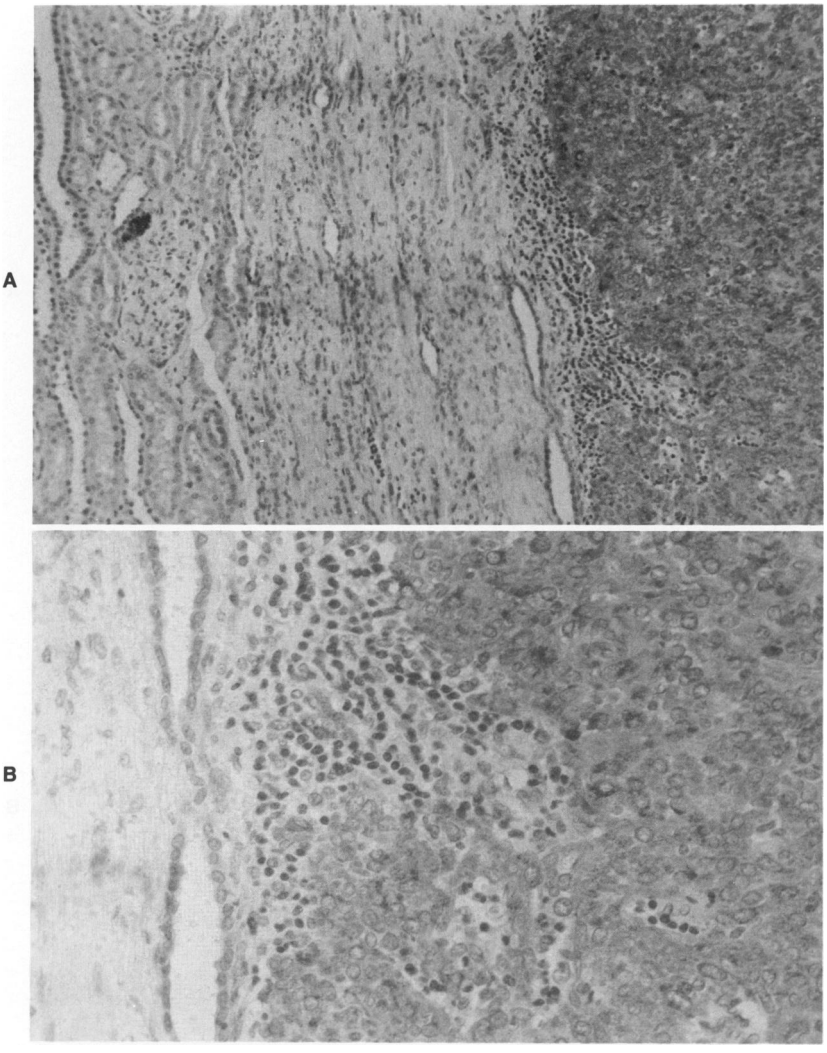

Figure 2 — JG tumor. By immunohistochemical staining for renin, the JG apparatus adjacent to the glomerulus is darkly stained, whereas the tumor cells are less, but diffusely, stained. (**A**, ×125; **B**, ×625)

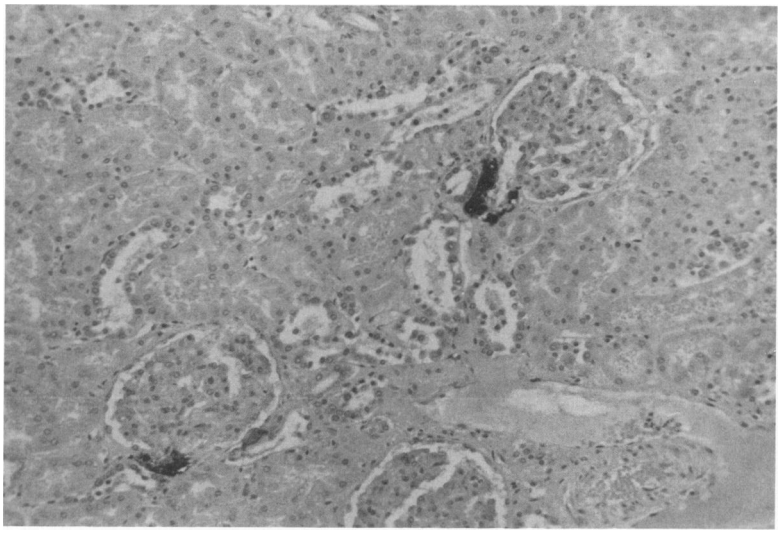

Figure 3 — Normal renal cortex adjacent to the JG tumor. By immunohistochemical staining, strongly renin-positive cells are apparent in the JG apparataus adjacent to two glomeruli. (×250)

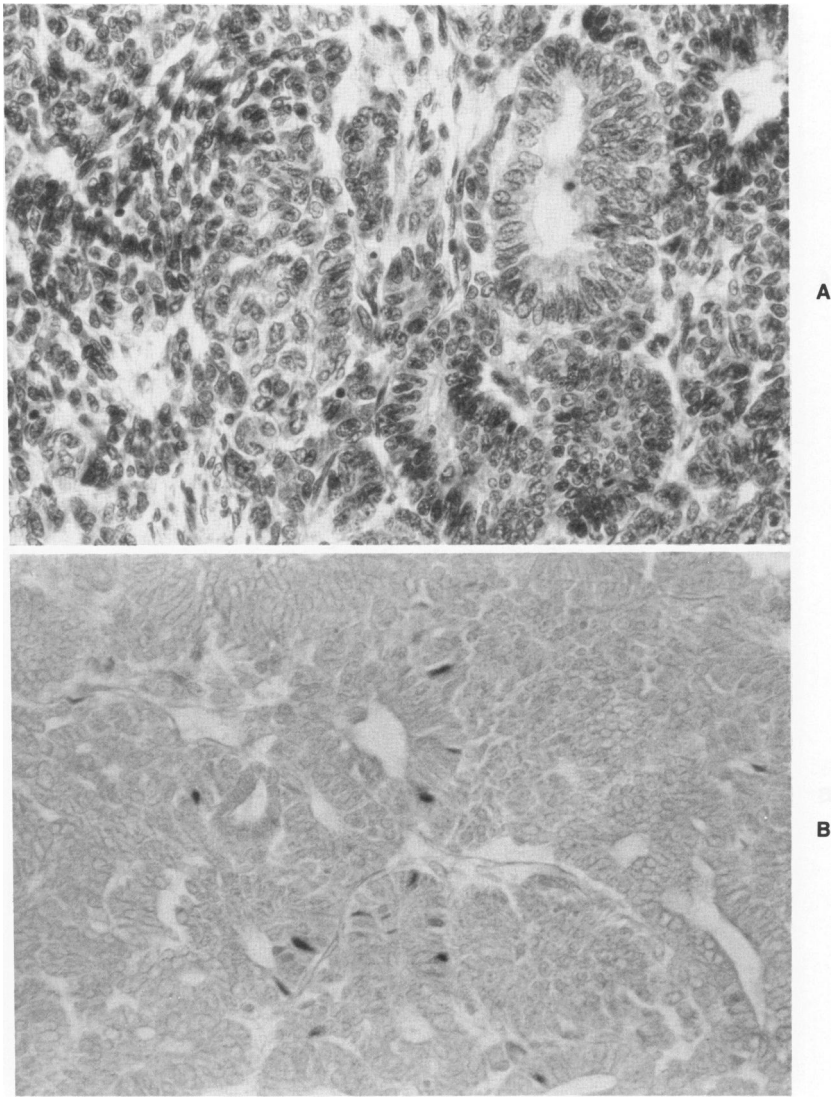

Figure 4 — Wilms' tumor, Case 1. This tumor is an admixture of glandular and stromal components. Both have scattered, but more numerous, renin-containing cells than the adjacent normal renal cortex. The positive renin staining appears spindled in shape along the longer axis of the cytoplasm. (**A**, H&E, **B**, immunostained for renin, ×625)

Wilms' tumor originates primarily from the cells of metanephritic blastema.[16] Typically, Wilms' tumor consists of an admixture of three components, epithelial, stromal, and blastematous,[16] although any of these may be predominant. In five cases of Wilms' tumors, only one case of predominantly blastematous tumor revealed abundant renin-containing cells by immunohistochemistry. The total renin concentration in the Wilms' tumor was about 2% that of the adjacent kidney, whereas the inactive renin concentration was higher than 50% of the total renin.

This situation is also probably true for renal oncocytomas and renal adenocarcinomas. Two of the latter tumors were studied for renin activity by radioimmunoassay, revealing about 1–2% of the renin activity of that found in the renal cortex, despite occasional renin-containing cells seen in one case by immunohistochemistry. Lindrop et al reported that as many as 40% of Wilms' tumors studied contained renin as seen by immunohistochemistry,[17] which also warrants further study by subclassifying Wilms' tumors as to epithelial, stromal, and blastematous differentiation with regard to renin production. The presence of renin in renal oncocytomas and renal adenocarcinomas also presents the question of the histogenesis of these tumors. Both tumors appear to arise from proximal convoluted tubular cells.[16] The reasonable speculation of histogenesis of renin-containing tumor cells is that the bulk of tumor cells differentiate into tubular cells but also partially develop into renin-containing cells as well either by 1) differentiating from metanephritic blastematous cells or 2) reverting into more pluripotent blastematous cells.

It is well known from the study of other tumors that

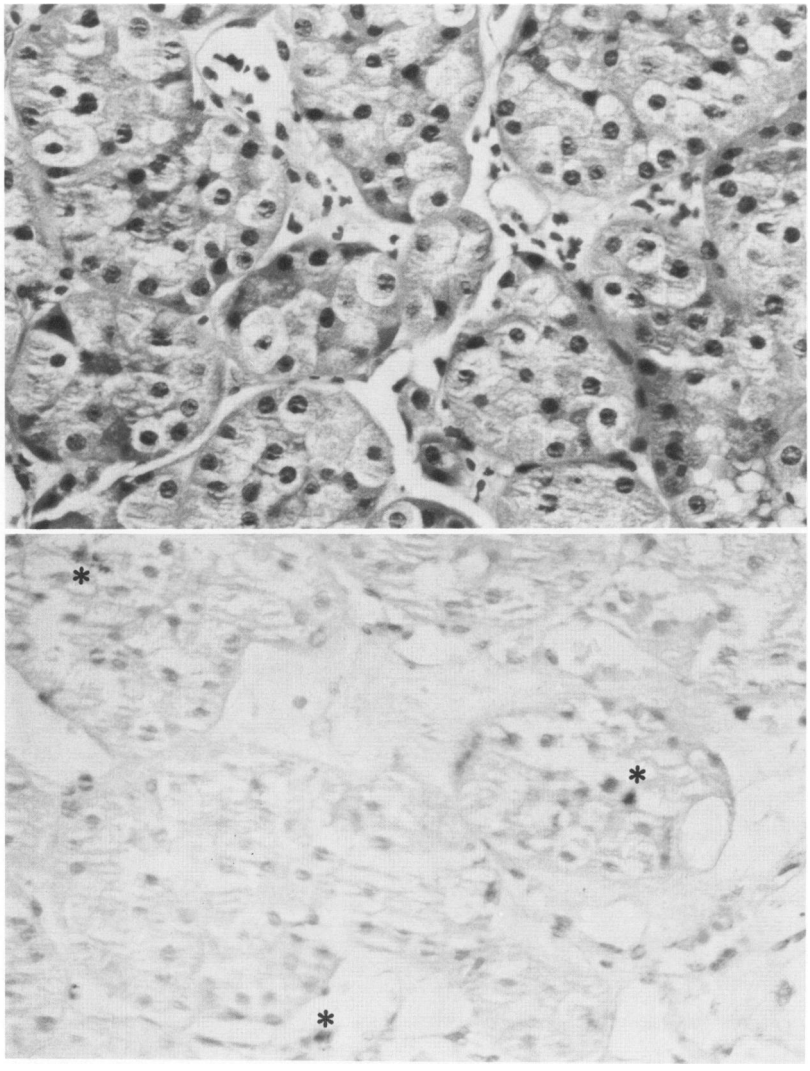

Figure 5—Oncocytic adenoma, Case 1. The tumor consists of relatively uniform tubular patterns with round nuclei and plump, eosinophilic, and granular cytoplasm. There are scattered, focally renin-positive tumor cell cytoplasms (*). **A**, H&E, **B**, immunostained for renin, ×625)

elaboration of peptides, including renin, appears to be a part of progressive cell differentiation in tumors.[18] Furthermore, JG cells were detected by electron microscopy in an angiomyolipoma,[19] which supports the nature of JG cells as "myoendocrine" cells with features of endocrine and smooth muscle cells.[20] The recently recognized atrial natriuretic peptide in the heart also supports the conceptional presence of "myoendocrine" cells.[21-23] JG cells are also present in the extraglomerular mesangium, and they increase in number under certain conditions such as adrenectomy and renal artery constriction.[20]

Reninlike substances have been detected in extrarenal tissues, including salivary gland,[24] brain,[25] and fetal membranes.[26] In the latter case, renin is almost entirely in the form of inactive renin. Inactive renin was also found in a case of hydatidiform mole.[27] Thus, there are also possibilities of renin (or inactive renin) production by extrarenal tissue and its tumors.[28] Although once attributed to the nonspecific proteolytic activity of cathepsin D,[29] recent studies using specific antirenin serum distinguished renin from other proteolytic enzymes in various tissues.[24-26,29] The presence of renin in extrarenal tissue raises an intriguing hypothesis that there exists a tissue renin–angiotensin system which can locally regulate tissue function in extrarenal tissue and its tumors.[28] By immunohistochemistry, alveolar soft part sarcomas were reported to contain renin[30]; however, renin activity was not studied by radioimmunoassay. We have also studied alveolar soft part sarcoma, which was negative for renin with our antiserum. Antisera have to be fully evaluated for any possible cross-reactivity with other antigens prior to performing immunohistochemical study. The confirmation of renin is not sufficient by immunohistochemical study alone, but

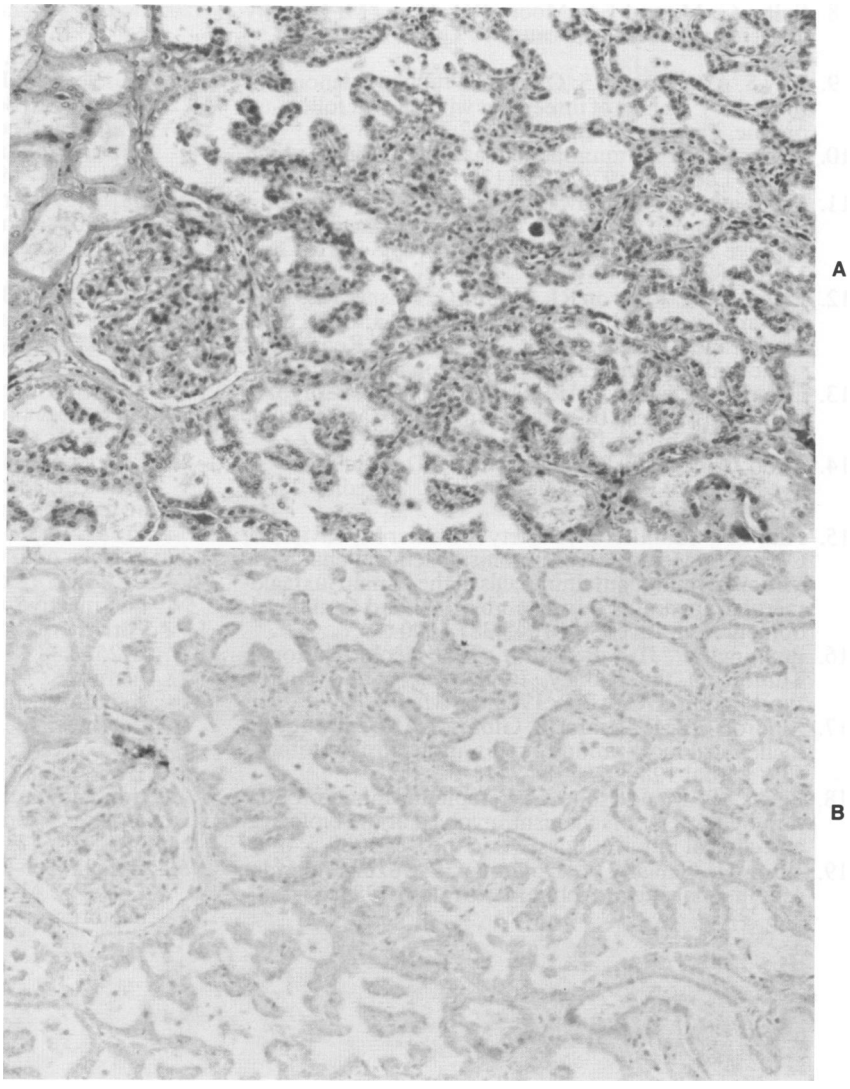

Figure 6—Cortical adenoma, Case 6. The trabecular, basophilic tumor cells are uniformly negative for renin, in contrast to the strongly positive JG cells adjacent to the glomerulus. (**A**, H&E, **B**, immunostained for renin, ×250)

Table 2—Renin Activity of Tumor and Kidney (mGoldblatt U/g tissue)

Case	Active renin	Inactive renin	Total renin
A. JG tumor			
1. Tumor	221,000	0	221,000
B. Wilms' tumor			
1. Kidney	2710	0	2710
Tumor	23.6	24.4	48.0
C. Renal adenocarcinoma			
3. Kidney	2708	0	2708
Tumor* −1	23.6	24.4	48.0
−2	3.9	20.3	24.2
5. Tumor	8.7	13.5	22.2

*Two portions of the same tumor were studied.

also requires the quantitative value of renin activity by radioimmunoassay.

References

1. Heptinstall RH: Renin-secreting renal tumors, Pathology of the Kidney. 3rd edition. Boston, Little, Brown, 1983, pp 282–285
2. Lebel U, Talbet J, Grose J, Morian J: Adenocarcinoma of the kidney and hypertension. J Urol 1977, 118:923–927
3. Bloodworth JMB: Juxtaglomerular cell tumors, Endocrine Pathology, Baltimore, Williams and Wilkins, 1982, pp 746–747
4. Mitchell JD, Baxter TJ, Blair-West JR, McGredie DA: Renin levels in nephroblastoma (Wilms' tumor). Arch Dis Child 1970, 45:376–389
5. Gangly A, Gribble J, Tore B, Kempson RL, Luetscher JA: Renin secreting Wilms' tumor with severe hypertension. Ann Intern Med 1973, 79:835–837
6. Stine K, Goertz K, Poisner AM, Lowman J: Congestive heart failure, hypertension and hyperreninemia in bilateral Wilms' tumor: Successful medical management. Med Ped Oncol 1986, 14:63–66
7. Smith DR: Tumors of genitourinary tract, General Urology, Los Altos, Lange Medical Pub. 1984, pp 306–323

8. Sulfin G, Mirand EA, Moore RH, Chu TM, Murphy GP: Hormones in renal cancer. J Urol 1977, 117:433–438
9. Kay S, Armstrong KS: Oncocytic tubular adenoma of the kidney: Report of three cases with 28 year follow-up on one. Prog Surg Pathol 1980, 2:259–268
10. Taylor CR: Immunoperoxidase techniques. Arch Pathol Lab Med 1978, 102:113–121
11. Yokosawa H, Yokosawa N, Inagami T: Specific antibody to human renal renin and its cross reactivity with inactive human plasma prorenin. Proc Soc Exp Biol Med 1980, 164:466–470
12. Poisner AM, Johnson RL, Hanna G, Poisner RB: Activation of renin in human amniotic fluid and placental membranes, Heterogeneity of Renin and Renin-Substrate. Amsterdam, Elsevier, 1981, pp 335–347
13. Galen FX, Devaux C, Honot AM, Menard J, Corvol P: Renin biosynthesis by human tumoral juxtaglomerular cells. J Clin Invest 1984, 73:1144–1155
14. Elrod JA, Warady BA, Beatty EC Jr, Duggan E: Severe hypertension in an adolescent girl. J Pediat 1982, 10:409–411
15. Phat VN, Camilleri JP, Bariety J, Galtier M, Baviera E, Corvol P, Merad J: Immunohistochemical characterization of renin-containing cells in the human juxtaglomerular apparatus during embryonal and fetal development. Lab Invest 1981, 45:387–390
16. Bennington JL, Beckwith JB: Nephroblastoma, Tumors of the Kidney, Renal Pelvis, and Ureter. Washington, DC, AFIP, 1975, pp 31–78
17. Lindrop GBM, Fleming S, Gibson AAM: Immunocytochemical localization of renin in nephroblastoma. J Clin Pathol 1984, 37:738–742
18. Baylis SB, Mendelsohn G: Ectopic hormone production by tumors: Mechanism involved at the biological and clinical implications. Endocrine Rev 1980, 1:45–77
19. Yum M, Ganguly A, Donohue JP: Juxtaglomerular cells in renal angiomyolipoma. Urology 1984, 24:283–286
20. Berajas L: Anatomy of the juxtaglomerular apparatus. Am J Physiol 1979, 237:F333-F343
21. Jamieson JD, Palade GE: Specific granules in atrial muscle cells. J Cell Biol 1964, 23:151–172
22. De Bold AJ, Borenstein HB, Veress AT, Sonnenberg H: A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci 1981, 28:89–94
23. Burnett JC, Jr, Kao PC, Hu DC, Heser DW, Heublein D: Atrial natriuretic peptide elevation in congestive heart failure in the human. Science 1986, 231:1145–1147
24. Gresik EW, Barka T: Epidermal growth factor, renin and protease in hormonally responsive duct cells of the mouse sublingual gland. Anat Rec 1983, 205:169–175
25. Inagami T: Renin in the brain and neuroblastoma cells: An endogenous and intracellular system. Neuroendocrinology 1982, 35:475–482
26. Poisner AM, Wood GW, Poisner R, Inagami T: Localization of renin in trophoblasts in human chorion laeve at term pregnancy. Endocrinology 1981, 109:1150–1155
27. Poisner AM, Cheng HC, Tomita T, Poisner R, King CR: Report of a hydatidiform mole containing renin, inactive renin, progesterone and HCG (Abstr) 7th International Congress on Endocrinology 1984, p1251
28. Naruse K, Murakoshi M, Osamura Y, Inagami T: Immunohistochemical evidence for renin in human endocrine tissue. J Clin Endocrinol Metab 1985, 61:172–177
29. Hackenthal E, Hackenthal R, Hilgenfeld V: Purification and partial characterization of rat brain acid proteinase (isorenin). Biochim Biophys Acta 1978, 522:561–573
30. DeSchryver-Kecskemeti K, Kraus FT, Engleman W, Lacy PE: Alveolar soft part-cell sarcoma: A malignant angioreninoma. Am J Surg Pathol 1982, 6:5–8