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Renin Localization in Segmental Renal Hypoplasia

Immunohistochemical Demonstration in Two Cases

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Summary. The distribuation of renin in two cases of segmental renal hypoplasia was investigated by immunofluorescence and the peroxidase anti-peroxidase (PAP) method using an anti-human renin antiserum. Renin-containing cells were found only in hypoplasic segments in the vicinity of altered glomeruli and small arteries. Well-preserved renal cortex and areas of chronic atrophic pyelonephritis failed to show any demonstrable site of renin production.

Whatever is the mechanism of the disease, the characterization of large numbers of renin-containing cells in the affected kidney support a role for the renin-angiotensin system stimulation in this form of hypertension.

Key words: Renin – Kidney – Segmental renal hypoplasia – Hypertension – Immunofluorescence – Peroxidase anti-peroxidase (PAP)

Introduction

Segmental renal hypoplasia (SRH) is a rare clinico-pathological entity predominantly affecting girls and frequently associated with vesicoureteral reflux (Ask-Upmark 1929; Habib et al. 1965). It is characterized by small sized kidneys with one or several scarred lobes sharply delimited from the normal tissue. SRH is a well known cause of hypertension in children and young adults. Hypertension may be related to a disturbance of the renin-angiotensin system as increased plasma renin activity has been demonstrated in peripheral blood and in the renal vein of the affected side (Favre 1967; Godard et al. 1973; Rosenfeld et al. 1973; Barajas et al. 1977). In addition, large numbers of juxtaglomerular granular cells (JGC) have been observed by conventional histochemistry and electron microscopy in the abnormal tissue (Barajas et al. 1977). These findings make plausible thy hypothesis that hypertension in SRH is secondary

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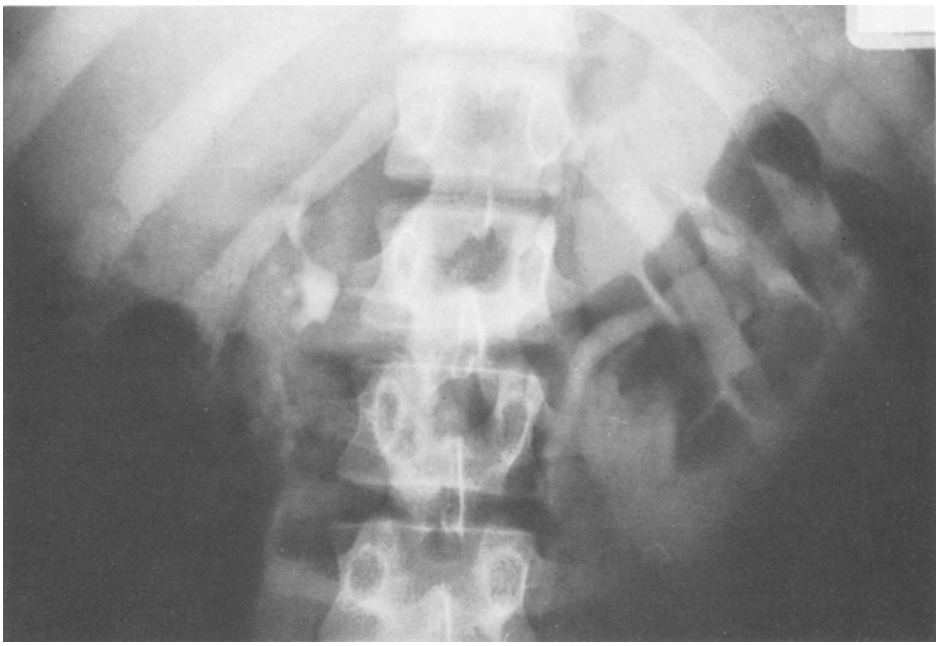

Fig. 1. Case 1. Intravenous pyelogram showing the small size of the right kidney and the deformities of the calices. Normal looking left kidney

to renin hypersecretion by the hypoplasic tissue. However a direct immunohistochemical demonstration of this phenomenon has not been achieved so far.

In this report we are presenting findings documenting the presence of renin-containing cells in the hypoplasic tissue by immunofluorescence (IF) and by the peroxidase anti-peroxidase method (PAP). The precise localization of these cells and their relationship with other structures of the renal cortex are described.

Case Report

Case 1. A 15 year old girl was noted to be hypertensive (180/120 mmHg) during a routine medical check up. Past history was unremarkable except for enuresis until age 14. Creatinine clearance was 86 ml/min. A fundi examination and an electrocardiogram were normal. An intravenous pyelogram revealed a small right kidney with pyelocaliceal deformities (Fig. 1). The controlateral kidney was normal. Radioactive mercury fixation was markedly diminished in the right kidney. Cystography did not show vesico-ureteral reflux. Peripheral blood renin activity was 5.8 ng/ml·h (normal less than 3 ng/ml·h) while the 24 h urinary sodium excretion was 148 nmol. After furosemide administration, renin activity was 12 mg/ml·h in the right renal vein and 8.6 ng/ml·h in the left one. A right nephrectomy was performed.

Case 2. A 21 year old girl was admitted because of sudden onset of intense headaches. Physical examination disclosed an elevated blood pressure (148/102 mmHg). In the past, she had complained of intermittent headaches for many years. During childhood she had two episodes of cystitis successfully resolved by antibiotherapy. Pyelograms were not performed at that time. Creatinine clearance was 65 ml/min. Urinary sediment, fundi and electrocardiogram were normal. An intravenous pyelogram revealed an irregularly shaped small left kidney with a thin cortex (Fig. 2). The right kidney

Fig. 2. Case 2. Tomography showing prominance of the leions in the middle and upper parts of the left kidney

was also irregular in shape and two notches, one in each pole, were noted. A cystogram revealed major vesico-ureteral reflux. Radioactive mercury fixation was 25% of normal in the left kidney and 135% in the right one. Peripheral blood plasma renin activity was 3.5 ng/ml·h while the 24 h urinary sodium excretion was 69 nmol. After furosemide plasma renin activity was 6.0 ng/ml·h in the left renal vein and 5.8 ng/mg·h in the right one. A left nephrectomy was performed.

Material and Methods

Renin Antiserum. Human renin was purified from a juxtaglomerular cell tumor. The physico-clinical characteristics and the criteria of purity used have already been reported (Galen et al. 1979a). Anti-renin antibodies were raised in rabbits by method already described (Galen et al. 1979b). The antiserum with the highest anti-renin activity was selected for immunochemistry. At a 1:80,000

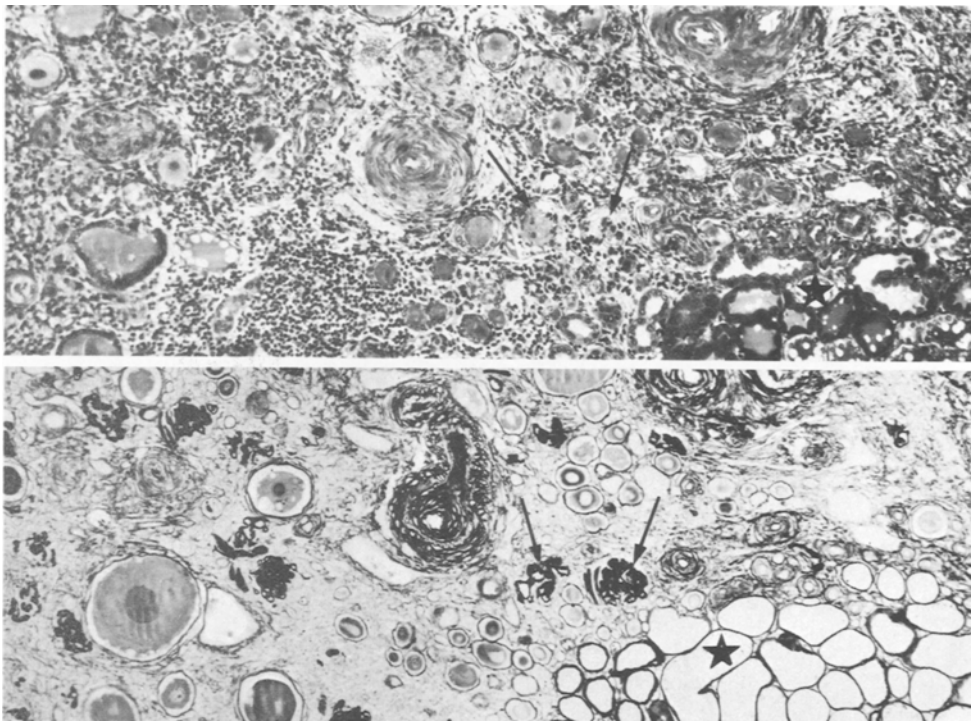

Fig. 3. Hypoplasic area in case 2. Matched sections of the same area stained with Masson's trichrome (*upper part*) and Marinozzi's silver methenamine (*lower part*). Atrophic tubules and altered vascular channels are present. Glomeruli are difficult to distinguish on trichome stain (*arrows*) and are readily appearent on silver methenamine impregnation (*arrows*). *Star*=spared renal parenchym. G=135 X

dilution, it inhibited the enzymatic activity of 25 microGoldblatt units of renin. In immunodiffusion, it produced a smooth precipitation line without spur formation against renin isomorphs indicating the immunological identity of these isorenins. By immunoelectrophoresis, a single precipitation line against each isorenin was seen (Galen et al. 1979b). It did not cross-react with renin from dog and rat, human albumin, pepsin, and cathepsin D. Renal renin, plasma renin and acid-activated plasma renin were inhibitd by this antiserum and behaved identically in the human renin radioimmunoassay.

Kidney Specimens. In both cases, the excised kidneys were considerably reduced in size (Case 1= 40 g; Case 2=30 g), and several coarse scars were apparent on the outer surface. On cut sections hypoplasic segments had no recognizable pyramids and were sharply delimited from the normal looking tissue. Sagittal sections passing through the hilus were fixed in Bouin's alcoolic fluid, embedded in paraffin and serially sectioned every 3 μm. Consecutive sections from a ribbon were picked up on glass slides, numbered, and stained with haematoxylin eosin safran, Masson's trichrome, Marinozzi's silver methenamie and orcein. In order to better establish the location of renin in relation to glomerular and vascular structures, sections stained with Masson's trichrome or silver methenamine were compared with alternate sections incubated with antibodies to renin by either immunofluorescence or the PAP procedure.

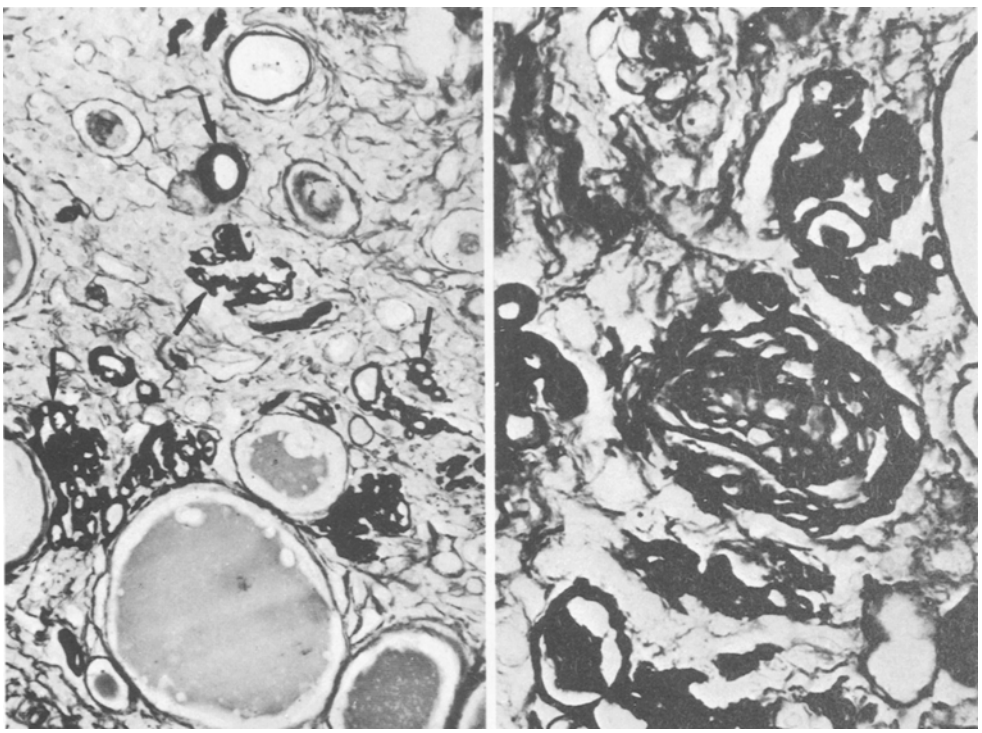

Fig. 4. Same hypoplasic area showing dilated tubules and glomeruli revealed as conglomerates of loops (*arrows*) with thickened and rigid basement membrane (*left*). Detail of an interlobular artery surrounded by a network of thickened basement membranes (*right*). Bowman's capsules are not clearly visible. Left part G=340×, right part G=480×

Immunohistochemical Methods. After removing paraffin with xylene, sections were passed through successive alcohol baths and washed in phosphate buffered saline (PBS). Indirect immunofluorescence was carried out as follows: sections were exposed to unlabelled rabbit renin antiserum at 1:50 dilution for 30 min. After repeated washing tissue sections were incubated with fluoresceinated goat anti-rabbit immunoglobulin for 30 min (Hyland Laboratories). The slides were mounted in buffered gelatin an examined under ultraviolet light with a Leitz Ortoplan Ortomat microscope.

Immunoperoxidase staining was performed by the unlabelled antibody peroxidase-antiperoxidase (PAP) procedure according to Sternberger (1974). Sections were incubated with normal rabbit serum at a 1:5 dilution for 30 min. Washing in PBS was followed by overnight incubation with the renin anti-serum diluted to 1:10,000; then, slides were soaked in normal swine serum at 1:5 dilution for 5 min. Excess serum was removed without washing and sections were treated in succession, with swine anti-rabbit serum (1:10, 30 min) and rabbit peroxidase-antiperoxidase complex (1:50, 30 min). Thereafter peroxidase was revealed by incubation in 3.3′ diamino-benzidine tetrahydrochloride and hydrogen peroxide for 20 min. (Graham and Karnowsky 1966). All antibodies and the PAP complex were obtained from Sebia.

In control sections, the anti-renin antiserum was omitted or replaced by rabbit normal serum. The specificity of immunohistochemical reaction was tested by incubating sections with antiserum which previously had been absorbed with pure human renin (20 μg per ml of original serum).

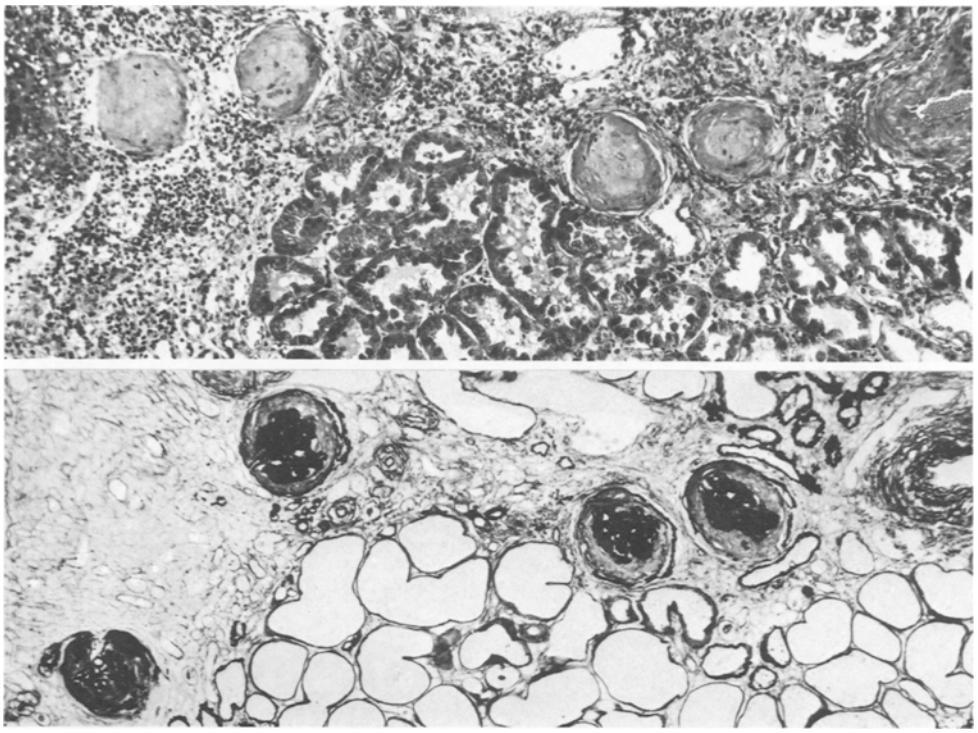

Fig. 5. Chronic atrophic interstitial nephritis in case 2. Matched sections of the same area stained with Masson's trichrome (*upper*) and Marinozzi silver methenamine (*lower*). Note periglomerular fibrosis. After silver impregnation Bowman's capsules remain identifiable. Compare with Fig. 3. G=135×

Results

Light Microscopy

In both cases the light microscopic appearance of the hypoplasic tissue was similar (Fig. 3). There were band-shaped areas of corticomedullary scarring sharply delimited from the surrounding tissue. The overlying cortex was thinned and retracted and showed advanced interstitial sclérosis and tubular atrophy; other tubules were markedly dilated and contained abondant proteinaceous material. A mononuclear interstitial infiltrate with a predominance of lymphocytes and presence of mast cells could be seen. Owing to their marked tortuosity the interlobular arteries gave the impression of being more numerous. With Masson's trichrome stain, glomeruli were identified with difficulty; they appeared as small masses made up of amorphous hyalin material and clusters of apparently endothelial cells. The seemed to be more numerous and of decreased size. With silver methenamine stain glomeruli were readily appearent as small conglomerates of 2 to 4 loops with thickened and rigid basement mem-

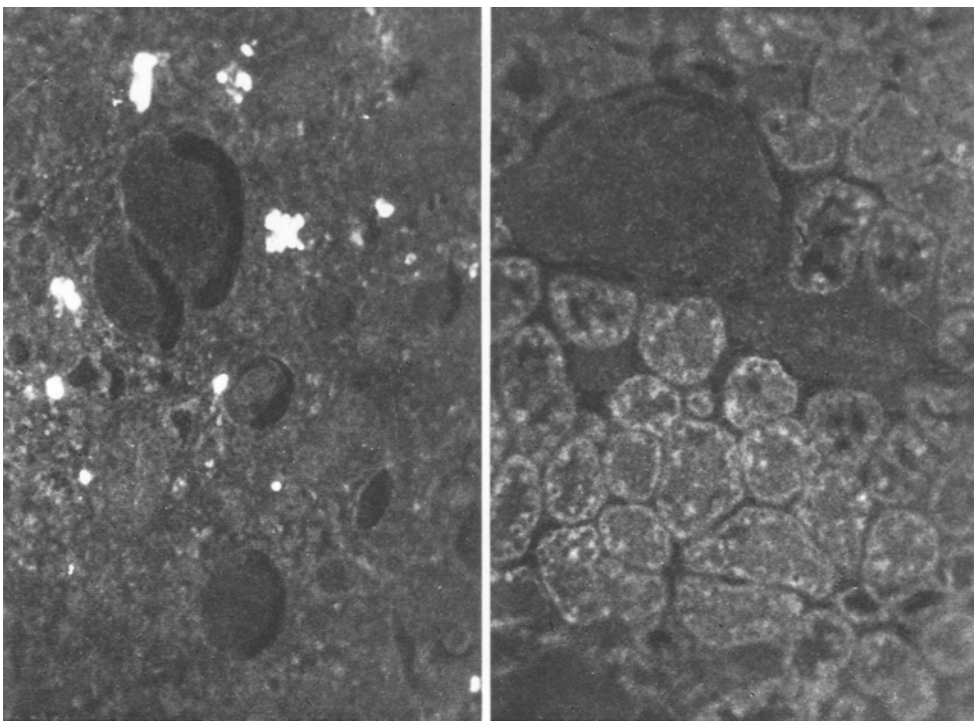

Fig. 6. Immunofluorescence in case 1 showing small groups of renin-containing cells scattered throughout the hypoplasic area (*left*). Normal area with two well recognizable glomeruli (*right*). No fluorescence is observed in the non hypoplasic tissue except in some convoluted proximal tubules

brane and barely visible lumina. Glomeruli were devoid of Bowman's capsule and seemed to arise abruptly from small arteries (Fig. 4).

In the non hypoplasic tissue of both cases some glomeruli showed ischaemic features but they were otherwise unremarkable. Hyalin subendothelial deposits were seen in more arterioles. Foci of chronic interstitial nephritis were frequent near the renal capsule, particularly in case 2. In these areas inflammatory cellular infiltration was more intense than hypoplasic parenchyma. There was periglomerular fibrosis and after impregnation Bowman's capsules were readily identifiable (Fig. 5).

Immunohistochemical Findings

By immunofluorescence, renin containing cells were found only in the hypoplasic segments (Fig. 6). Positive cells showed diffuse or granular cytoplasmic fluorescence and usually occured in small groups of 2 to 6 cells irregularly distributed throughout the cortex, being more numerous immediately below the capsule.

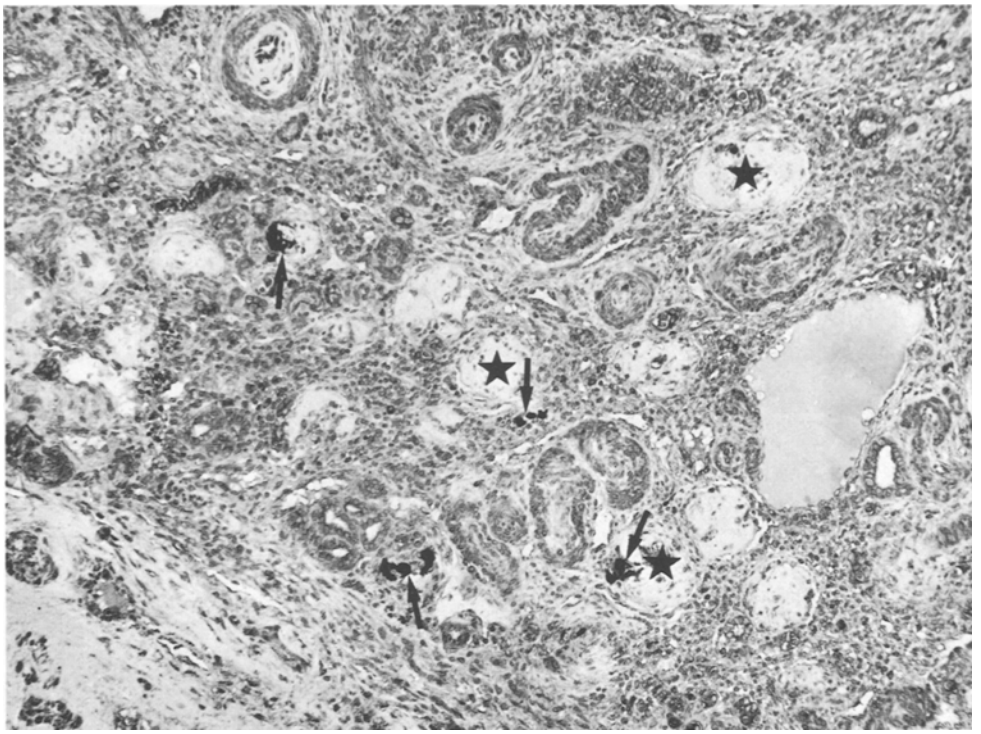

Fig. 7. Hypoplasic area revealed after indirect PAP-staining with renin antiserum. Note scattered foci of renin containing cells (*arrows*). Stars: hyalinized glomeruli. G=150×

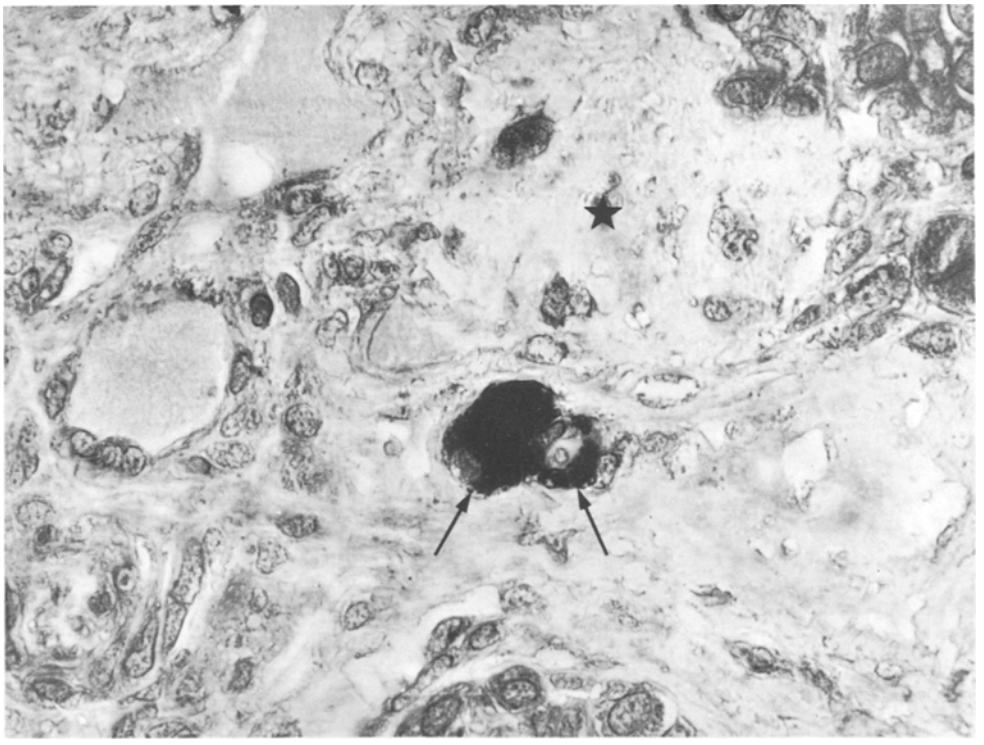

Fig. 8. Detail of a group of renin-containing juxtaglomerular cell involving a small arteriole showing well defined dark cytoplasm (*arrows*). *Star*: glomerulus. Indirect PAP-reaction. G=1.130×

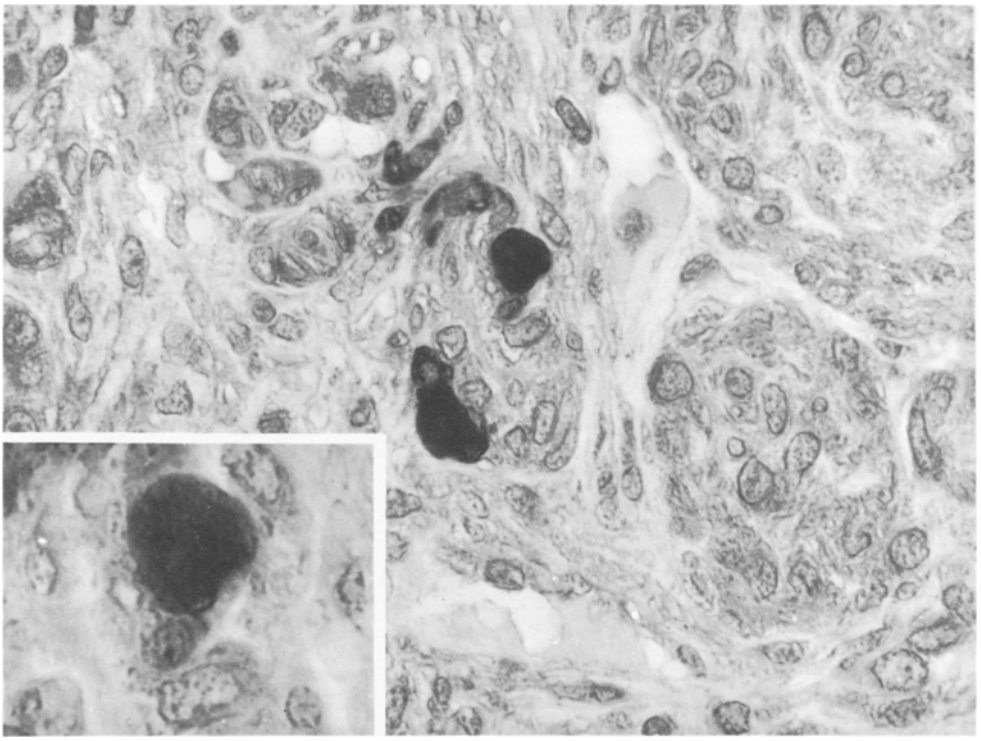

Fig. 9. Detail of an interlobular artery showing two renin-containing cells. Indirect PAP-staining. G=1.130×. *Inset*: high magnification of a renin-containing cell. The reaction product appears coarsely granular. G=2.300×

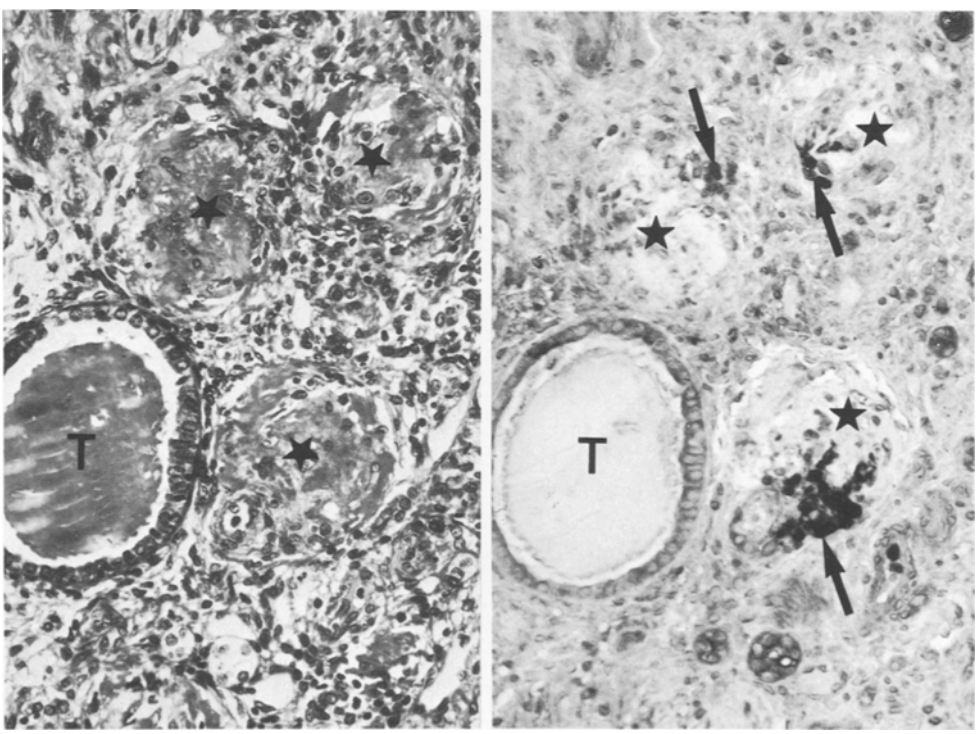

Fig. 10. Hypoplasic area. Matched sections stained with Masson's trichrome (*left*) and indirect PAP-reaction (*right*). Note three groups of renin-containing cells (*arrows*) in close vicinity to three hyalinized glomeruli (*stars*). G=300×

In the spared tissue and the areas of chronic interstitial nephritis of Case 2 no fluorescence was observed except in the brush borders of some convoluted proximal tubules.

By PAP method the distribution of positive cells overlapped with that seen by immunofluorescence (Fig. 7). The cells labelled by the renin antiserum showed well defined dark brown cytoplasm (Fig. 8). In some of them the reaction product appeared coarsely granular (Fig. 9). In addition, examination of serially sectionned slides alternatively stained by the PAP method and trichrome stain allowed us to establish that renin producing cells were always in direct relationship with either glomeruli or small arteries (Fig. 10).

By both immunofluorescence and the PAP method we observed, far away from glomeruli, scattered isolated positive cells within the muscular wall of interlobular arteries.

The control series were consistently negative.

Discussion

The clinical, radiological and pathological features of the cases reported above are those characteristically described in SRH (Habib 1979). The histological diagnosis of this entity is based on the presence of bands of corticomedullary scarring sharply delimited from the spared tissue. In the hypoplasic areas the outstanding changes occur in blood vessels and glomeruli. Arteries and arterioles are extremely tortuous giving the impression of being more numerous. That tortuosity has been confirmed by microangiographic studies (Ljunquist and Lagergren 1961). On Masson's trichrome stained slides, the glomeruli are difficult to identify. But with Marinozzi silver impregnation they appear to be increased in number and display a rudimentary appearance with the Bowman's capsules being usually absent. Such a histological pattern is quite different from that observed in chronic atrophic interstitial nephritis, specially the glomerular changes (Kincaid-Smith 1975; Kissane 1976). The lesions observed in chronic interstitial nephritis may have the same segmental topography as in SRH but show periglomerular fibrosis and extensive interstitial inflammatory infiltrates; the blood vessels are never as modified as in SRH. It is unlikely that the histological features noted in SRH can be the end-result of a process of chronic atrophic pyelonephritis. However, as seen in case 2, the two kinds of changes can be associated in the same Kidney.

As far as we know, immunomorphological studies, using antihuman renin antibodies to localize renin in SRH, have not been reported. The method of preparation and the criteria attesting for the reliability and specificity of the antiserum used in this study have been reported elsewhere (Camilleri et al. 1980). In this study, immunofluorescence and the PAP method proved to be equally efficient to reveal the renin-producing cells. However the PAP technique enhanced greatly our capacity to determine topographic relation of such cells. Similar data are available in mouse kidney (Taugner et al. 1979). As suspected by others by histochemistry and electron microscopy in SRH, renin is formed in the hypoplasic areas, thus providing an anatomical basis for the source

of increased plasma renin activity in renal vein blood of the affected kidney as seen in our patients and in other reported cases (Barajas et al. 1977). The PAP method was useful in determining some characteristics of the renin-containing cells, particularly when they were compared with serially sectioned slides stained with trichrome stain and silver methenamine. The renin-containing cells were not randomly dispersed in the interstitium as described by Barajas (1977); we observed that such cells were always seen in clusters directly related to vascular structures. Positive cells in the wall of medium size arteries were scarce. As a rule rudimentary looking glomeruli were seen in close vicinity to the renin-containing cells. We also observed that the reaction product was always intracytoplasmic and diffuse or granular in appearance.

Thus, hypoplasic areas are recognizable by histological and immunomorphological characteristics. The absence of renin production in normal tissue, as shown by our techniques, is somewhat comparable to that seen by immunofluorescence in non-ischaemic areas of infarcted kidneys (Camilleri et al. 1980). The positivities seen in some convoluted proximal tubules of these portions suggest tubular reabsorption of ultrafiltrated renin. We did not observe renin-containing cells in the associated areas of chronic atrophic interstitial nephritis, and hypoplasic tissue in the same kidney can be also differenciated on immunohistological grounds. Those data are consistent with the fact that in four cases of chronic atrophic pyelonephritis we could not detect a site of renin localization by immunofluorescence (unpublished data). On the other hand, hypertension is rarely seen in patients with chronic pyelonephritis (Holland et al 1975).

Whatever is the mechanism of SRH, the localization of large amounts of renin in some specific sites of the diseased kidney is a new agreement to support the responsability of renin-angiotensin system stimulation in this form of hypertension. Altered glomeruli with their adjacent renin-containing cells can be looked on as a human equivalent of the non-filtering kidney which has been used by Blaine and Davis to study the mechanism of renin secretion in dogs (1971). The signal which is the primary mechanism to stimulate renin synthesis in the altered glomeruli remains unknown. A decrease of the perfusion pressure in the afferent arterioles could be secondary to the lesion in the walls of the medium-sized and small arteries. It is also possible that the defect in glomerular growth is associated with a normal growth or a proliferation of the endocrine arteriolar cells; permanent renin synthesis and release would be due to the structural distorsion of the renal glomerulus.

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