THE GLOMERULAR PERIPOLAR CELL

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DECLARATION

Most of the histological and immunohistochemical preparations used in this research were made by various members of the technical staff, Department of Pathology, Western Infirmary. However, I was responsible for all of the technical preparations in the section of work on the normal human peripolar cell. In addition I am completely familiar with the other techniques, of which I have previous practical experience. Dr.Chris Kenyon, of the Medical Research Council Blood Pressure Unit performed or supervised most of the animal experiments. Otherwise the work which I present is entirely my own.

The section on peripolar cells in the normal human kidney describes the work which I completed in part fulfilment for the degree of BSc in Pathology. Some of this work has already been published:

(1) Gardiner DS, Lindop GBM. The granular peripolar cell of the human glomerulus: a new component of the juxtaglomerular apparatus? Histopathology 1985; 9: 675-685.

(2) Gardiner DS, More IAR, Lindop GBM. The granular peripolar cell of the human glomerulus: an ultrastructural study. Journal of Anatomy 1986; 146: 31-43.

(3) Kelly G, Downie I, Gardiner DS, More IAR, Lindop GBM. The peripolar cell: a distinctive cell type in the mammalian glomerulus. Morphological evidence from a study of sheep. Journal of Anatomy 1990; 168: 217-227.

(4) Gardiner DS, Downie I, Gibson IW, More IAR, Lindop GBM. The glomerular peripolar cell - a review. Histology and Histopathology 1991; (in press).

SUMMARY

The peripolar cell is a recently described glomerular epithelial cell, which may be an additional secretory component of the juxtaglomerular apparatus (JGA). I have described the human peripolar cell. Human peripolar cells were sparse and difficult to find. They were found in 12% of glomeruli on average. They were situated at the vascular pole of the glomerulus, between the visceral and parietal epithelial cells. Peripolar cells contained prominent intracytoplasmic granules, which showed similar staining reactions to the adjacent renin-containing cells of the JGA. However, immunohistochemical studies with an antiserum to human renin, showed that the human peripolar cell does not contain renin.

By electron microscopy, the most striking feature was the intracytoplasmic secretory-type granules. Other organelles were sparse, although there were frequently complex membrane invaginations. Peripolar cells formed junctional complexes with adjacent parietal and visceral epithelial cells.

Sheep peripolar cells were prominent and easily found. They were present in 64% of glomeruli on average, and were packed with cytoplasmic granules. Peripolar cells were sparse in rat kidneys, being

present in only 6% of glomeruli on average. They were similar in appearance to human peripolar cells.

Peripolar cells were most numerous in superficial cortical glomeruli, similar to renin-containing cells. In states of hyperplasia, renin-containing cells were also present in juxtamedullary JGA's, but this could not be confirmed for peripolar cells.

Using immunohistochemical techniques, I have demonstrated plasma proteins in the granules of human and sheep peripolar cells. I have used a variety of antisera and monoclonal antibodies, but have been unable to demonstrate an immunophenotype specific for peripolar cells.

I have examined the reactions of peripolar cells to stimuli, which are known to affect renin-secretion by the JGA. In rats, sodium-depletion or sodium-loading resulted in no alteration in numbers of peripolar cells. I have also examined 11 autopsy cases of Addison's disease, and found that peripolar cells were unaltered despite immunohistochemical evidence of hyperplasia of renin-containing cells.

I investigated the response of the peripolar cell to a reduction in renal perfusion pressure by examining the 2-kidney 1-clip model of experimental hypertension

in rats, and 10 human nephrectomy specimens with renal artery stenosis. In experimental renovascular hypertension, there was no alteration in numbers of peripolar cells, however in the unclipped kidney other glomerular epithelial cells acquired granules. There was hyperplasia of peripolar cells in a minority of cases of renal artery stenosis, but no significant alteration as a group. There was hyperplasia of renin-containing cells in both rats and humans.

I examined 12 autopsy cases of malignant hypertension and found increased numbers of peripolar cells. In addition, other glomerular epithelial cells became granulated and their numbers correlated with peripolar cells. There was hyperplasia of renin-containing cells in some of the cases.

Lastly, I investigated the reactions of peripolar cells in human renal disease. I examined 242 renal biopsies affected by 19 different disaeses. Peripolar cells were especially prominent in membranous glomerulonephritis, mesangioproliferative glomerulonephritis, and focal segmental glomerulosclerosis. Other granulated glomerular and tubular epithelial cells were most prominent in different diseases. This suggests that peripolar cells react specifically in certain immune-complex mediated renal diseases.

ABBREVIATIONS

I have used the following abbreviations:

Abbreviation	Meaning
JGA	Juxtaglomerular apparatus
RCC	Renin-containing cell
A I	Angiotensin I
A II	Angiotensin II
PPI	Peripolar cell index
PPI(r)	Peripolar cell index (random)
ECDs	Epithelial cell droplets
ECDI	Epithelial cell droplet index
ECDI(r)	Epithelial cell droplet index (random)
THDI	Tubular hyaline droplet index
GN	Glomerulonephritis

INTRODUCTION

The anatomy of the juxtaglomerular apparatus (JGA) has been studied in great detail. It was surprising therefore when the peripolar cell, was described for the first time in 1979 (1). It was suggested that it was a secretory cell, which may be an additional component of the juxtaglomerular apparatus (JGA). I commenced this study in 1983. At that time, only four papers concerning peripolar cells had been published. These were: the original report of peripolar cells and the suggestion that they may be part of the JGA (1); a description of peripolar cells in two amphibian species (2); peripolar cell embryogenesis in sheep (3); and an article which described exocytosis of peripolar cell granules in sheep (4). I will first give a description of the JGA, to place the peripolar cell in historical perspective.

(1) THE ANATOMY OF THE JUXTAGLOMERULAR APPARATUS

The JGA consists of the renin-secreting cells of the afferent and efferent glomerular arterioles, the macula densa and the extraglomerular mesangium (5) (Figure 1). The granular "myoepithelioid" cells of the afferent arteriole were first described by Ruyter in 1925 (6), and have subsequently been shown to be the main source of the hormone renin (7,8,9,10). Renin granules stain positively with conventional stains such as Lendrum's Martius scarlet blue, the periodic acid-

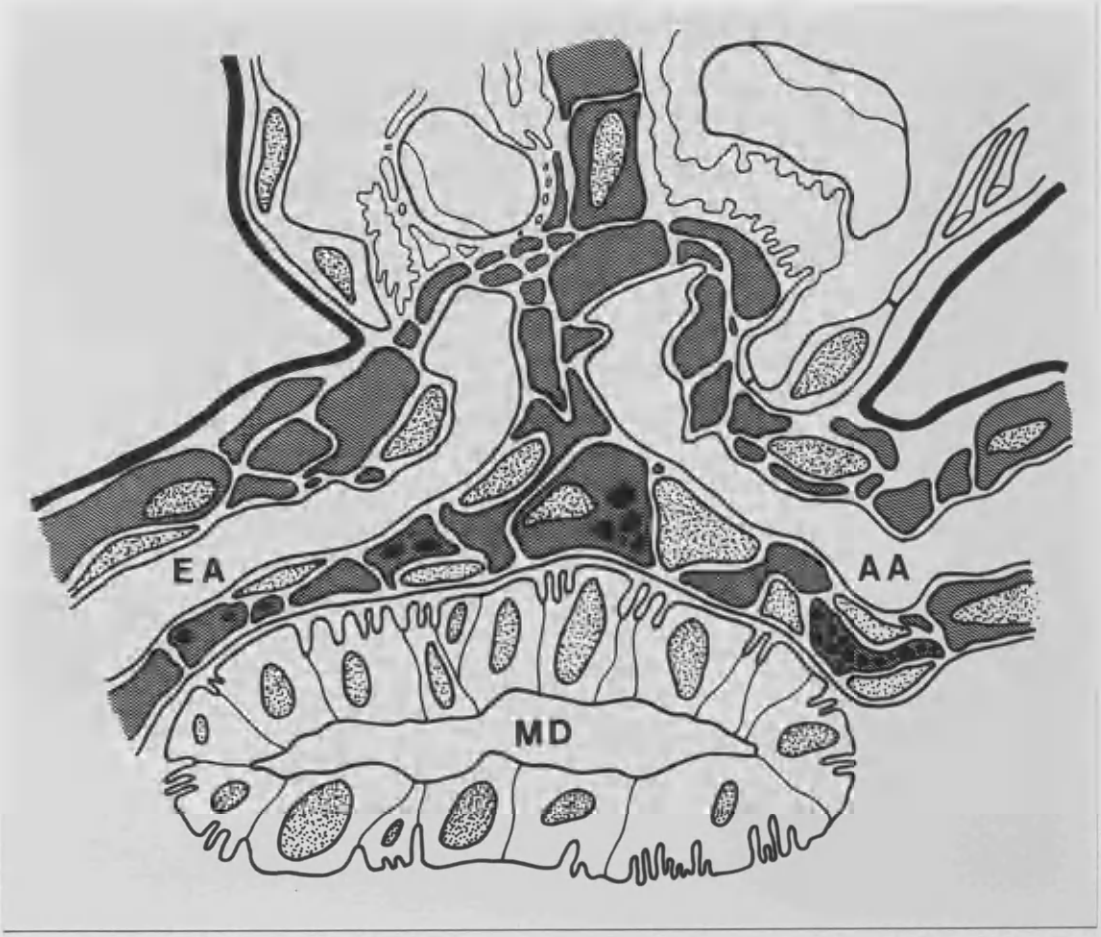


Figure 1. Diagram of the juxtaglomerular apparatus before the discovery of the peripolar cell. Note the myoepithelioid cells, which contain renin granules, in the walls of the afferent (AA) and efferent (EA) arterioles. MD = macula densa.

Schiff technique, toluidine blue and Bowie's stain. However all of these traditional methods are relatively non-specific and have been replaced by immunohistochemical techniques using antibodies to renin (7,8,9,10).

The macula densa is an anatomicaly distinct portion of the distal tubule, which is closely related to the glomerular arterioles, especially the efferent arteriole (5). It is recognised on light microscopy by its closely packed pallisading nuclei. The extraglomerular mesangium occupies a pyramidal space, which has its base overlying the macula densa and its apex in continuity with the intraglomerular mesangium at the vascular pole. Its function is unknown.

(2) FUNCTIONAL ASPECTS OF THE JUXTAGLOMERULAR APPARATUS

Renin plays an important role in the regulation of systemic blood pressure and sodium metabolism. It is a proteolytic enzyme which cleaves angiotensin I (A I) from the α 2 globulin angiotensinogen. A I is then hydrolysed by angiotensin converting enzyme, to the biologically active octapeptide angiotensin II (A II). This peptide has a multitude of biological effects, the most important of which are vasoconstriction and the stimulation of aldosterone production from the adrenal gland (11).

In 1939, Goormatigh first suggested that the granular myoepithelioid cells of the JGA were the source of renin (12). Based on histological study of the JGA, he postulated that the renin-containing cells were the effector arm of a secretory unit of which the macula densa was the sensor. The macula densa cells lack surface uromucoid (Tamm-Horsfall protein), which covers the surface of the other distal tubular cells (13). This glycoprotein is thought to hinder the passage of water from the tubular lumen, hence the macula densa probably represents a permeable area of the ascending thick loop of Henle, which is adapted to monitor the tubular fluid. The exact mechanism by which the macula densa is stimulated and sends a signal to the renin-containing cells is controversial. However it appears that an increased tubular sodium chloride concentration stimulates renin secretion (14).

The other major stimulus for renin secretion is a decrease in arterial perfusion pressure via a baroreceptor mechanism (15). This would apply in particular to renin-containing cells in the proximal afferent arteriole and interlobular arteries, which are less likely to be affected by the macula densa mechanism. The JGA has a dense sympathetic innervation (16), and many studies have shown that ß-adrenergic agents stimulate renin secretion (17). In addition, many humoral factors also affect renin release from the

JGA. These include vasopressin, prostaglandins, calcium ion, and angiotensin II (15).

The intrarenal renin-angiotensin system

Renin and its biologically active products also have local effects on the kidney. Renin-containing cells are situated on the external aspects of the arterioles (10,18), and there is good experimental evidence that renin is secreted into the renal interstitium, rather than directly into the lumen of the arteriole. It then gains access to the blood via the peritubular capillaries (19,20). All of the components of the renin-angiotensin system are present in the kidney (18). Following renin secretion, A II is generated within the kidney; indeed A II has even been identified within the renin storage granules of the myoepithelioid cells, and is probably generated intracellularly (21,22). Locally produced A II may alter renal haemodynamics and so regulate glomerular filtration within individual nephrons, partly by causing contraction of mesangial cells (23). In addition, A II stimulates sodium reabsorption by tubular cells (24), and releases vasoactive lipids and ecosanoids from medullary interstitial cells (25).

(3) THE GLOMERULAR PERIPOLAR CELL - MORPHOLOGY

(a) Light microscopy

Peripolar cells may be identified by their intracytoplasmic granules and their position in the reflection of Bowman's capsule at the vascular pole, where they are interposed between the visceral and parietal epithelium (1) (Figure 2). The granules stain blue with toluidine blue, purple with aldehyde fuchsin and Bowie's stain, usually red but occasionally blue with Lendrum's Martius scarlet blue, and magenta with the periodic acid-Schiff technique (1). Peripolar cells and the renin-containing cells of the JGA are often separated only by the basement membrane of Bowman's capsule and care is required to distinguish the two. Peripolar cells are most prominent in sheep but are present in smaller numbers in almost all other mammals studied (26,27), although the human peripolar cell has not been specifically studied. Peripolar cells have also been identified in chickens (28), amphibians (2) and in elasmobranch fish (29).

Examination of serial sections of individual glomeruli is a reliable method of demonstrating peripolar cells (28). By light microscopy up to four granulated peripolar cells encircle the vascular pole of the sheep glomerulus (1). In this species, peripolar

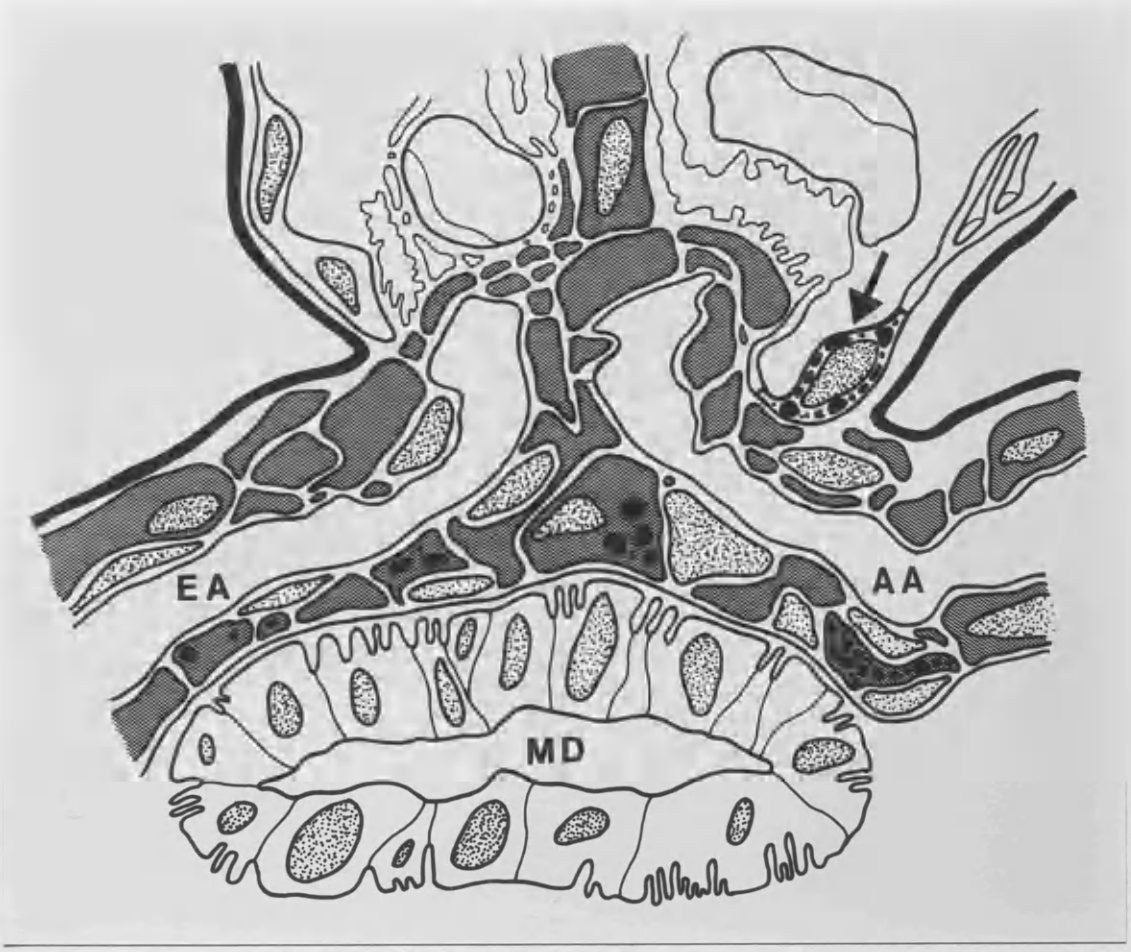


Figure 2. The peripolar cell (arrow) is situated at the vascular pole of the glomerulus, within Bowman's capsule. It contains prominent cytoplasmic granules.

cells are seen in an average of 12% of glomeruli in random histological sections (26). There is wide variation in numbers of peripolar cells, not only between apparently normal animals of the same species (26,30,31), but also between glomeruli in the same kidney. In many species, such as rats, mice, rabbits, and humans, peripolar cells are sparse and difficult to find (26).

(b) Transmission electron microscopy

In all species, peripolar cells rest on the basement membrane of Bowman's capsule between the parietal and visceral glomerular epithelium, with their surface exposed to the urinary space (1,2,4). Peripolar cells form junctional complexes with visceral and parietal epithelial cells and also adjacent peripolar cells (1,2,4). The peripolar cell contains the organelles associated with the synthesis and secretion of protein; namely rough endoplasmic reticulum, Golgi apparatus, free ribosomes, and microtubules. In sheep, peripolar cells have surface microvilli and single cilia (4).

The intracytoplasmic granules are generally round but sometimes have a more irregular shape. There is a wide variation in size; they measure 100-2000 nm in diameter in most species but they may be as large as 4000 nm in the axolotl (2) and newborn lamb (32). The

granules are membrane-bound and some granules contain small vesicles adjacent to the investing membrane (28). They usually have a dense fibrillogranular substructure, but some have a pale matrix. A paracrystalline substructure has been noted in the axolotl (2) and elasmobranch fish (29). Granule contents are secreted by exocytosis into the urinary space (4,30).

(c) Scanning electron microscopy

Scanning electron microscopy (SEM) shows that peripolar cells are a distinct population of cells which are applied to the vascular pole, especially the afferent arteriole (33,34). In sheep, up to 10 peripolar cells can be found forming a complete collar around the vascular pole of the glomerulus. They are easily distinguished from both podocytes and parietal epithelial cells. In this species, peripolar cells are large cells with a bossellated surface due to the closely packed cytoplasmic granules bulging into the cell membrane (33).

In rats peripolar cells have a bipolar, dendritic shape (34). Long tapering processes extend from ovoid or pyramidal cell bodies and embrace the glomerular arterioles. They have a relatively smooth surface, often covered with abundant microvilli. SEM shows at

least one peripolar cell in over 40% of glomeruli and up to 3 cells can be found around a single vascular pole. However, by light microscopy granulated peripolar cells are sparse in rat glomeruli (26). This suggests that there is a larger population of non-granulated peripolar cells in the rat (and possibly other species) which are identifiable by SEM but not by light microscopy.

(d) Distribution of peripolar cells in the renal cortex

There are well known structural and functional differences between superficial and juxtamedullary glomeruli (35), for instance in some species there are significantly more renin-containing cells in superficial JGAs than deep JGAs (36). In sheep and rats, peripolar cells are larger and more numerous in outer cortical glomeruli (26,34). However in chickens there is no difference in numbers of peripolar cells between superficial and juxtamedullary glomeruli (28). Therefore, there is no agreement over the distribution of peripolar cells in the renal cortex at present.

Conclusions from morphological studies

Peripolar cells can be recognised by light microscopy by their position at the vascular pole and

by the presence of intracytoplasmic granules. Transmission electron microscopy has demonstrated granules which undergo exocytosis into the urinary space, suggesting a secretory function. In rats, scanning electron microscopy reveals a large population of peripolar cells, which are not recognised by light microscopy, hence a population of non-granulated peripolar cells may also exist.

(4) THE GLOMERULAR PERIPOLAR CELL - FUNCTION

(a) Granule content

Studies in sheep and chickens have found no immunoreactive renin in peripolar cells (28,37). Recently, kallikrein mRNA has been localised by in situ hybridisation at the vascular pole of rat glomeruli, although peripolar cells were not specifically identified (38). Despite an earlier report (39), neither kallikrein nor kallikrein mRNA have been identified in sheep peripolar cells (40). Sheep peripolar cell granules also contain albumin, immunoglobulins and IgG F(ab)2. It has been suggested that peripolar cells could reabsorb these proteins from the glomerular filtrate (40).

If peripolar cell granules do contain reabsorbed protein, do their numbers relate to

proteinuria? Other glomerular epithelial cells become granulated in most models of proteinuria. The prominence of these granules usually correlates with the degree of proteinuria (41,42,43), although not always (44,45). Podocytes take up excessive filtered protein by micropinocytosis (41,42,46). The absorbed protein is then incorporated into phagolysosomes which are either digested or extruded into the urinary space (41). Peripolar cells have not been studied in experimental proteinuria.

Epithelial cell granules or "hyaline droplets" are also found in the tubular epithelium. Albumin is taken up readily by both glomerular and tubular epithelial cells (45,47). Tubular epithelial granules contain a variety of lysosomal enzymes including acid phosphatase, ß glucuronidase, ribonuclease and cathepsin (48). Glomerular epithelial cells contain cathepsin B (49), although this is disputed (28). Cathepsins C, D and H (28) and acid phosphatase (40) are undetectable in glomerular epithelial cell granules. No lysosomal enzymes have been demonstrated in peripolar cells (28,40). Therefore there are differences in the enzyme content of granules in tubular epithelial cells, glomerular epithelial cells and peripolar cells. In addition, glomerular epithelial cell show a zonal partitioning of plasma proteins

within their granules, which does not occur in tubular epithelial cells (50).

This circumstantial evidence for absorptive activity by peripolar cells does not however preclude a secretory function. Cathepsins and other lysosomal enzymes are found in the renin-containing granules of juxtaglomerular myoepithelioid cells and these granules take up exogenous tracers (48,51). Acid phosphatase and proteases are common in a variety of secretory granules (52,53,54,55). Therefore there is good evidence that the granules in tubular epithelial cells are lysosomes, but the evidence is not as strong for glomerular epithelial cells, including peripolar cells.

(b) Animal experiments

Sodium and water depletion increase peripolar cell numbers in chickens (28), but studies in sheep have produced equivocal results (30,31). Granule exocytosis into the urinary space has been observed most frequently in sodium depleted animals (30). Therefore, the role of the peripolar cell in sodium metabolism remains unclear.

Granules occur in both podocytes and parietal epithelial cells in some models of experimental hypertension (28,56,57). In Goldblatt hypertension,

this occurs in the unclipped kidney but not in the clipped kidney (56). In DOC-salt hypertension in chickens the numbers of peripolar cells are unchanged, however many podocytes and parietal epithelial cells acquire granules which resemble those of peripolar cells (28).

(c) Peripolar cells in the neonate

In fetal sheep peripolar cells appear at 53 days gestation (3); this precedes the appearance of the renin-secreting cells of the JGA. Peripolar cells appear to arise from the lower limb of the S-shaped body and form a cuff around the vascular pole in newborn lambs.

Peripolar cells are increased in number from 12 hours after birth until up to 4 days postpartum (26,32). This increase is greater in fetal sheep which are treated in utero with dexamethasone (32). Peripolar cells are also prominent in newborn rats on the first day after birth (26), regardless of the period of gestation (58). In fetal and neonatal rats, other glomerular epithelial cells become granulated, initially adjacent to the vascular pole, but later in foetal life and after birth granulated epithelial cells are identified distant from the hilum. Dehydration or cortisone administration increases the number of

granular epithelial cells (58). The numbers decrease after maternal adrenalectomy (58). The granulation of glomerular epithelial cells may therefore be dependent on adrenal function.

In summary, peripolar cells increase in number immediately after birth. Other glomerular epithelial cells may also become granulated. Whilst these changes presumably reflect the dramatic alterations in physiology which occurs following birth, their significance is unknown.

(d) The peripolar cell in human disease

Glomerular parietal and visceral epithelial cells become granulated in several pathological states, for example malignant hypertension (60,61) and pre-eclampsia (62,63). In a renal biopsy study of pre-eclampsia, peripolar cells and granulated epithelial cells could not be distinguished by light or electron microscopy (63). In the definitive account of the pathology of pre-eclampsia, Sheehan and Lynch described granules in visceral and parietal glomerular epithelial cells in approximately half of their cases. These were often situated close to the vascular pole, although at this time peripolar cells had not been described (62). Their incidence diminished rapidly 4-5 days postpartum. The authors concluded that proteinuria

per se could not be the only factor in the production of the granules.

(5) AIMS OF THIS WORK

My aims are as follows:

(1) To look for peripolar cells in human kidneys and study their morphology, and to compare them with peripolar cells of other species.
(2) To study the distribution of peripolar cells in the renal cortex in normal kidneys and in disease states.
(3) To characterise peripolar cells and their granules using immunohistochemical methods.
(4) To test the hypothesis that the peripolar cell could be part of the JGA.

MATERIALS AND METHODS

(1) NORMAL HUMAN KIDNEYS

(a) Light microscopy

Full-thickness blocks of renal cortex were taken from six human nephrectomy specimens. Four of these kidneys were removed because of renal cell carcinoma; material was taken from normal kidney distant from the tumour. One kidney was removed for a renal artery aneurysm, and another was removed for pain following a previous repair of a pelviureteric junction stricture. All of the kidney blocks showed normal histology.

The tissue was immersion fixed in 15% picric acid in 1% glutaraldehyde for 24 hours. The blocks were dehydrated through graded alcohols and propylene oxide, and embedded in Transmit resin (Taab Laboratories Ltd). Serial 2 μm sections were cut on a Reichert-Jung 1140-Autocut and mounted on chrome alum-gelatin coated slides. The sections were stained after removal of the resin using a saturated solution of sodium ethoxide. Toluidine blue was used mostly, although in selected cases Dawes and Hilliers trichrome, aldehyde fuchsin, Bowie's stain and the periodic acid-Schiff technique were also used.

(b) Transmission electron microscopy

Tissue was trimmed into 2mm cubes and fixed overnight in 15% picric acid in 1% glutaraldehyde, followed by 1% osmium tetroxide. The tissue was then dehydrated and embedded in Araldite resin. Serial 1 μm sections were cut on an LKB Ultratome 1, stained with Toluidine blue and examined for granular peripolar cells. When a peripolar cell was identified, 90 nm ultrasections were cut serially through the entire cell. The sections were mounted on copper grids, "stained" with uranyl acetate and lead citrate and examined in a Phillips EM301 G electron microscope at 80 kV.

(2) NORMAL SHEEP KIDNEYS

Kidneys from seven sheep were obtained from a commercial abattoir. The animals were stunned electrically and then exsanguinated. Blocks were selected and fixed by immersion in Bouin's fluid. The tissue was then dehydrated and embedded in paraffin wax. 50 serial 3μm sections were cut and stained by the MSB trichome method.

(3) NORMAL RAT KIDNEYS

Kidneys from 6 adult male Sprague-Dawley rats were examined. The animals were killed by an overdose of pentobarbital. The aorta was clamped below the diaphragm and cannulated immediately above the aortic bifurcation. The kidneys were then perfused at a pressure of 120 mmHg with heparinised isotonic saline followed by 2% glutaraldehyde in Millonig's buffer. Transverse slices of each kidney were taken and further fixed by immersion for 24 hours in the glutaraldehyde solution.

The kidney blocks were dehydrated and embedded in paraffin wax, and fifty serial 3 μm sections were cut and stained with Toluidine blue or by Lendrum's MSB technique.

(4) EXPERIMENTAL ANIMALS

(a) Experimental alteration in sodium balance

Twelve male Sprague-Dawley rats were fed a low sodium diet (Special diet services Ltd., Witham, Essex) for four weeks. Six were given free access to 0.2% normal saline as drinking water, and therefore became sodium depleted. The other six were given free access to 2% normal saline, and consequently were sodium

loaded. The kidneys were fixed and processed for light microscopy as before.

(b) Experimental renovascular hypertension

Ten young adult (180-200g) male Sprague-Dawley rats were used. In five animals, hypertension was induced by applying a clip to the left renal artery. Under anaesthesia, the abdomen was opened and a silver clip (internal diameter 0.2 mm) was applied to the left renal artery, close to the aorta. The right kidney was left untouched. After the procedure, the animals were allowed free access to drinking water and were fed a standard rat diet (41B Oxoid). The five control animals were sham-operated. Systolic blood pressure was measured weekly in all animals, using a tail cuff method. After 8 weeks, the animals were killed by a lethal injection of sodium pentobarbitone.

Immediately after death, the kidneys were fixed by perfusion with a 2% glutaraldehyde solution, as described above for normal rats. The perfusion pressure used was equivalent to the last recorded blood pressure. Each kidney was weighed and then processed for light microscopy.

(5) PATHOLOGICAL HUMAN KIDNEYS

(a) Addison's disease

11 cases of Addison's disease were examined. Six autopsy kidneys were examined from patients who died during Addisonian crisis. In five patients this was the presentation of Addison's disease, whilst the other patient had shown poor compliance with treatment. In all cases the adrenals were atrophied and showed histological evidence of autoimmune adrenalitis. Five cases of treated Addison's disease who died during Addisonian crisis were also included. All of these patients had been treated with corticosteroids long term, but died shortly after admission to hospital. Eleven age and sex-matched kidneys from medico-legal autopsies for sudden death were used as a control group.

All kidneys had been formalin fixed, embedded in paraffin wax and processed routinely for diagnostic histology. I selected blocks which included full-thickness renal cortex.

(b) Renal artery stenosis

I examined kidneys from 10 patients with renal artery stenosis. The nephrectomies were performed to

relieve renovascular hypertension. Nine surgically removed kidneys which were histologically normal were used as controls. Five of these were removed because of renal stones, three were removed for trauma, and one for unexplained haematuria.

All kidneys had been formalin fixed, embedded in paraffin wax and processed routinely for diagnostic histology. I selected only blocks which included the full width of the renal cortex, avoiding medullary rays and columns of Bertin.

(c) Malignant hypertension

I examined 12 autopsy cases of malignant hypertension. They constituted all cases of malignant hypertension from 1960-1975, for which blocks were available in the files of the Department of Pathology, Western Infirmary, Glasgow. In all cases malignant hypertension was the primary cause of death, and typical histological features were present in the kidneys. The highest recorded systolic and diastolic blood pressures, and the time between these and death, were obtained from the case-notes. A paraffin-wax embedded block of kidney was selected from each case.

The 11 autopsy kidneys which were used as a contol group for the Addison's disease study were used as controls for these cases.

(d) Renal biopsies

I examined 242 renal biopsies from 19 different renal diseases. These were taken from the files of the Department of Pathology, Western Infirmary, Glasgow. I examined only biopsies which contained at least 10 glomeruli and excluded those cases in which there were multiple pathologies or diagnostic uncertainty. They had been classified previously by a single pathologist (Dr IAR More), taking account of ultrastructural and immunofluorescence findings and the clinical context. Three consecutive 3 μm sections of each biopsy were examined. These had been previously stained by the MSB trichrome method, the periodic acid-Schiff technique and Masson's trichrome.

(6) QUANTIFICATION OF PERIPOLAR CELLS

Peripolar cell index (PPI)

Glomeruli were examined in their entirety by following individual glomeruli through at least 50 serial sections. For each kidney a peripolar cell index (PPI) was derived by expressing the number of glomeruli

in which at least one granulated peripolar cell could be identified as a percentage of the total number of glomeruli examined. At least 30 glomeruli were examined in each kidney, from all areas of the renal cortex.

Peripolar cell index (random)

$$PPI(r) = \frac{\text{Number of peripolar cells}}{\text{Number of glomeruli}} \times 100$$

This index was used only to quantify peripolar cells in renal biopsies, from which 3 consecutive sections were available. If a single peripolar cell appeared on more than one section, it was counted as one cell. The number of glomeruli was counted on the section with most glomeruli.

(7) QUANTIFICATION OF OTHER GRANULATED GLOMERULAR EPITHELIAL CELLS

Epithelial cell droplet index (ECDI)

Glomerular epithelial cells which are not in a peripolar position may also acquire granules. The granules in these cells are commonly referred to as "epithelial cell droplets". I examined at least 30 glomeruli in their entirety through serial sections, and counted the number of granulated glomerular epithelial cells. I calculated an epithelial cell

droplet index (ECDI) by expressing the number of granulated cells as a percentage of the total number of glomeruli examined.

Epithelial cell droplet index (random)

$$ECDI(r) = \frac{\text{Number of granulated glomerular epithelial cells}}{\text{Number of glomeruli}} \times 100$$

This index was calculated only for renal biopsies, from which three consecutive sections were available. A cell appearing on more than one section was counted only once. The number of glomeruli was counted on the section with most glomeruli.

Distribution of granulated glomerular epithelial cells in the glomerular tuft

The position in the glomerular tuft of granulated glomerular epithelial cells was assessed by measuring with an eyepiece graticule, the distance from the centre of the vascular pole to the granulated cell and the distance from the vascular pole to the edge of the glomerular tuft, in the same straight line. The two measurements were then expressed as a ratio, where 1 represents the edge of the glomerular tuft and 0 represents the centre of the vascular pole. The position of each cell was then plotted on a scatter-

diagram. This assessment was only possible, when the vascular pole was in the plane of section.

(8) QUANTIFICATION OF GRANULATED TUBULAR EPITHELIAL CELLS

Tubular hyaline droplet index (THDI)

Granulated tubular epithelial cells were assessed in the renal biopsies only, on a semi-quantitative basis. The granules in these cells are commonly referred to as "hyaline droplets". These were assessed on an arbitrary scale from 1 to 4, where 1 represents minimal granulation of tubules, whilst 4 represents maximal granulation. One section from each biopsy was examined for this assessment.

(9) PERIPOLAR CELL IMMUNOHISTOCHEMISTRY

Tissue was obtained from the normal parts of human and sheep kidneys, as described above. Blocks were immersion fixed in 10% neutral buffered formalin or Bouin's fluid and then embedded in paraffin wax, using standard techniques. In addition, blocks of sheep kidney was also frozen in liquid nitrogen for immunofluorescence.

Localisation of peripolar cells

Since peripolar cells are sparse in human kidneys they were first identified by examining serial 3μm sections, stained by the MSB trichrome method. When a peripolar cell was found, it was photographed and its position on the section was noted. The coverslip was removed and the section was stained with the immunoperoxidase technique (The MSB stains were removed in this process).

By contrast, peripolar cells are very prominent in sheep kidneys (1,26,33). They could be identified in immunoperoxidase-stained sections with haematoxylin counterstaining alone. Frozen sections were "stained" using an indirect immunofluorescent technique and then examined with interference contrast microscopy. When a glomerulus containing a peripolar cell was identified, it was photographed and its position on the section was marked. The same glomerulus could then be found again and was examined by fluorescence microscopy.

Antibodies used

A number of monoclonal and polyclonal antibodies were used (Table 1). Immunoperoxidase techniques were used to localise the following antigens in human and sheep kidney: Cam 5.2, PKK1, vimentin, desmin,

Table 1. Details of antisera and monoclonal antibodies used for immunohistochemistry.

Reagent	Polyclonal/ monoclonal (p/m)	Manufacturer
Cam 5.2	m	Becton-Dickinson
PKK 1	m	Labsystems
vimentin	m	Amersham
desmin	m	Boehringer-Mannheim
epithelial membrane antigen (EMA)	m	Dakopatts
human milk factor globulin (HMFG I & II)	m	Unipath
S-100	p	Dakopatts
neurone-specific enolase (NSE)	p	Dakopatts
neurofilament	m	Eurodiagnostics
chromogranin	p	Biogenesis
human albumin	p	Dakopatts
human IgG	p	Dakopatts
α 1 antitrypsin	p	Dakopatts
sheep albumin	p	Cappel
sheep IgG	p	Scottish Antibody Production Unit / The Binding Site
sheep IgM	p	Cappel
sheep C3	p	Cappel

epithelial membrane antigen (EMA), human milk factor globulin I and II (HMFG I and II), S-100 protein, neuron-specific enolase (NSE), neurofilament (NF) and chromogranin. For human kidneys, antisera to human albumin, IgG and α1-antitrypsin were also used. For sheep kidneys, antisera to sheep albumin, IgG, IgM and C3 were used with both immunoperoxidase and immunofluorescence techniques. The immunoperoxidase-stained sections were counterstained either with haematoxylin or with the periodic acid-Schiff technique. Negative controls were performed by replacing the primary antibody with a buffer solution.

(10) RENIN IMMUNOHISTOCHEMISTRY

(a) Human kidneys

The peroxidase-antiperoxidase (PAP) technique was used to localise renin. The method was based on that of Sternberger & Cucullis (64). For resin-embedded tissue, the primary antiserum was raised in rabbits against human renin, which had been purified from a juxtaglomerular cell tumour (65). The antiserum detects both inactive and active renin (66), and has been used to localise renin in normal (8) and pathological (8,67) kidneys.

The resin was removed from the sections by treating them with 2.5% sodium ethoxide for 20 minutes. They were then washed with four changes of absolute ethanol for 8 minutes, followed by rinsing in 70% ethanol, distilled water and phosphate buffered saline (PBS). Endogenous peroxidase was removed by immersing the sections in 0.5% hydrogen peroxide in PBS for 30 minutes. The slides were washed in distilled water and phosphate buffered saline (PBS), then treated with normal swine serum (NSS), diluted 1/5 in PBS for 20 minutes. The NSS solution was drained and the sections were covered with the rabbit anti-human renin antiserum, diluted 1/1250 in PBS. After an incubation period of 72 hours at 4 °C, the antiserum was washed off with 3 changes of PBS in 30 minutes. The sections were then treated with swine anti-rabbit immunoglobulin, diluted 1/50 in 1/25 NSS/PBS for 30 minutes at room temperature. This was washed off with 3 changes of PBS over 30 minutes. Finally, the sections were treated with rabbit peroxidase-antiperoxidase (PAP) complex, diluted 1/100 in 1/25 NSS/PBS for 30 minutes at room temperature. The reaction was visualised by immersing the slides in a 20 mg % w/v solution of diaminobenzidine in PBS, which also contained 0.001% hydrogen peroxidase. After a thorough wash in tap water, the sections were counterstained with the periodic acid-Schiff technique, or with Mayer's haemalum alone. The sections were then

dehydrated through graded alcohols and xylene, and mounted in synthetic resin.

Negative controls were performed by replacing the primary antiserum with PBS or normal rabbit serum.

Sections in which peripolar cells had already been identified using conventional stains were subsequently treated with the immunoperoxidase technique to identify renin.

For paraffin-embedded human kidneys, I used a different antiserum to human renin. The antiserum was raised to pure human renin and detects both inactive renin (prorenin) and active renin (71,72,73). The methodology of the immunoperoxidase technique was the same as that used to localise renin in resin-embedded human kidneys, with the exception that the resin-removing stages were not necessary, but the paraffin wax was removed with xylene, after dehydration.

(b) Rat kidneys

A polyclonal antiserum to mouse renin, which cross-reacts with rat renin, was used. The antiserum was a gift from Dr. K. Poulsen, and has previously been used extensively for the immunohistochemical localisation of mouse and rat renin (7,22,36,68,69,70).

The methodology of the immunoperoxidase technique was the same as that used to localise renin in human kidneys. Appropriate negative control sections were included as before.

(c) Quantification of renin-containing cells

A semi-quantitative assessment of renin-containing cells (RCCs) was made, similar to the method described by Nochy *et al* (67). The following indices were calculated:

$$\text{JGA+} = \frac{\text{Glomeruli with renin-positive JGAs}}{\text{Number of glomeruli}} \times 100$$

A section of the normal human JGA contains at most four or five renin-containing cells, so like Nochy *et al* (67), hyperplasia of these cells was assessed as follows:

$$\text{JGA++} = \frac{\text{JGAs with more than 6 RCCs}}{\text{All renin-positive JGAs}} \times 100$$

The presence of renin-containing cells in the walls of arteries was assessed as follows:

$$\text{A+} = \frac{\text{Arterial sections with RCCs}}{\text{Number of arterial sections}} \times 100$$

The following index was also calculated:

$$\text{Renin cell index (RCI)} = \frac{\text{Total number of RCCs}}{\text{Total number of glomeruli}} \times 100$$

At least 100 glomeruli in all layers of the renal cortex were examined.

(11) THE DISTRIBUTION OF PERIPOLAR CELLS, OTHER GRANULATED GLOMERULAR EPITHELIAL CELLS AND RENIN-CONTAINING CELLS IN THE RENAL CORTEX

The position in the human renal cortex of each glomerulus which contained a peripolar cell, other granulated epithelial cells, or with a renin-positive JGA, was recorded by measuring the distance from the corticomedullary junction to the relevant glomerulus and the distance from the corticomedullary junction to the renal capsule, measured in the same straight line. The corticomedullary junction was defined as an imaginary line which joins the deepest glomeruli in the cortex. The two measurements were then expressed as a ratio, where a ratio of 1 represents the renal surface, and a ratio of 0 represents the corticomedullary junction. The position of each glomerulus was then plotted on a scatter-diagram.

In human kidneys, the distances were measured in

X 40 microscopic fields. In rat kidneys, the distances were measured using an eyepiece graticule, because the cortex is narrower.

(12) ASSESSMENT OF HYPERTENSIVE DAMAGE

For the experimental renovascular hypertension and human malignant hypertension kidneys, one of the serial sections from each kidney was selected at random and examined to quantify the amount of hypertensive damage, as described previously (68). Glomerular abnormalities which were noted were tuft contraction, fibrinoid necrosis, cellular proliferation and crescent formation. Arterial abnormalities were hyaline arteriolosclerosis, fibrinoid necrosis and concentric cellular thickening. At least 100 glomeruli and 100 blood vessels were examined. The numbers of abnormal glomeruli and abnormal arteries/arterioles were expressed as a percentage of the total number of glomeruli and arteries/arterioles, respectively.

For the cases of malignant hypertension, an assessment of the degree of chronicity was also obtained. The number of blood vessels displaying fibrinoid necrosis was recorded and taken to reflect acute damage. The number of blood vessels showing intimal cellular proliferation was taken to represent more chronic damage. A ratio of the two was calculated

for each case. Whilst the ratio itself has little meaning, it was correlated with PPI and ECDI, to investigate whether either of these indices were related to the stage of the disease.

(13) CLINICAL DATA

The following preoperative data were noted for each patient: age, sex, blood levels of sodium, potassium, chloride, bicarbonate, calcium, urea, creatinine, and haemoglobin, creatinine clearance, urinary protein, systolic and diastolic blood pressure and drug therapy.

In addition, in most of the patients in the renal artery stenosis group, the following data were available: plasma renin, angiotensin II and aldosterone concentrations. The most immediate pre-operative measurement was taken.

(14) STATISTICAL ANALYSES

The Mann-Whitney U test was used to determine differences between means and Spearman's rank correlation coefficient was used to assess relationships between variables. For the biopsy study, the Kruskal-Wallis test was used to test the null

hypothesis that each of the histological indices were similar throughout all of the groups.

RESULTS

(1) NORMAL HUMAN KIDNEYS

(a) Light microscopy

(i) Peripolar cells

Peripolar cells were identified in all of the kidneys. They were generally sparse; the peripolar cell index (PPI) ranged from 3 to 27.8 (mean = 12.4) (Table 2). They were situated in the reflection of Bowman's capsule at the vascular pole of the glomerulus (Figures 3 and 4). The myoepithelioid cells of the afferent arteriole were frequently located immediately on the other side of Bowman's capsule from the peripolar cells (Figure 5). Occasionally, two peripolar cells were present in the same glomerulus. The peripolar cells varied in appearance; either flattened like parietal cells (Figure 5), or plump (Figure 3). In general, the cytoplasm appeared clearer than that of neighbouring epithelial cells. The peripolar cell nucleus was vesicular, with evenly dispersed chromatin and a single nucleolus.

The most conspicuous feature of the peripolar cell was the presence of cytoplasmic granules (Figures 3,4,5). The granules varied in size, the smallest being of a similar size to renin granules, whilst the largest were at least 1 μm in diameter.

Table 2. Mean peripolar cell index (PPI) and renin indices for the histologically normal parts of 6 human kidneys (see text for definitions of indices).

	PPI	JGA+	JGA++	A+
mean	12.4	20.1	26.2	9.4
(±SEM)	(±4.5)	(±3.7)	(±4.9)	(±1.5)

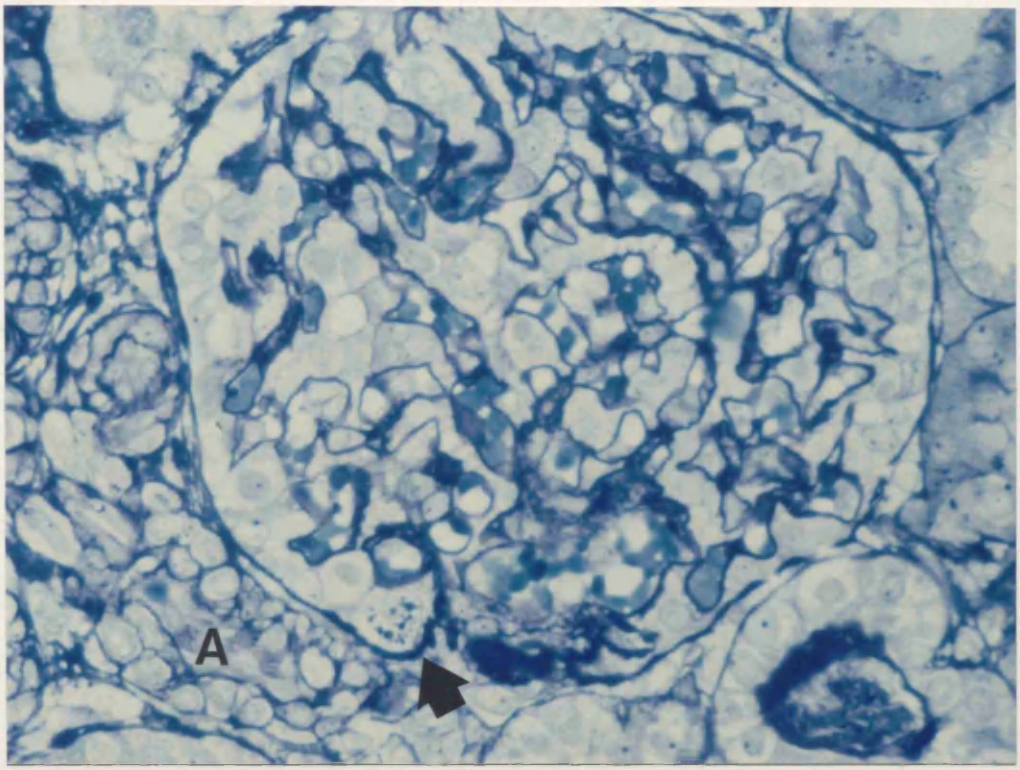


Figure 3. Resin-embedded section of human kidney, with a peripolar cell (arrow) at the vascular pole. A = afferent arteriole. Toluidine blue.

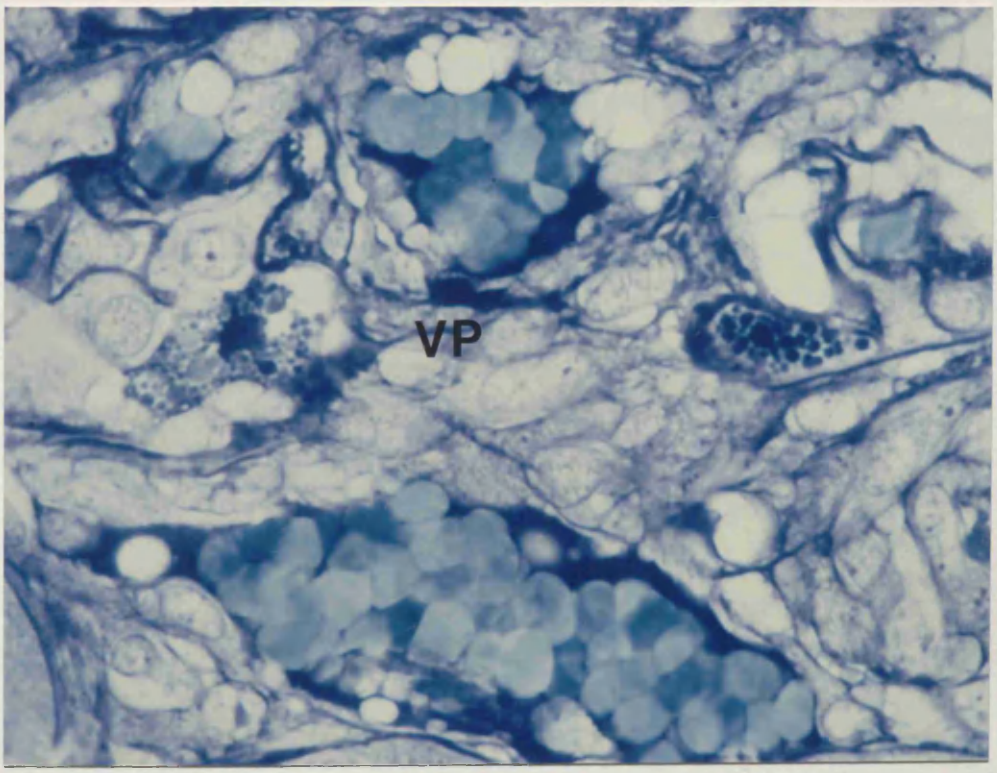


Figure 4. Resin-embedded section of human kidney with two peripolar cells on either side of the vascular pole (VP). Note the prominent cytoplasmic granules. Toluidine blue.

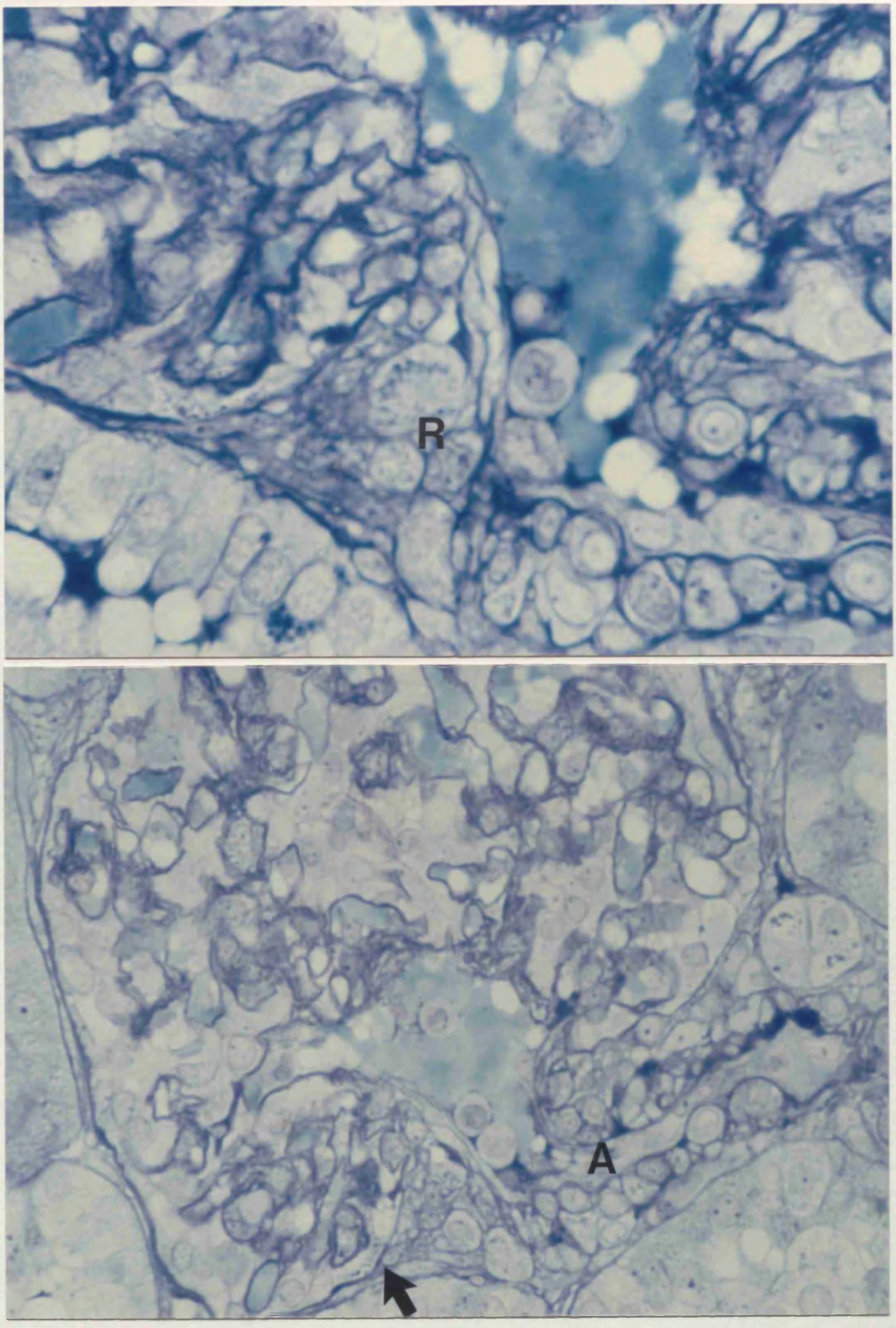


Figure 5. (upper) Resin-embedded section of human kidney, showing a peripolar cell (arrow) at the vascular pole. A = afferent arteriole.
(lower) At higher magnification, the peripolar is resting on the basement membrane of Bowman's capsule, with granular renin-containing cells (R) in close proximity. Toluidine blue.

There was no evidence of polarity towards either the basal or luminal aspects. The granules stained blue with toluidine blue, purple with aldehyde-fuchsin and Bowie's stain, and magenta with the periodic acid-Schiff technique, as did the renin granules. When peripolar cells and granular myoepithelioid cells were seen in the same section, the peripolar cell granules stained a deeper red with Dawes and Hillier's trichrome, the renin granules appearing more orange in colour. Occasionally in trichrome stained sections a few peripolar cell granules stained blue, although the majority stained red. Granules were never present in other glomerular epithelial cells. PPI did not correlate with any of the clinical data.

(ii) Renin-containing cells

The juxtaglomerular myoepithelioid cells stained strongly positively for renin with the PAP technique. However, immunoreactive renin was never detected in peripolar cells (Figure 6). The adjacent juxtaglomerular apparatus contained immunoreactive renin and therefore acted as a positive control. Renin was identified in JGAs and occasionally in the walls of proximal arterioles. The values of the renin indices are illustrated in Table 2; JGA+ ranged from 10.6 to 33.1, JGA++ from 7.7 to 41.5, and A+ from 3.4 to 12.5. PPI did not correlate with any of the renin indices.

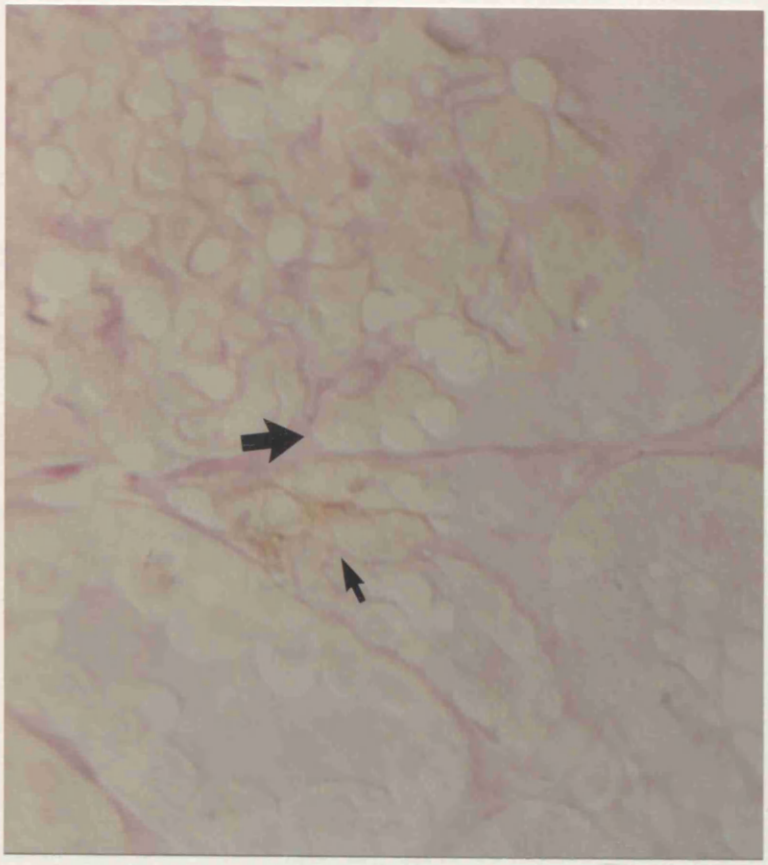


Figure 6. Immunoperoxidase-stained section of human kidney, using an antiserum to human renin. There is positive staining of renin-containing cells in the arteriole (small arrow), however the peripolar cell (large arrow) is negative. Its granules are faintly stained with the PAS counterstain.

However examination of serial sections showed that the majority of glomeruli with peripolar cells also had renin-positive JGA's.

(b) Transmission electron microscopy

Peripolar cells were found at the glomerular vascular pole, where their borders abutted on to neighbouring parietal epithelial cells, Bowman's capsule, the visceral epithelial cells and the urinary space (Figure 7). The glomerular afferent arteriole was also closely related to the peripolar cells. Peripolar cells were often elongated, with a curved profile as they rested on the inner surface of Bowman's capsule. They varied in size from 10 - 13.5 μm in diameter (mean = 11.2 μm). The nucleus was round, with finely dispersed chromatin and a peripheral rim of condensed heterochromatin. Nucleoli were not identified.

The most striking feature was the presence of multiple membrane-bound, electron-dense granules (Figures 7,8,9,10). In general, the granules were round and varied in size between 400 and 2150 nm in diameter (mean = 1050 nm). The majority had a homogeneous electron density, however a few granules were pale with some collapse of the investing membrane (Figure 8). Some of these paler granules had a fibrillogranular substructure. Scattered small electron-opaque areas

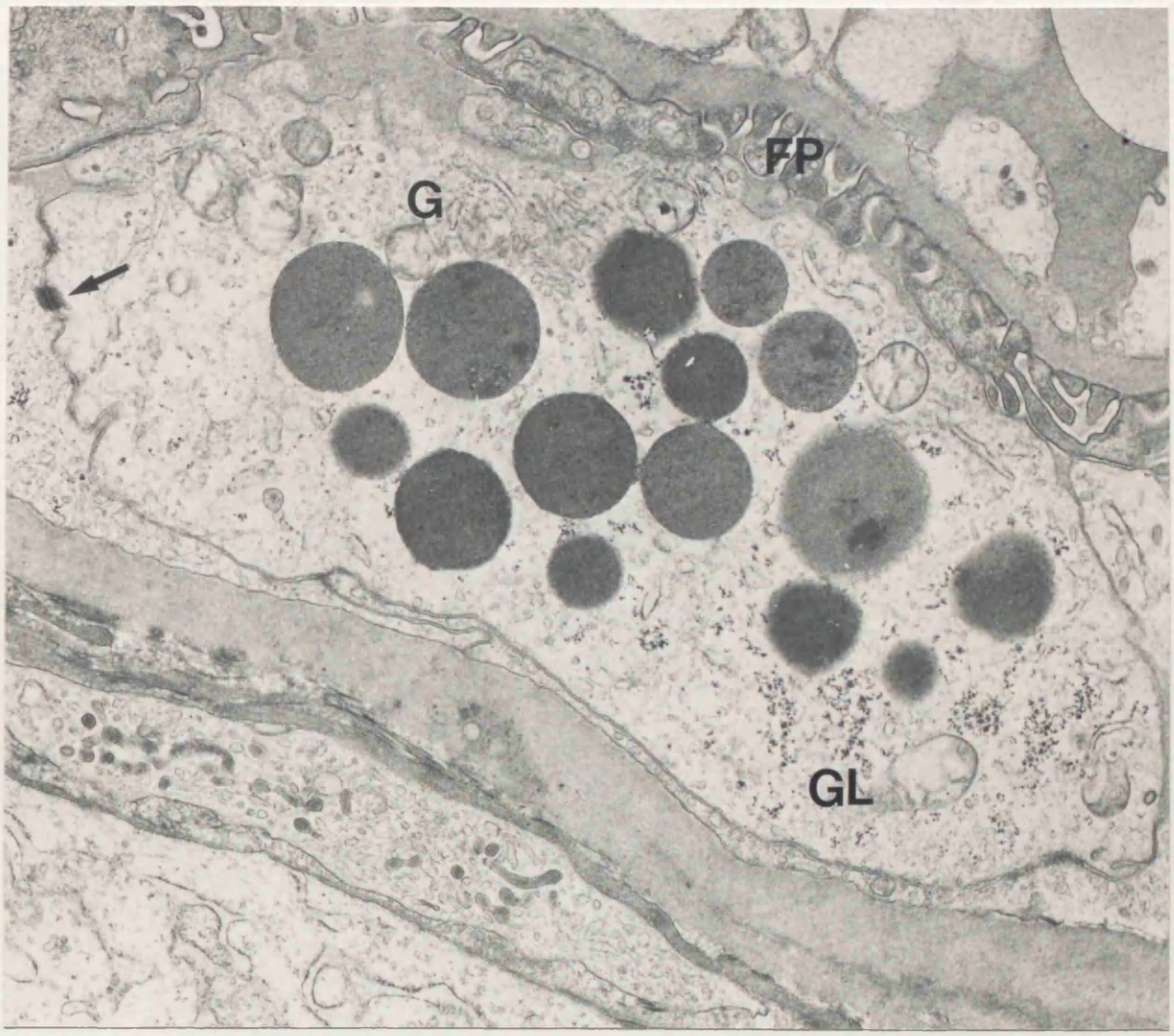


Figure 7. Transmission electron micrograph of a human peripolar cell. Note the cytoplasmic granules, Golgi apparatus (G), glycogen (GL) and a desmosome (arrow), joining it to an adjacent parietal epithelial cell. The peripolar cell is separated from the basement membrane of Bowman's capsule by extensions of cytoplasm from adjacent epithelial cells. FP = foot processes of a podocyte. X 16870.

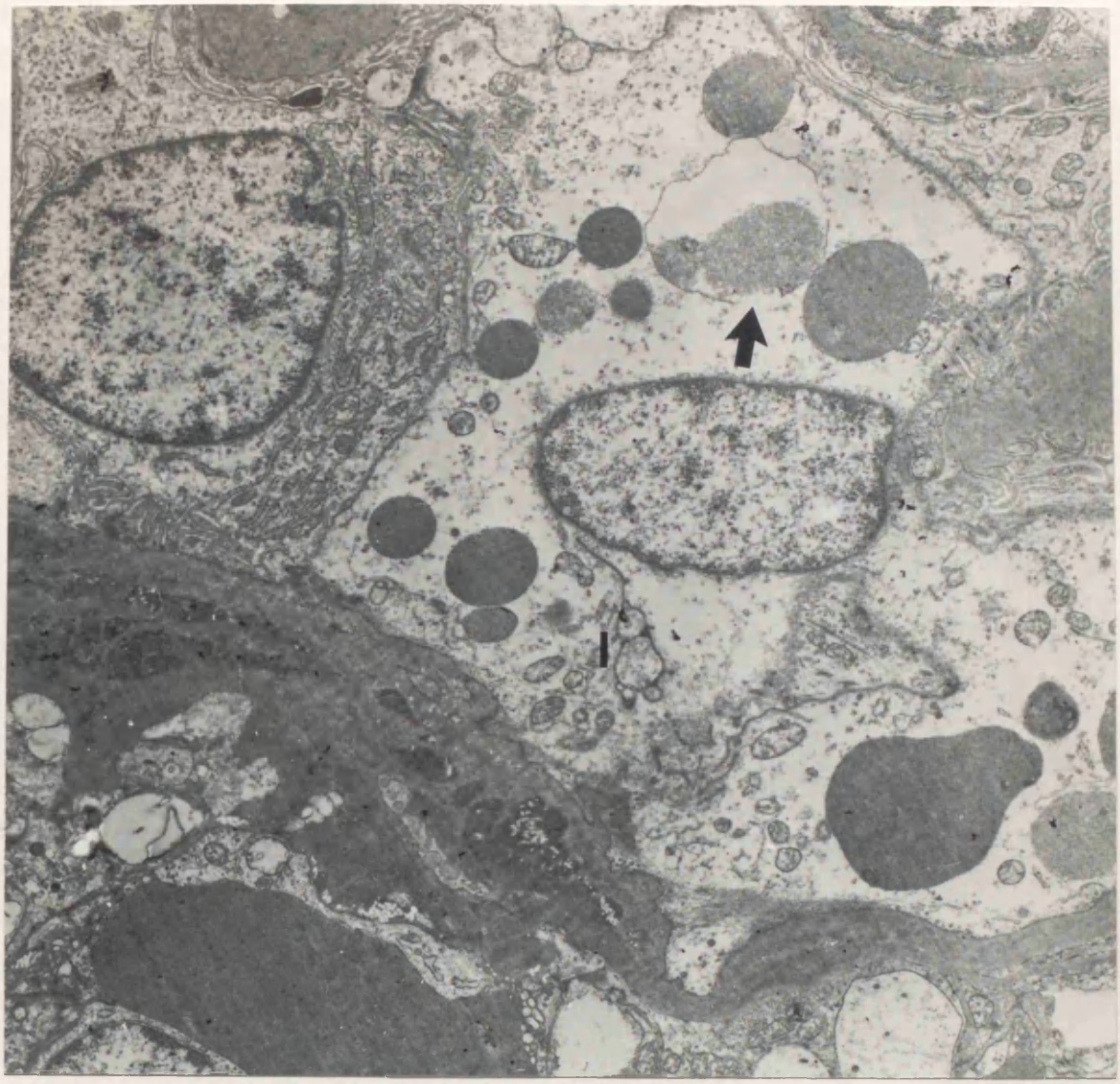


Figure 8. In this peripolar cell, the granules display heterogeneity of staining, and one granule (arrow) is undergoing dissolution. There are also complex membrane invaginations (I). The nucleus has a peripheral ring of condensed heterochromatin. X 8100.

were present in the granules, often in close association with the investing membrane (Figures 7 and 10). Small vesicles were present in the cytoplasm in such cases. No crystalline or paracrystalline areas were seen, and although granules were occasionally in contact, there were no definite examples of granule fusion or exocytosis.

Other organelles were relatively sparse. A few cisternae of rough endoplasmic reticulum were seen but this was not a prominent feature (Figures 7 and 10). Mitochondria were small, simple, rounded and few in number. Golgi complexes were present, but scanty. Glycogen granules were present focally in small amounts and occasional multivesicular bodies were seen. Microfilaments were found in association with the cell membrane but there were no microtubules. The adjacent parietal epithelium showed similar cytoplasmic features but contained no cytoplasmic granules. By contrast, the adjacent podocytes had abundant organelles (Figure 9): the Golgi apparatus was prominent and numerous cisternae of granular endoplasmic reticulum were seen, some of the latter containing flocculent material. Polyribosomes were apparent and microtubules were seen; however no cytoplasmic granules were identified.

The cell borders of the peripolar cell had a number of features. It was often separated from the

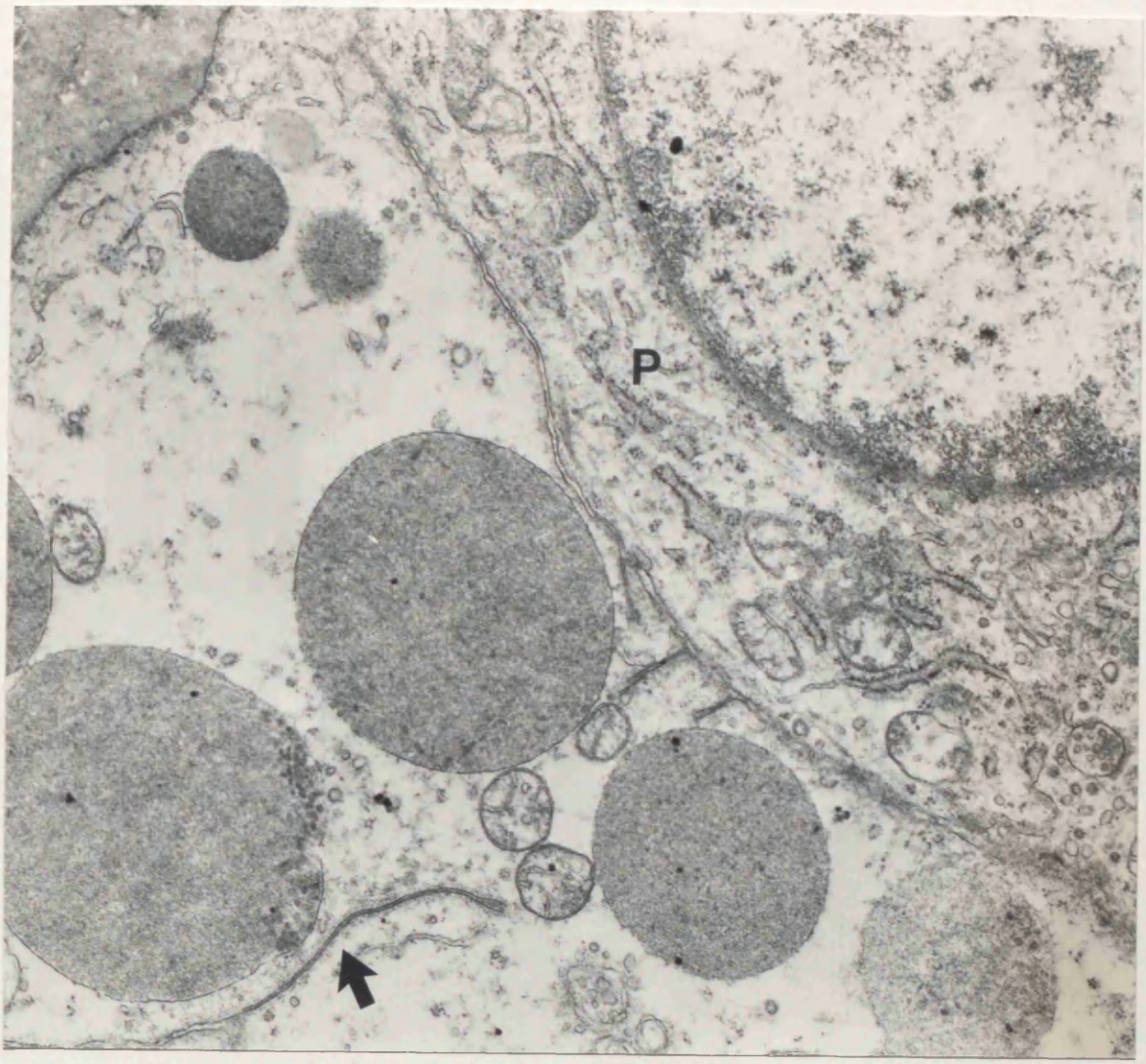


Figure 9. High power view of a peripolar cell demonstrates a long, branching membrane invagination (arrow). The peripolar cell has scant organelles, in contrast to the adjacent podocyte (P). X 29250.

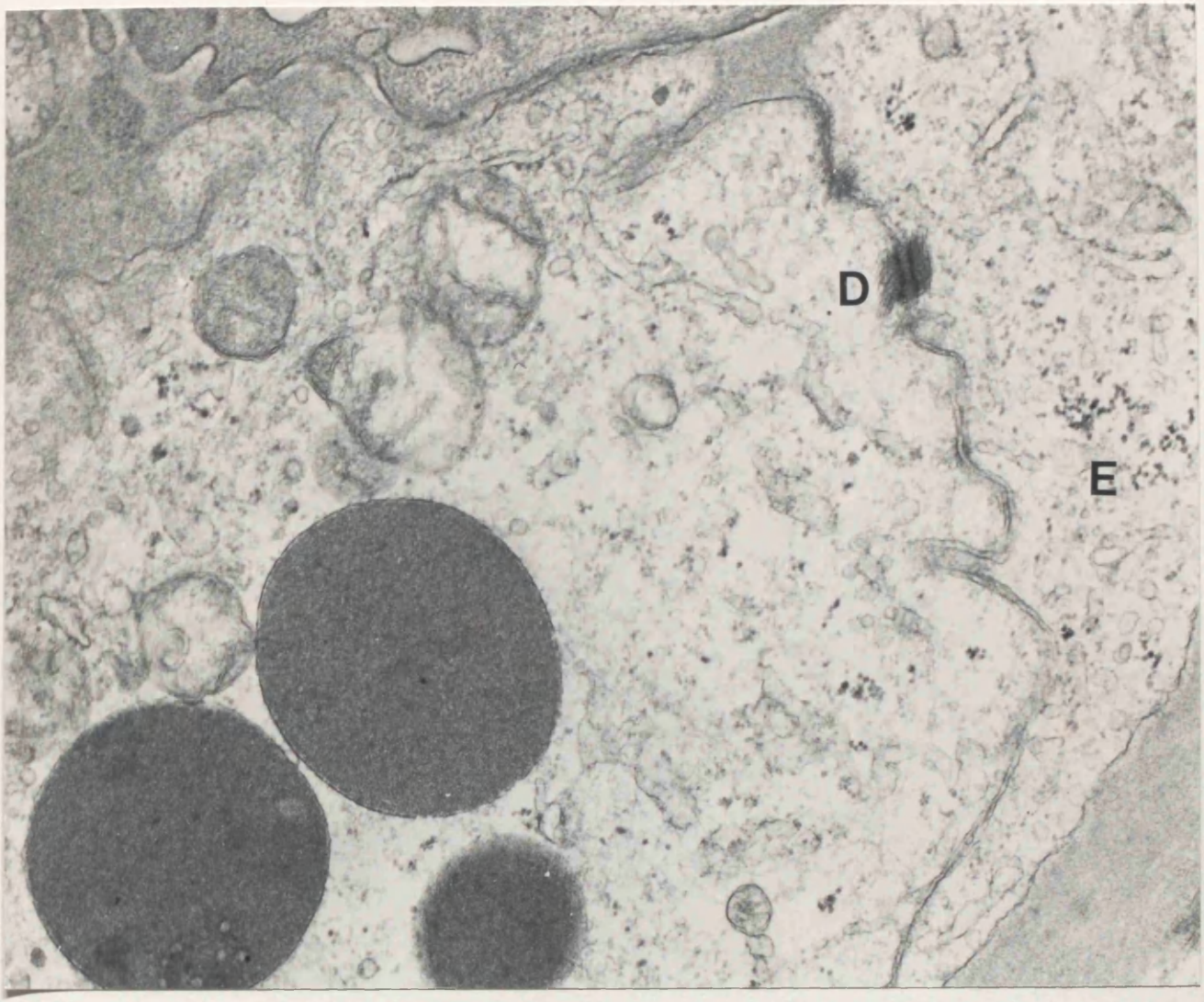


Figure 10. Junctional region of a peripolar cell and an adjacent parietal epithelial cell (E). Note the desmosome (D). X 40500.

basement membrane of Bowman's capsule by a thin rim of cytoplasm, extending from adjacent parietal epithelial cells (Figure 7). This rim of cytoplasm showed prominent micropinocytosis (Figure 7). Tight junctions and true desmosomes were found attaching the peripolar cell both to the adjacent parietal epithelial cells and to neighbouring podocytes (Figures 7 and 10). There were only a few areas of interdigitation between the cells. In some areas the peripolar cell membrane was folded in on itself to produce blunt ended invaginations into the cell cytoplasm. These invaginations varied from those which were short and straight to others of considerable length and complexity, extending almost to the nucleus (Figures 8 and 9). They were sometimes closely related to the granules. Electron-dense material was often present between the two opposed cell membranes. No microvilli or cilia were detected on the free surface of the peripolar cells which faced into the urinary space. No nerve fibres were seen adjacent to peripolar cells.

(2) NORMAL SHEEP KIDNEYS

Peripolar cells were identified in every animal. They were easily identified by their position within Bowman's capsule at the vascular pole and by their content of cytoplasmic granules which stained red with the MSB technique (Figures 11,12,13). The cells varied

Table 3. Approximate age, sex and peripolar cell index (PPI) for seven sheep.

SHEEP	AGE (years)	SEX	PPI
1	1	M	28
2	1	F	20
3	1	M	40
4	1.5	M	87.5
5	1.5	M	84
6	6-9	M	92
7	6-9	M	100
mean (±SEM)			64.5 (±12.8)

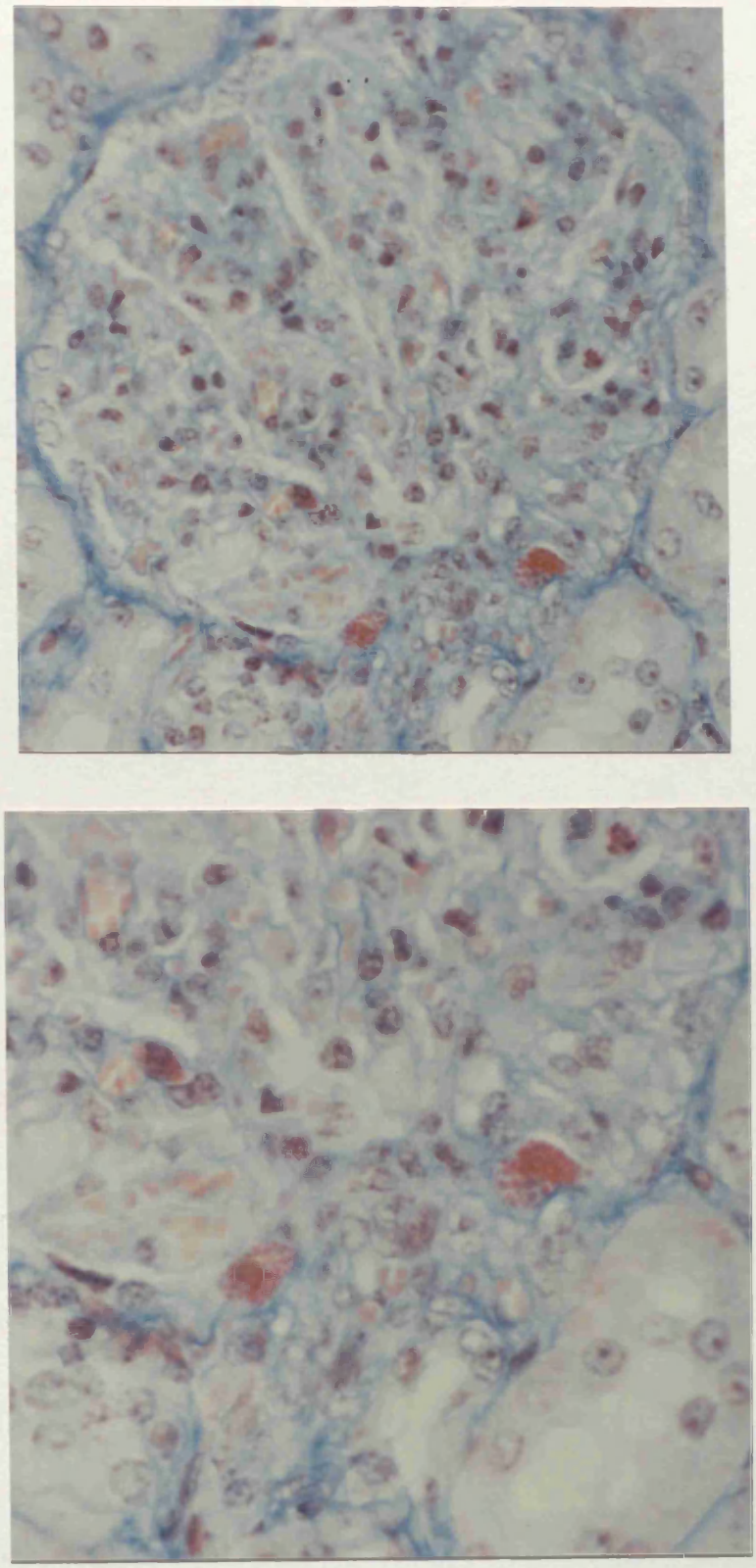


Figure 11. Low and high power micrographs of a sheep glomerulus. Two peripolar cells containing red-staining granules are situated in Bowman's space at the vascular pole. MSB trichrome.

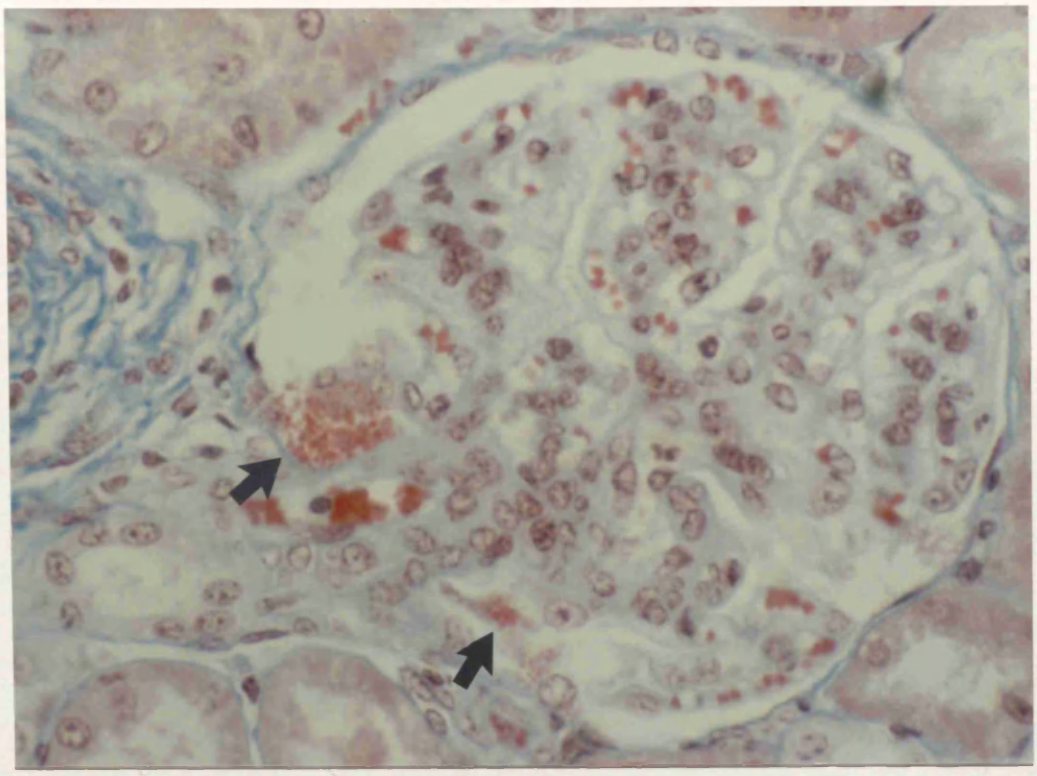


Figure 12. A sheep glomerulus containing two peripolar cells (arrows). A = arteriole. MSB trichrome.

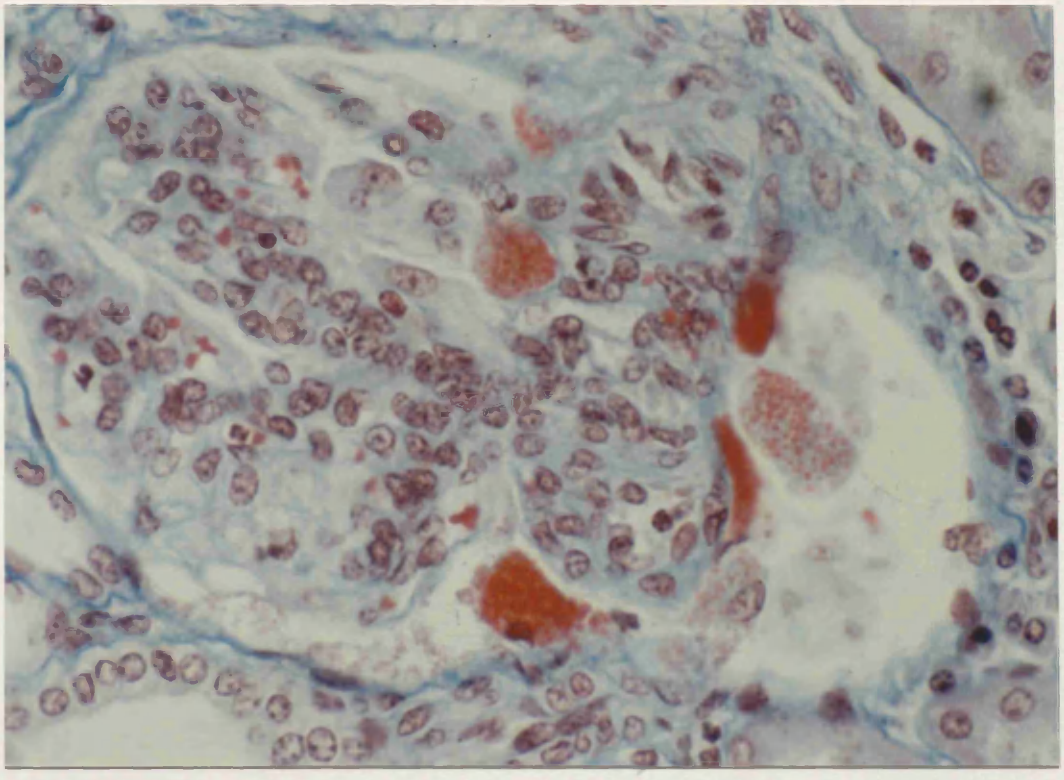


Figure 13. An oblique section of the vascular pole of a sheep glomerulus, reveals numerous peripolar cells. MSB trichrome.

in shape; some were round and plump, others were elongated and flat. Most peripolar cells were packed with cytoplasmic granules. The peripolar cell index (PPI) ranged from 20 to 100 (mean = 64.5) (Table 3). Up to 6 peripolar cells could be identified around a single vascular pole, although the median number of peripolar cells in each glomerulus was two. Peripolar cells were generally more prominent and numerous in older sheep (Table 3).

(3) NORMAL RAT KIDNEYS

(a) Peripolar cells

Peripolar cells were sparse and difficult to identify in rat kidneys. They were always closely related to the vascular pole (Figures 14 and 15). The granules stained blue with Toluidine blue and red with the MSB technique (Figures 14 and 15). Peripolar cells were often closely related to the granular myoepithelioid cells of the afferent glomerular arteriole. Only one granulated peripolar cell could be identified at each vascular pole. Granules were never identified in other glomerular epithelial cells.

Peripolar cells were identified in five of the six animals. The mean peripolar cell index (PPI) was 5.97, range = 0 - 12.5 (Table 4).

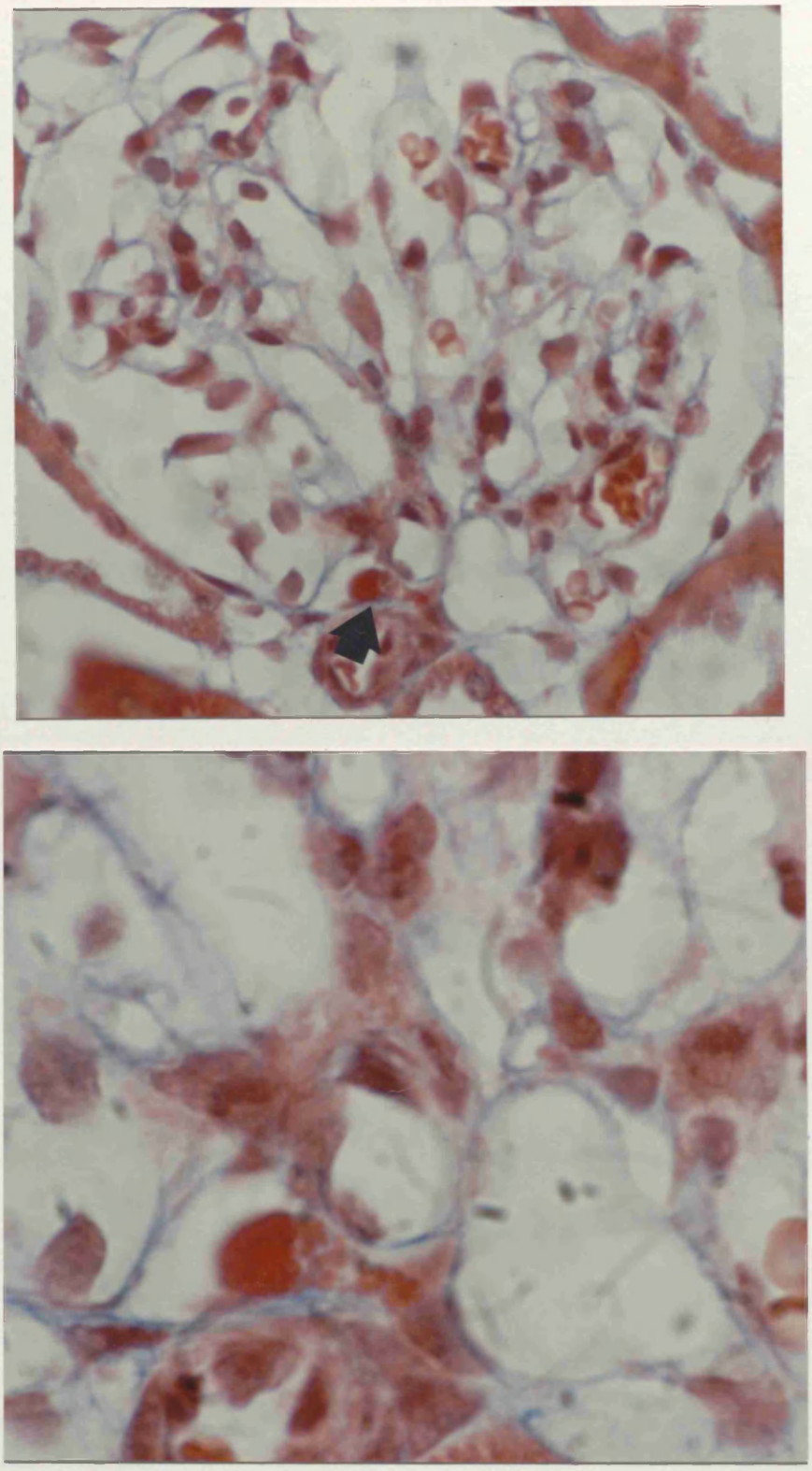


Figure 14. Low (upper) and high (lower) power micrographs of normal rat kidney, showing a peripolar cell (arrow) at the vascular pole. A = arteriole. MSB trichrome.

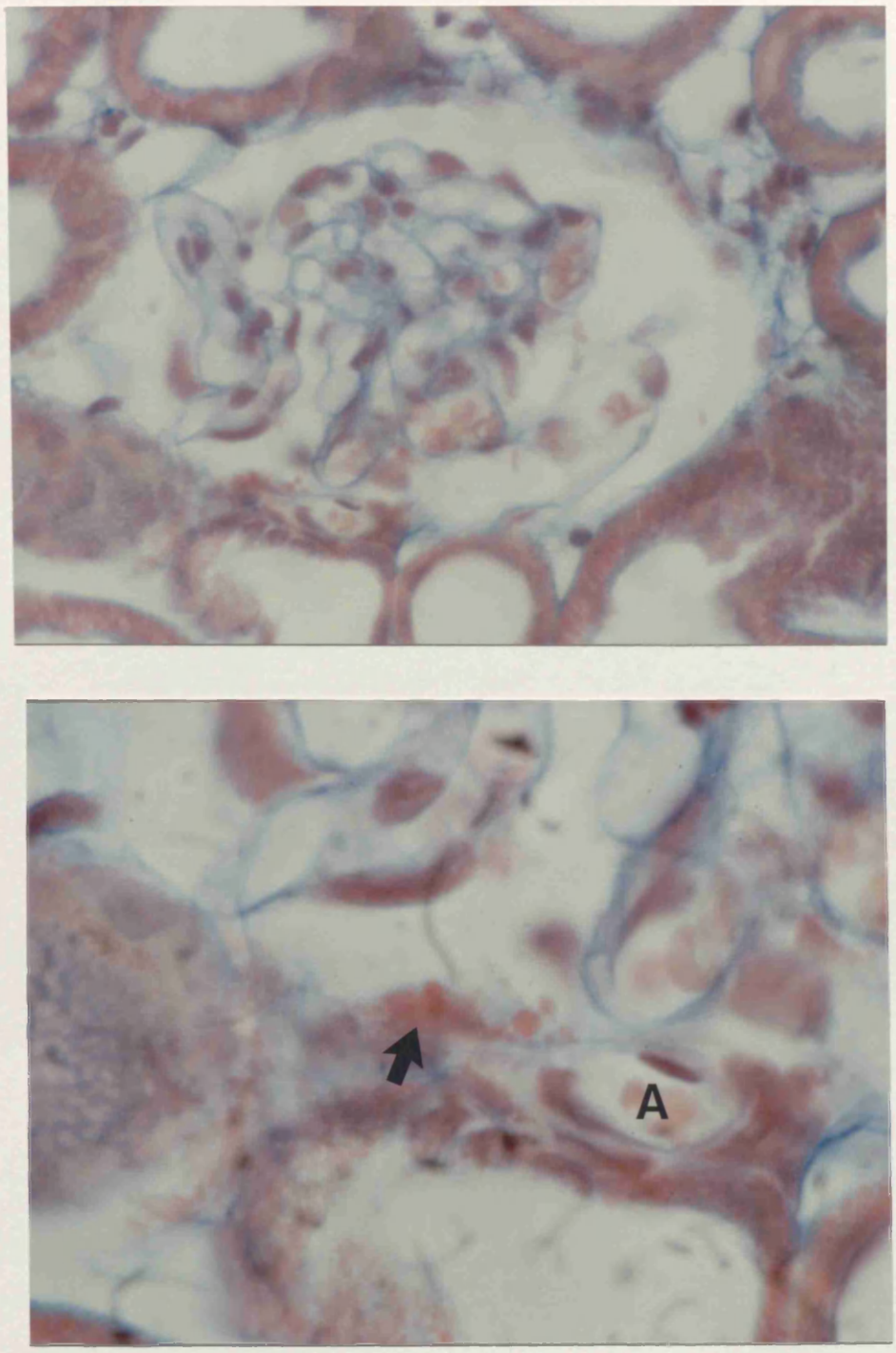


Figure 15. Low and high power micrographs of normal rat kidney, with a granulated peripolar cell (arrow) adjacent to the arteriole (A). MSB trichrome.

Figure 16. Rat kidney stained by the immunoperoxidase technique with an antiserum to mouse renin. Several renin-containing cells (arrow) are present in the afferent arteriole (A). Interference contrast illumination.

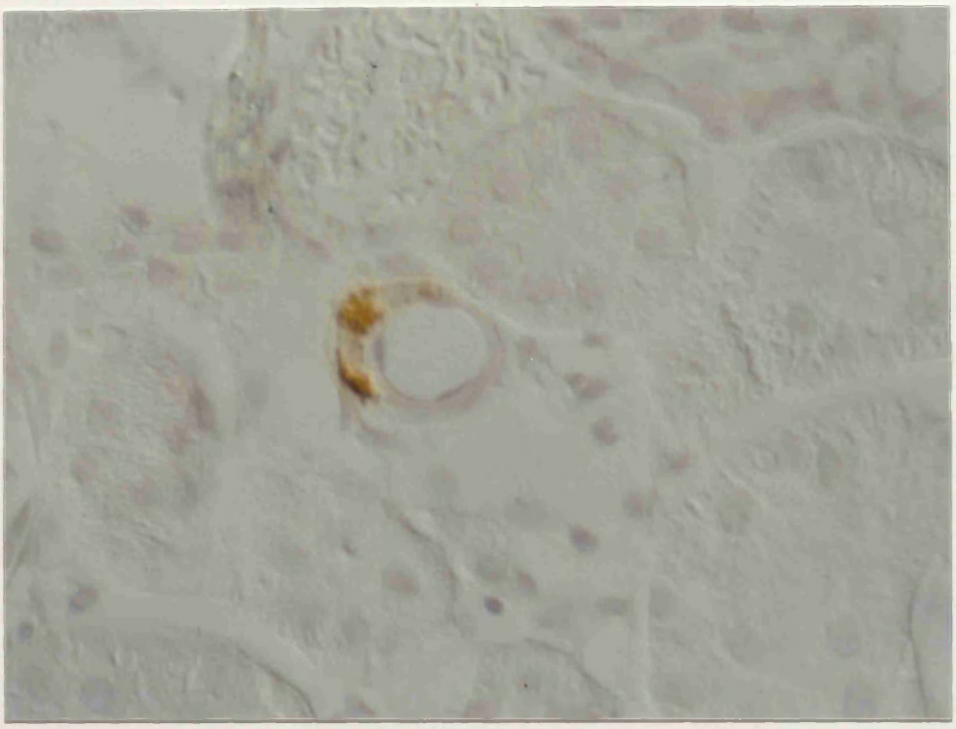


Figure 17. Renin-containing cells in an arteriole distant from the vascular pole. This is rare in normal rat kidneys. Immunoperoxidase, interference contrast illumination.

(b) Renin-containing cells

Renin-containing cells were identified in the walls of afferent arterioles and occasionally in efferent arterioles (Figure 16). Most of these cells were located close to the glomerulus, although sometimes they were present in the proximal afferent arteriole and interlobular arteries (Figure 17). The renin indices which were derived are shown in Table 4. There was no significant correlation between the renin indices and the peripolar cell index.

(4) THE DISTRIBUTION OF PERIPOLAR CELLS AND RENIN-CONTAINING CELLS IN THE RENAL CORTEX

(a) Peripolar cells

(i) Normal human kidneys

In both the resin-embedded and paraffin-embedded kidneys, peripolar cells were more frequent in superficial glomeruli, and rare in juxtamedullary glomeruli. This was more obvious in the resin-embedded group, where more peripolar cells were found (Figure 18). Peripolar cells were too sparse to plot in the autopsy control group.

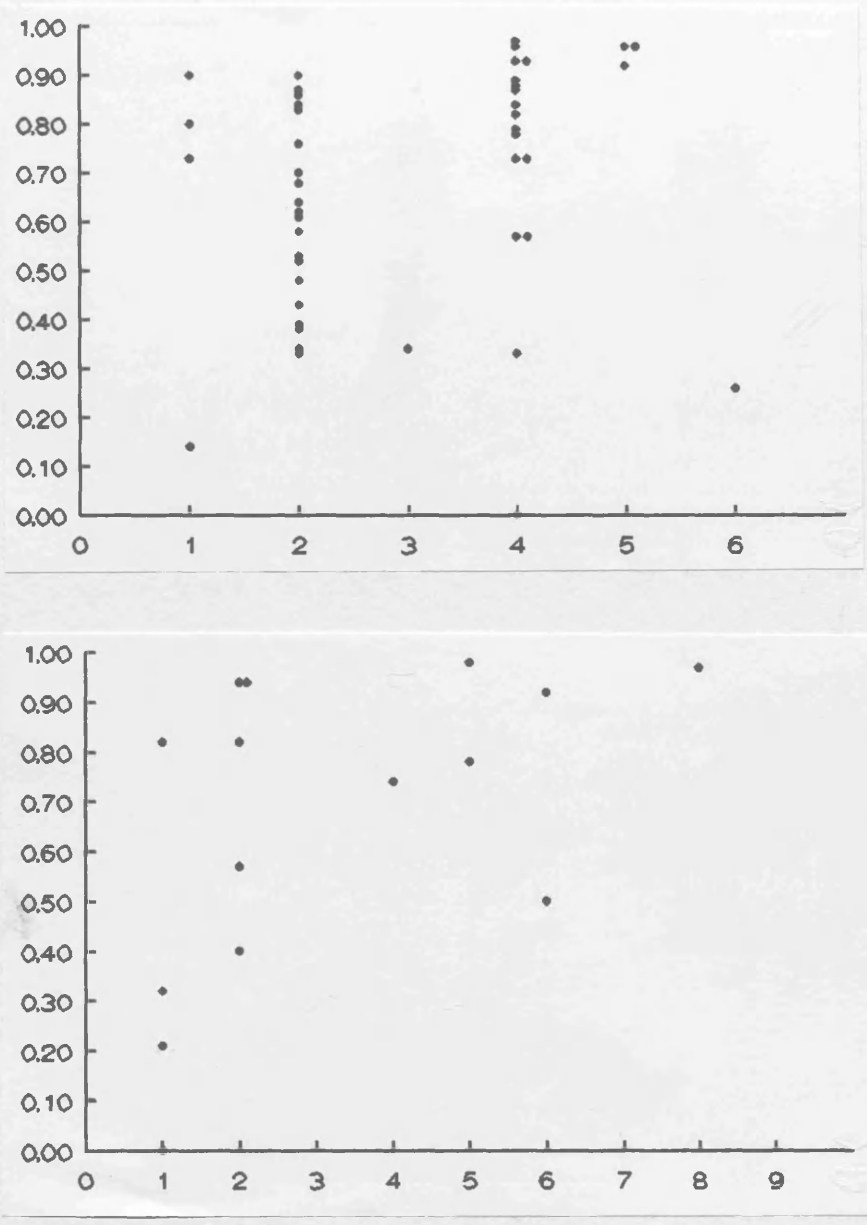


Figure 18. Scatter diagrams illustrating the distribution of peripolar cells in the renal cortex in normal human kidneys (upper = resin-embedded, lower = paraffin-embedded). 1 represents the renal surface, whilst 0 represents the corticomedullary junction. Each column represents a kidney. See text for details of measurements. Peripolar cells are more often located in superficial zones of the cortex.

(ii) Normal sheep kidneys

Figure 19 shows that more peripolar cells were found in the superficial renal cortex compared with the deep renal cortex. However, the distribution of all glomeruli in the renal cortex shows a similar superficial distribution. Therefore in sheep, the superficial distribution of peripolar cells is probably due to this sampling problem.

(iii) Normal rat kidneys

Five peripolar cells in total were identified in the six normal kidneys, therefore no conclusions regarding their distribution in the renal cortex can be drawn (Figure 20).

(iv) Response to alteration in sodium balance

The rats which were subjected to sodium-depletion or loading, and the Addison's cases, had insufficient peripolar cells to plot.

(v) Peripolar cells in hypertension

In experimental renovascular hypertension, peripolar cells were too sparse in any of the kidneys to allow interpretation, however glomeruli with other granulated epithelial cells were plotted, and these were evenly distributed (Figure 21).

In renal artery stenosis, peripolar cells were present mainly in the superficial renal cortex, except

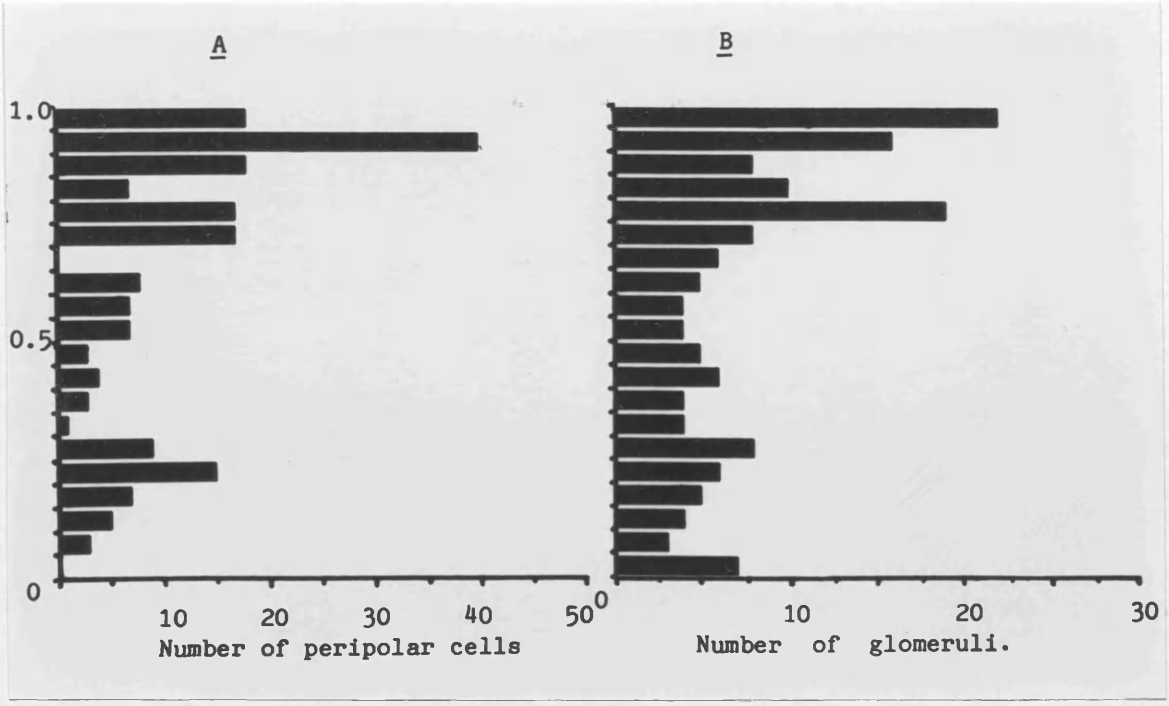


Figure 19. Distribution of peripolar cells (A) and all glomeruli (B) in the normal sheep kidney. In this case the apparent preponderence of peripolar cells in the superficial cortex is due to the greater density of glomeruli there.

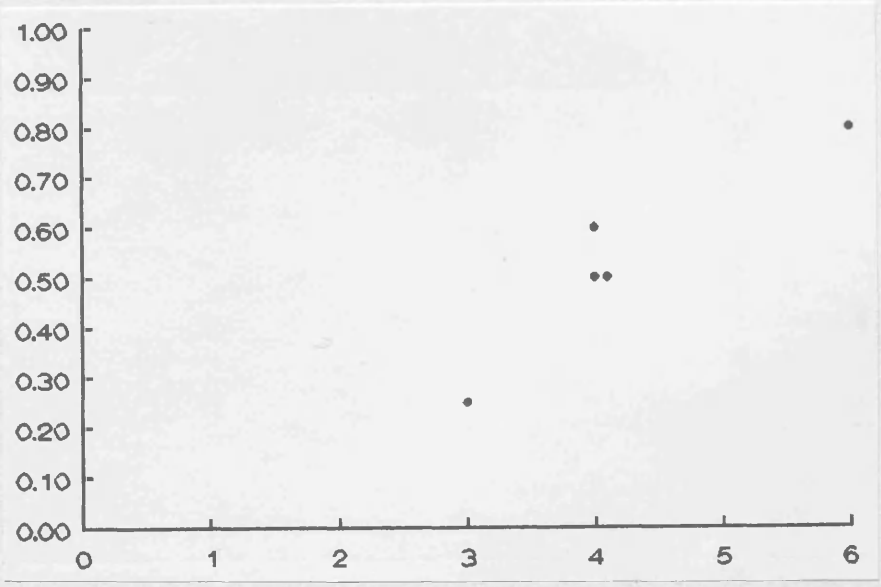


Figure 20. Distribution of peripolar cells in the renal cortex of normal rats.

for case 1, where the peripolar cells were spread evenly throughout the renal cortex (Figure 22).

In malignant hypertension, peripolar cells and other granulated epithelial cells were present mainly in the superficial renal cortex, and rarely in juxtamedullary glomeruli (Figure 23).

(b) Renin-containing cells

(i) Normal human kidneys

In both the resin-embedded and paraffin-embedded nephrectomy material, and in the autopsy controls, RCCs were present in greater numbers in the superficial cortex, and less often in the juxtamedullary region (Figure 24).

(ii) Normal rat kidneys

Most RCCs were present in the superficial renal cortex, with relatively few in juxtamedullary JGAs. (Figure 25).

(iii) Response to alteration in sodium balance

In sodium-depleted and sodium-loaded rats, RCCs were more evenly distributed in the renal cortex, compared with the normal kidneys (Figure 26).

In untreated Addison's disease, RCCs were more frequent in superficial zones of the kidney, however many juxtamedullary JGAs also contained RCCs. In

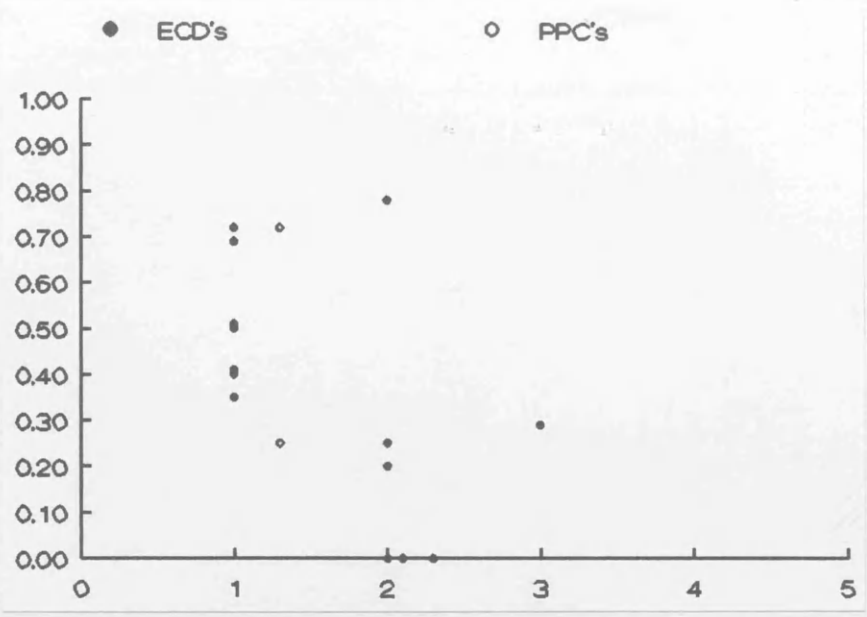


Figure 21. Distribution of peripolar cells (PPC's) and other granulated glomerular epithelial cells (ECD's) in the renal cortex of the clipped kidneys in experimental renovascular hypertension in rats. Peripolar cells are scant, however other granulated glomerular epithelial cells are evenly distributed.

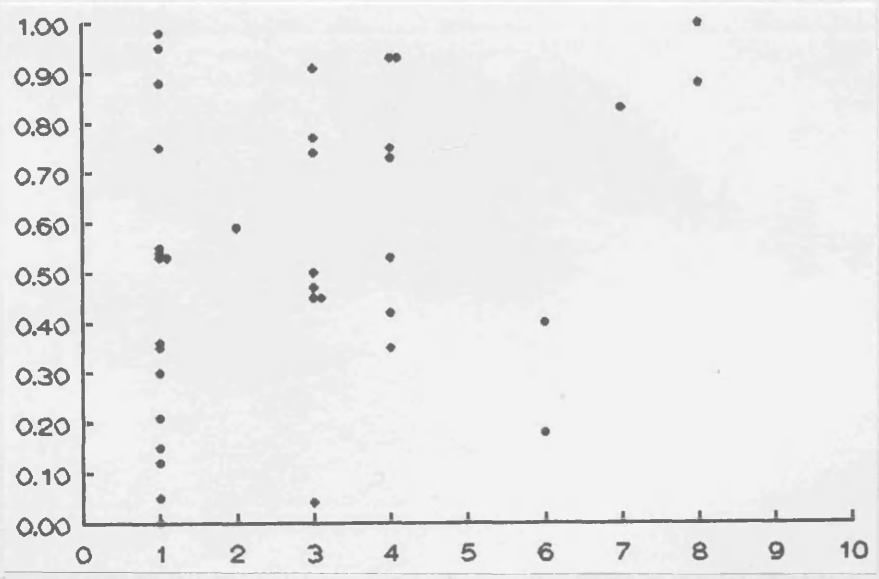


Figure 22. Distribution of peripolar cells in the renal cortex in renal artery stenosis. Generally peripolar cells are confined to the superficial layers of the cortex, however case 1 contains numerous peripolar cells, which are evenly distributed.

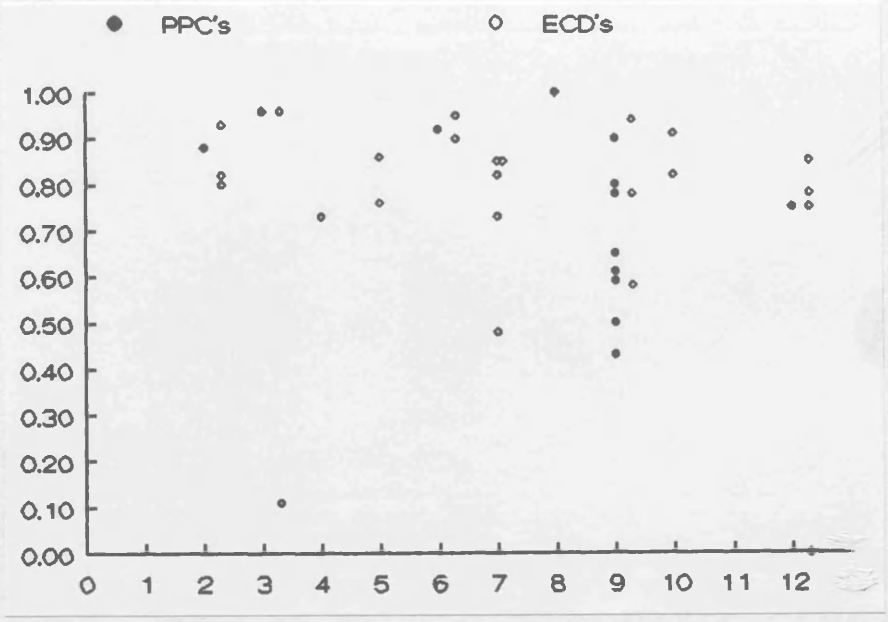


Figure 23. Distribution of peripolar cells (PPC's) and other granulated glomerular epithelial cells (ECD's) in the renal cortex in malignant hypertension. Both cell types are situated almost exclusively in the outer zones of the cortex.

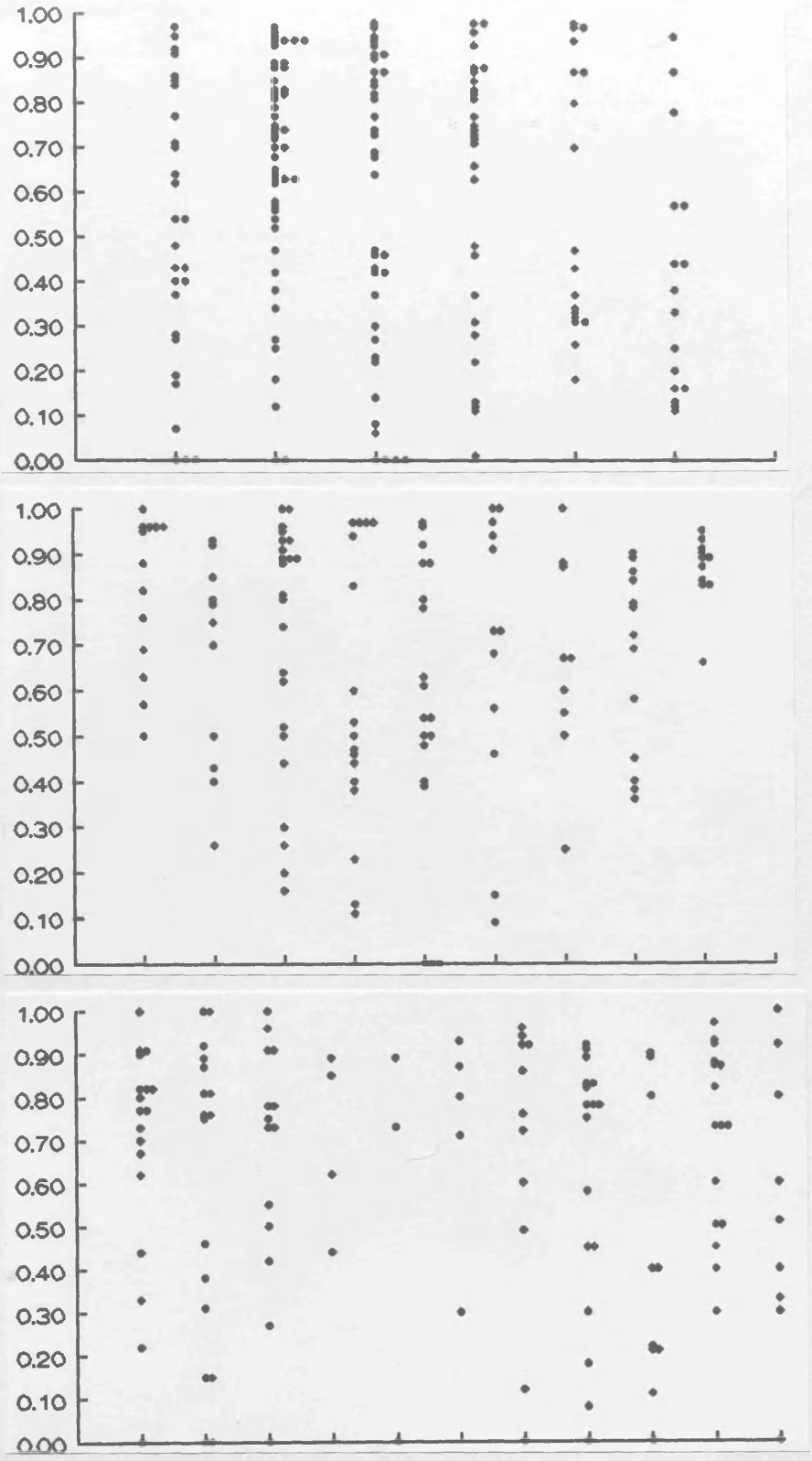
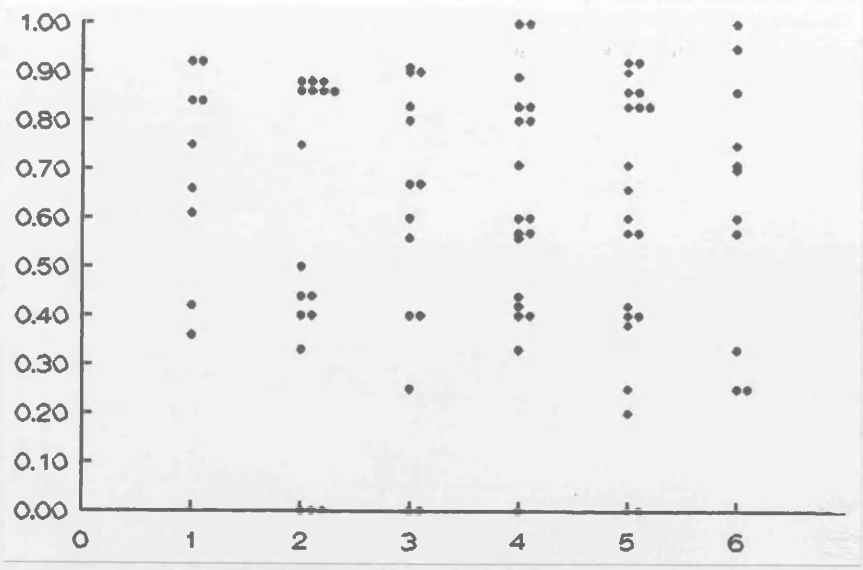


Figure 24. Distribution of renin-positive JGA's in normal human kidneys (upper = resin-embedded nephrectomies, middle = paraffin-embedded nephrectomies, lower = autopsy kidneys). Renin-containing cells are situated superficially, with relatively few in juxtamedullary JGA's.



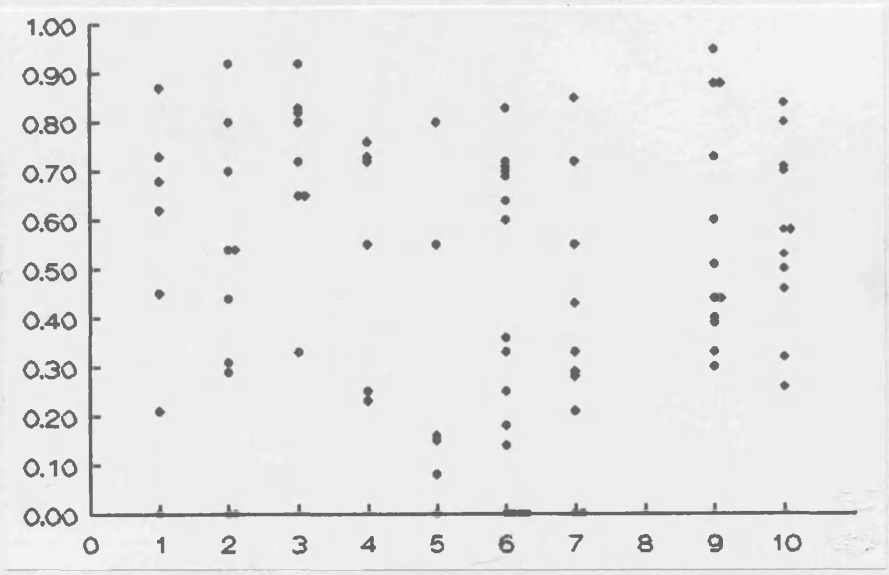


Figure 25. Distribution of renin-positive JGA's in normal rat kidneys. The lower scatter-diagram represents the control group of the experimental renovascular hypertension experiment. The renin-containing cells are mainly in the superficial zones, although the gradient is not as marked as in human kidneys.

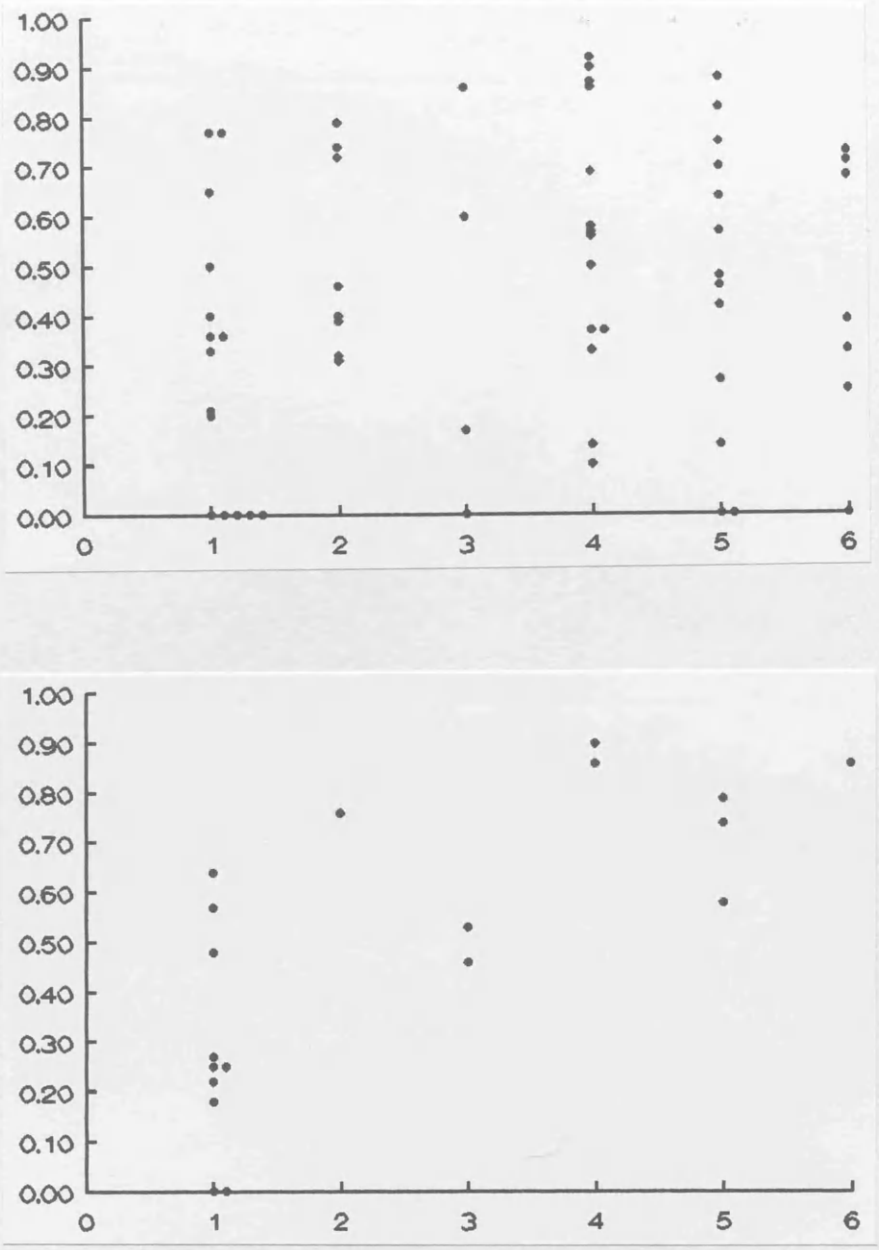


Figure 26. Distribution of renin-positive JGA's in the kidneys of sodium-depleted (upper) and sodium-loaded (lower) rats. In both groups there is an even distribution of renin-containing cells, with abolition of the normal gradient.

treated Addison's disease, the distribution was intermediate between that of untreated Addison's disease and the controls (Figure 27).

(iv) Renin-containing cells in hypertension

In the clipped kidneys of experimental renovascular hypertension, RCCs were evenly distributed in three of the kidneys but were more frequent in juxtamedullary JGAs in the other two (Figure 28).

For renal artery stenosis, RCCs still predominated in the superficial layers of the cortex, although the distribution was more even compared to normal human kidneys, with recruitment of juxtamedullary JGAs (Figure 28).

In malignant hypertension, there was an even distribution of RCCs, except for 2 kidneys (cases 1 and 10) where there were more RCCs in the deep cortex (Figure 29).

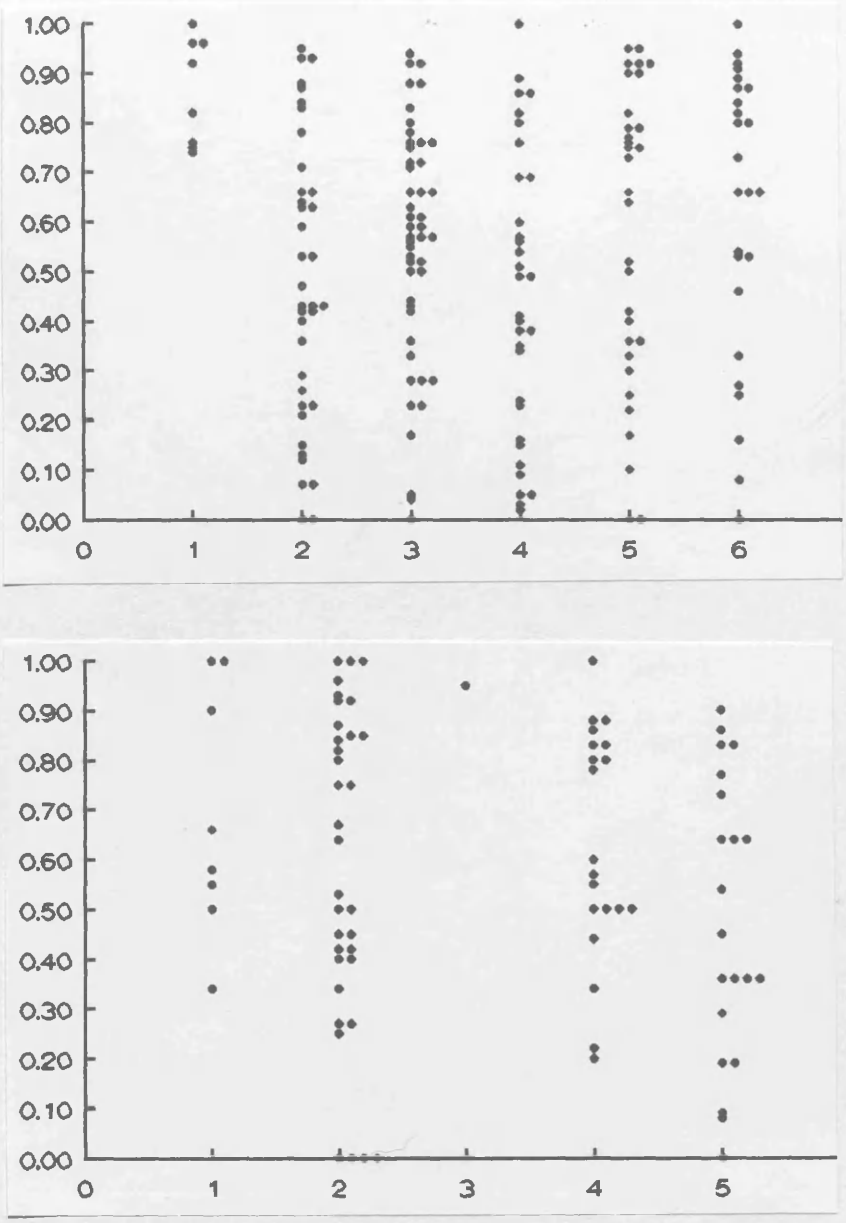


Figure 27. Distribution of renin-positive JGA's in the kidneys of patients with untreated (upper) and treated (lower) Addison's disease. Most renin-containing cells are situated in the superficial renal cortex, however significant numbers are also present in juxtamedullary JGA's. This is more marked in the untreated cases.

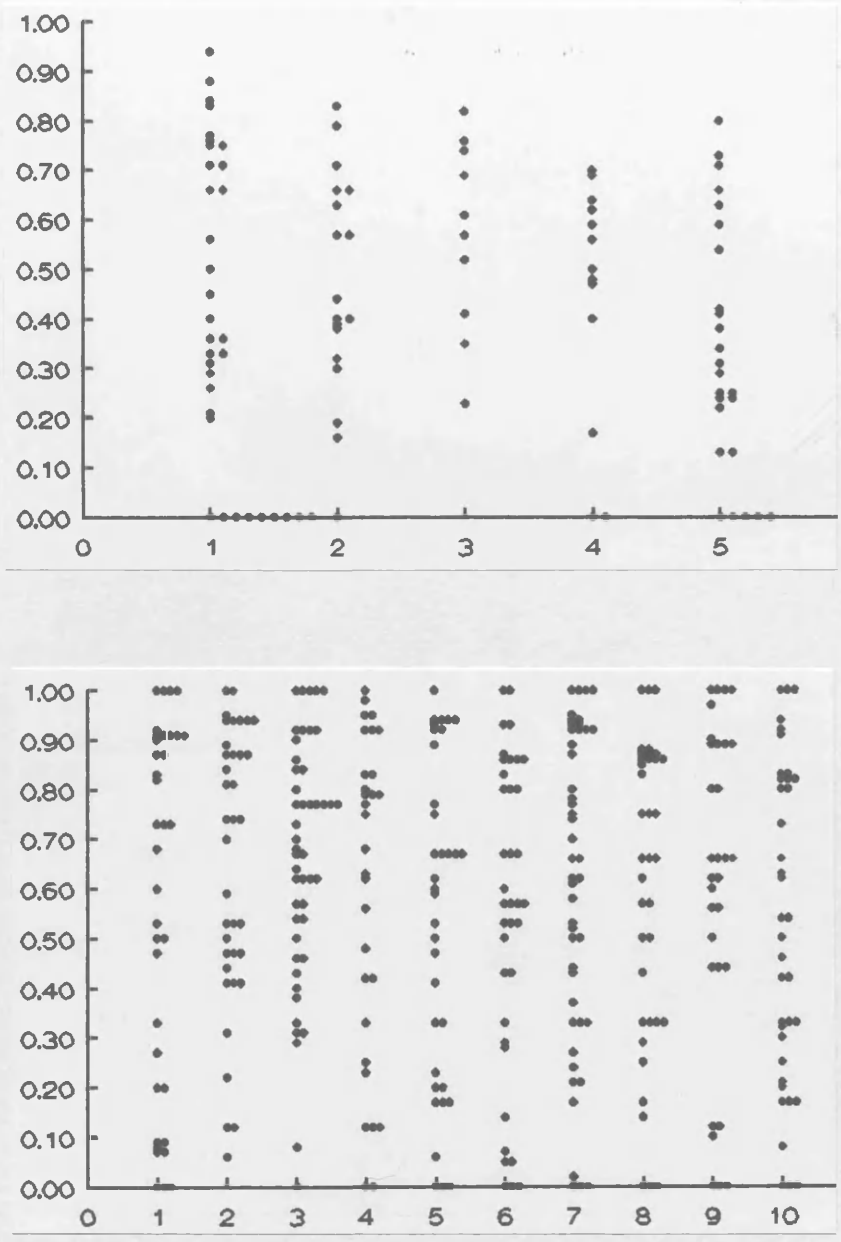


Figure 28. Distribution of renin-positive JGA's in the kidneys of "clipped" experimental renovascular hypertension in rats (upper) and renal artery stenosis (lower). In 2 of the rats (1 and 5) the normal gradient is reversed, whilst there is an even distribution in the other 3. In renal artery stenosis, renin-containing cells are still predominantly superficial, although many are also present in juxtamedullary JGA's.

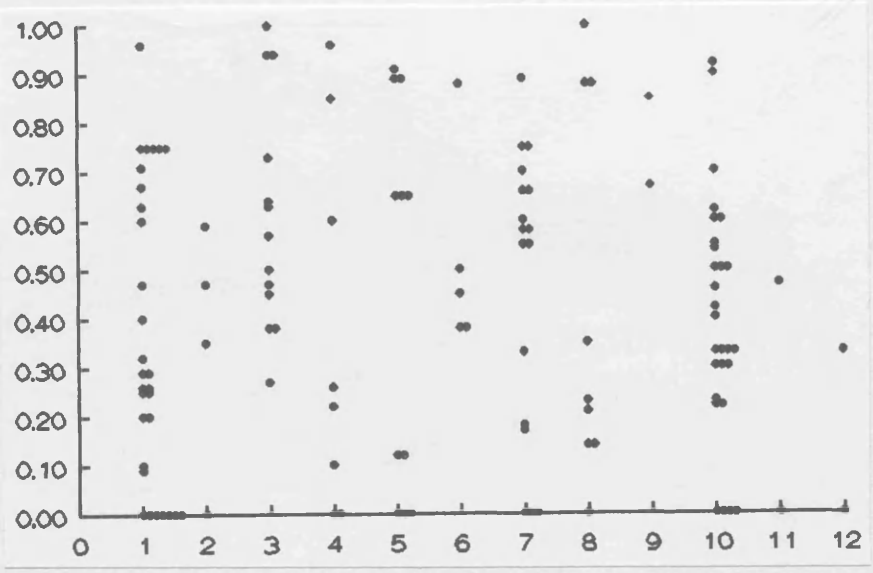


Figure 29. Distribution of renin-positive JGA's in kidneys affected by malignant hypertension. In most cases, renin-containing cells are evenly distributed in the renal cortex, but the normal gradient is reversed in cases 1 and 10.

(5) IMMUNOHISTOCHEMICAL STUDIES OF THE PERIPOLAR CELL

(a) Humans

These results are summarised in Table 5.

(1) Intermediate filaments

A minority (approximately 20%) of peripolar cells showed positivity for vimentin, usually along the basal aspect of the cell, but sometimes filling the entire cytoplasm, leaving the granules unstained (Figure 30). Peripolar cells were always negative with Cam 5.2, PKK1 and antibodies to desmin.

Podocytes always stained strongly for vimentin but were negative with Cam 5.2, PKK1 and for desmin (Figure 30). Occasional parietal epithelial cells showed positivity for vimentin, a minority were positive with PKK1 and approximately half were positive with Cam 5.2. Parietal cells were desmin-negative. The distal tubules were strongly positive with Cam 5.2 and PKK1 with weak staining of the proximal tubules. Vimentin positivity was noted in interstitial cells and occasional tubular epithelial cells (Figure 31). Vascular smooth muscle cells and interstitial cells stained positively for vimentin.

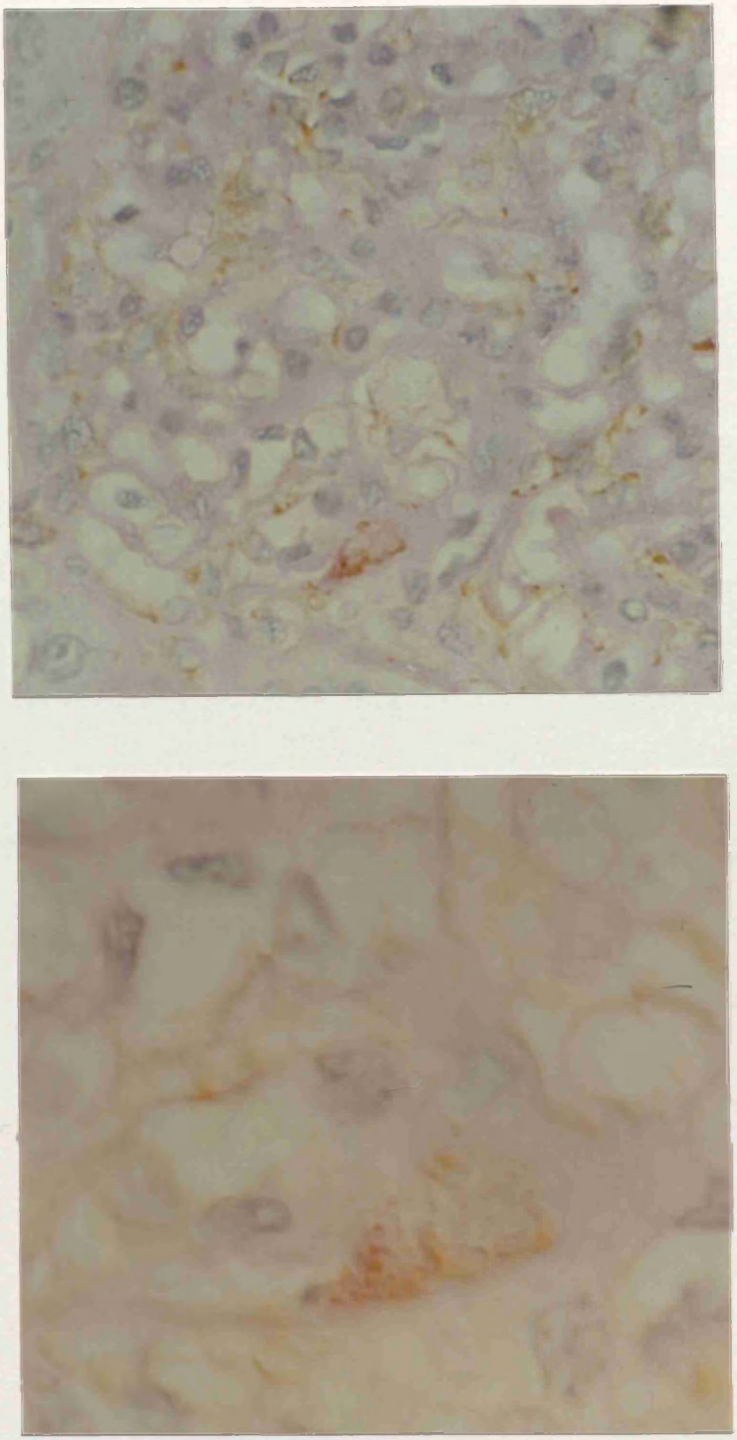


Figure 30. Human kidney stained by immunoperoxidase technique for vimentin. In the low power micrograph, podocytes are staining positively, and in the higher power field there is positive staining of a peripolar cell. The granules are stained by the PAS counterstain.

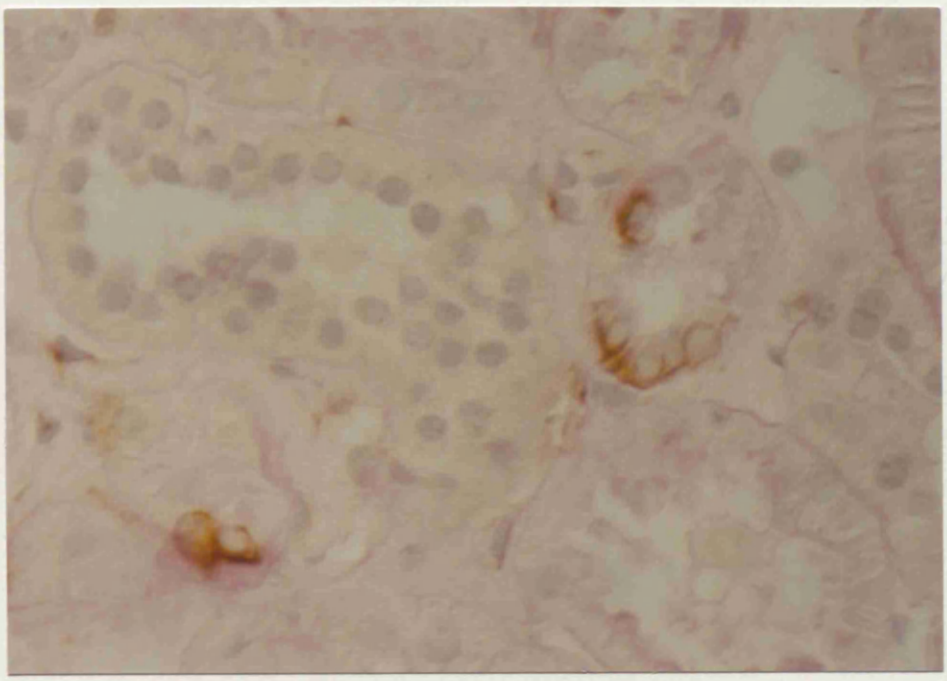


Figure 31. Human kidney stained by immunoperoxidase technique for vimentin. A minority of tubular epithelial cells are positive. PAS counterstain.

(2) Epithelial markers

Peripolar cells were negative for EMA and HMFG I and II. EMA antisera stained the luminal border of distal tubules. Antisera to HMFG I and II stained distal tubules strongly. There was no glomerular staining with any of these antisera.

(3) Neuroendocrine markers

Peripolar cells were negative for NSE, S-100, NF and chromogranin. All of these reagents stained renal nerves. In addition antibodies to NSE, S100 and NF lightly stained distal tubules.

(4) Plasma proteins

Peripolar cell granules exhibited positive staining for IgG, albumin and α1-antitrypsin (Figures 32,33). The positivity was mostly around the edge of the granules (Figure 32), although sometimes the granules were stained homogeneously (Figure 33). The peripolar cell cytoplasm was always negative. Light staining for all plasma proteins was also identified in the brush border of the proximal tubule.

Figure 32. Human peripolar cell staining positively with IgG antiserum. Note the peripheral pattern of granule staining. PAP technique, interference contrast illumination.

Figure 33. Human peripolar cell (arrow) showing positive staining for albumin. PAP technique, interference contrast illumination.

(b) Sheep

These results are summarised in Table 6.

(1) Intermediate filaments

Peripolar cells were negative for all of the intermediate filaments (Figures 34,35,36). Podocytes showed strong positivity for vimentin, but were negative with Cam 5.2, PKK1 and antibodies to desmin (Figure 34,35,36). Occasional parietal cells and tubular epithelial cells stained positively for vimentin only (Figure 34). The macula densa stained strongly positive with PKK1 antibody (Figure 35). Vimentin and desmin positivity was present in vascular smooth muscle cells and interstitial cells. In addition, the mesangium was always stained with the antibodies to desmin (Figure 36).

(2) Epithelial markers

No structures were positive for EMA. HMFG I and HMFG II were localised to the luminal aspect of most tubules (Figure 37).

(3) Neuroendocrine markers

Peripolar cells were negative for NSE, S-100, NF and chromogranin (Figure 38). All of these antibodies lightly stained distal tubules.

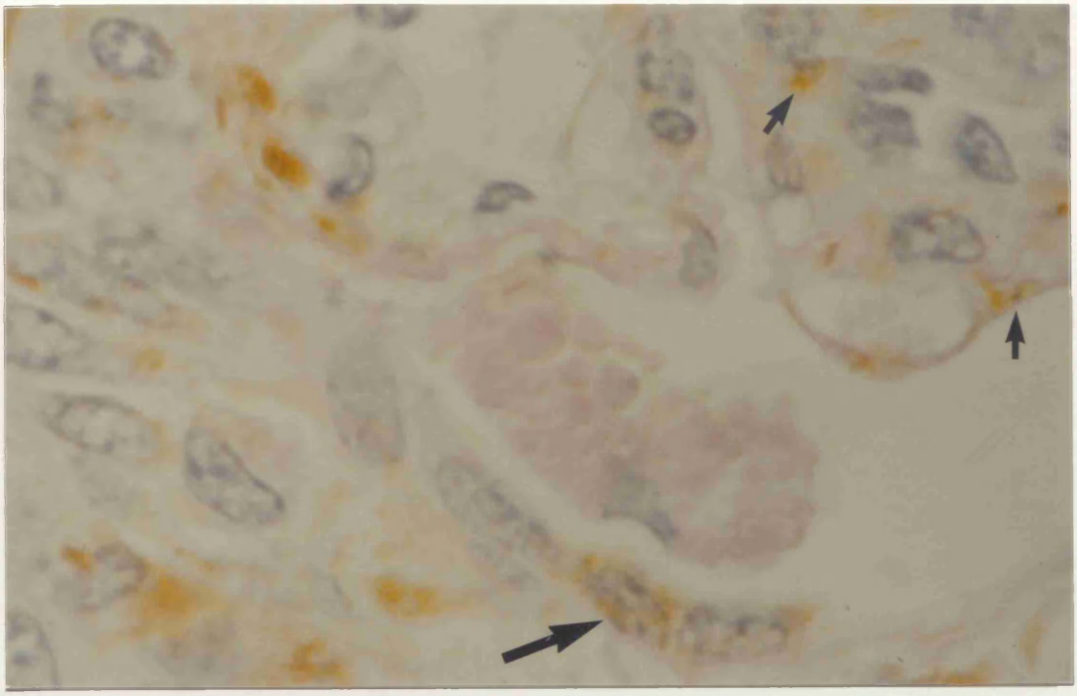


Figure 34. Sheep kidney stained with antibodies to vimentin. The peripolar cell is negative, but there is positive-staining of podocytes (small arrows) and some parietal cells (large arrow). PAS counterstain.

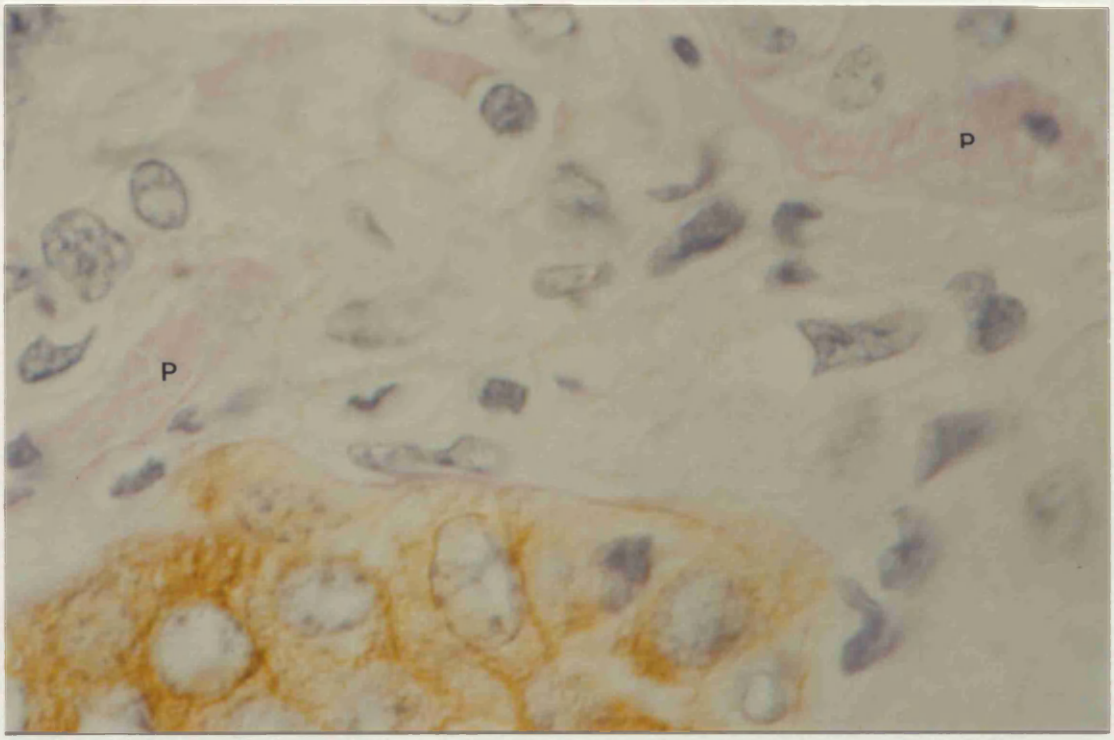


Figure 35. Sheep kidney stained with PKK1 antibody. The peripolar cells (P) are negative, but the macula densa is strongly positive. PAS counterstain, interference contrast illumination.

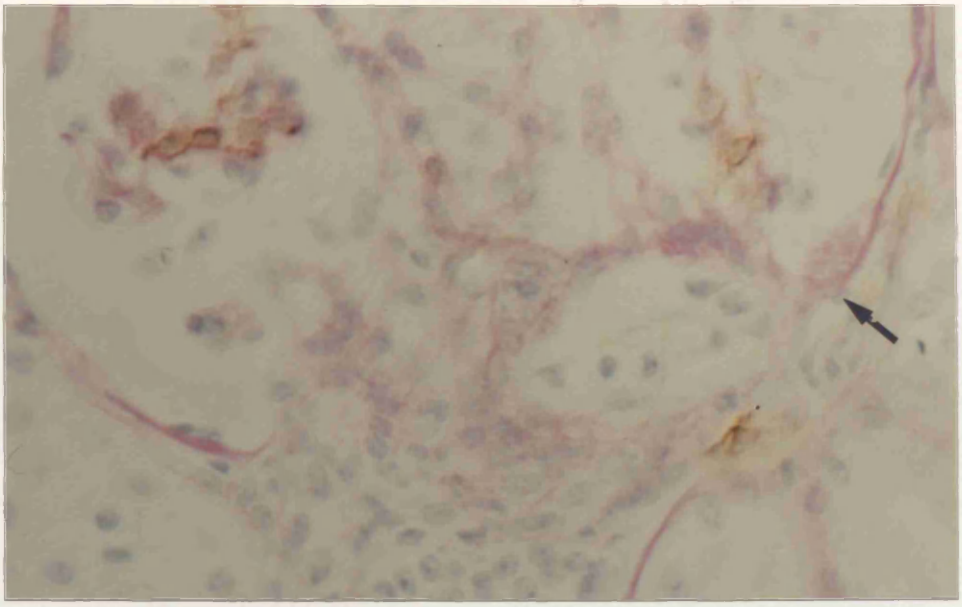


Figure 36. Sheep kidney stained with anti-desmin antibodies. There is mesangial and arteriolar staining, but the peripolar cell (arrow) is negative. PAS counterstain.

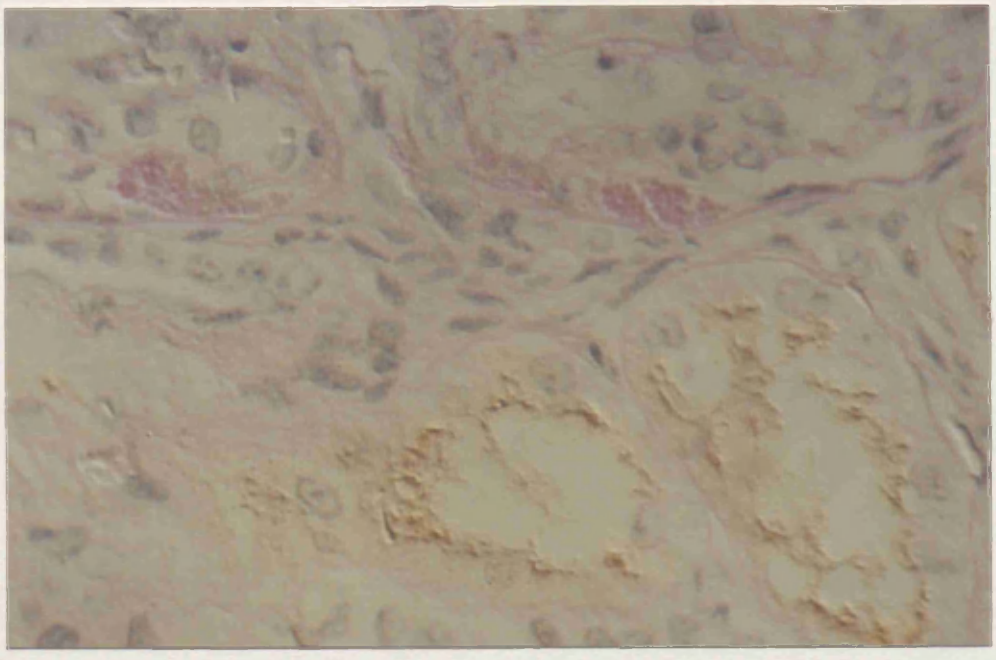


Figure 37. Immunoperoxidase stained sheep kidney with HMFG I antibody. There is light staining of the tubular brush borders, but the peripolar cells are negative. PAS counterstain.

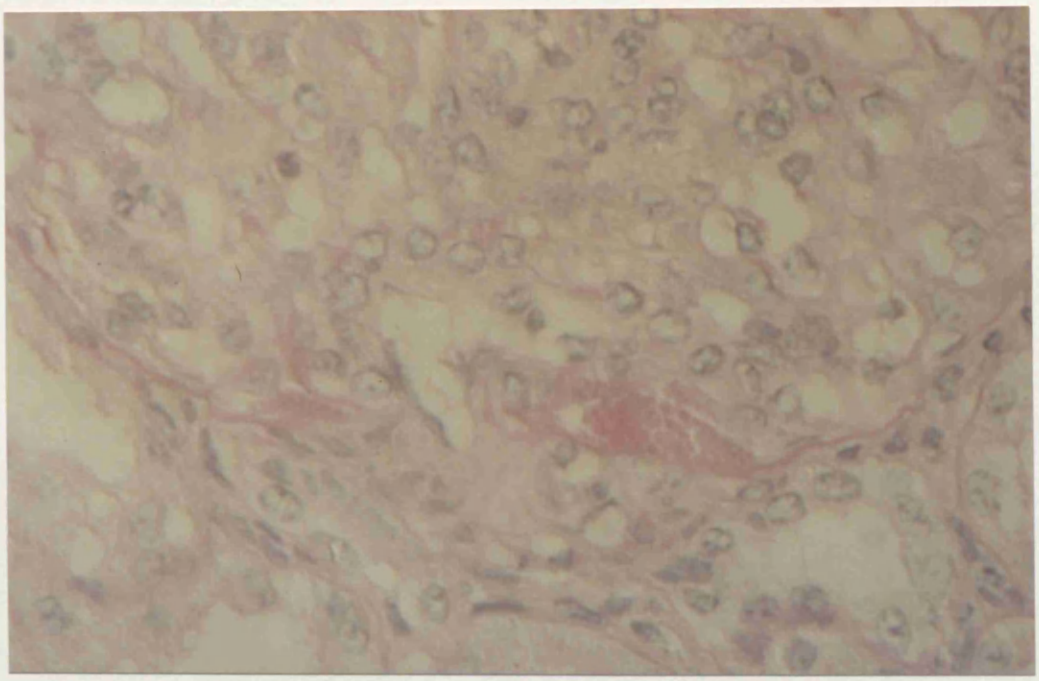


Figure 38. Immunoperoxidase-stained section for neurone-specific enolase. All structures, including peripolar cells are negative. PAS counterstain.

(4) Plasma proteins

Peripolar cell granules stained strongly positively for IgG, C3 and albumin but were negative for IgM (Figures 39 and 40). The staining pattern for IgG was similar to that observed in humans, however the granules were homogeneously stained by antisera to C3 and albumin. The peripolar cell cytoplasm was always negative. In addition there was weak positivity for C3, albumin and IgG within the glomerular tuft (Figures 39 and 40). This was due to staining of podocytes and in some instances the glomerular basement membrane. This was often accentuated in the region of the vascular pole, and sometimes extended into the extraglomerular mesangium (Figures 39 and 40). There was a similar pattern of positivity for IgM, but this often extended into the macula densa (Figure 41). The apical borders of most tubules stained positively for IgG, albumin and C3, but not IgM (Figures 39,40,41).

Immunofluorescence produced similar results with the antisera to plasma proteins, with the exception that albumin immunostaining was present in peripolar cell granules, but in no other glomerular cells (Figure 42).

Figure 39. IgG immunoperoxidase stained section of sheep kidney. The peripolar cell (arrow) shows strong granular positivity. There is also staining of brush borders. Interference contrast illumination.

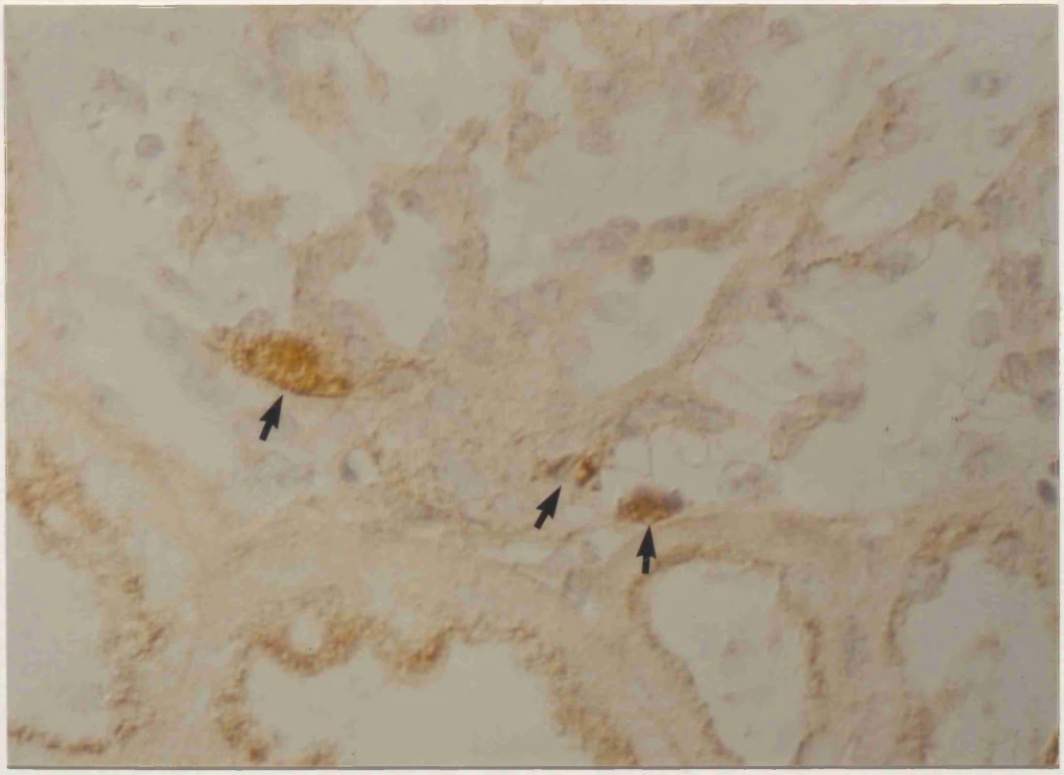


Figure 40. Sheep kidney stained for C3, by the immunoperoxidase technique. Three peripolar cells (arrows) and the tubular brush borders are positive.

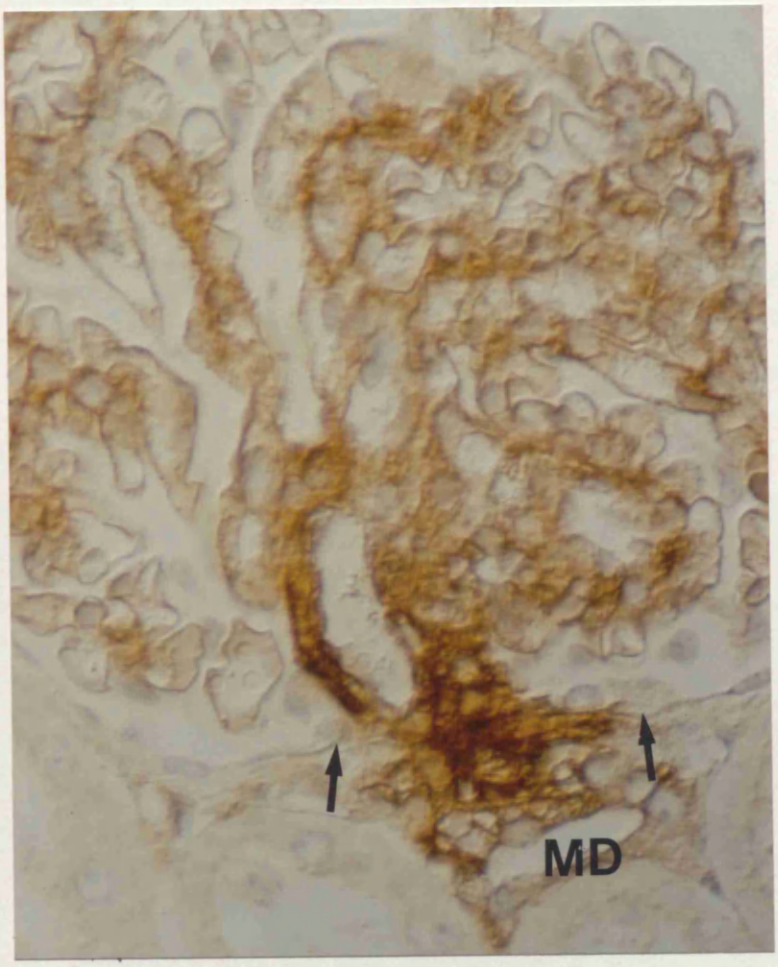


Figure 41. IgM immunoperoxidase-stained sheep kidney. There is staining of the mesangium, which is accentuated at the vascular pole and extends into the macula densa (MD). The peripolar cells (arrows) are negative.

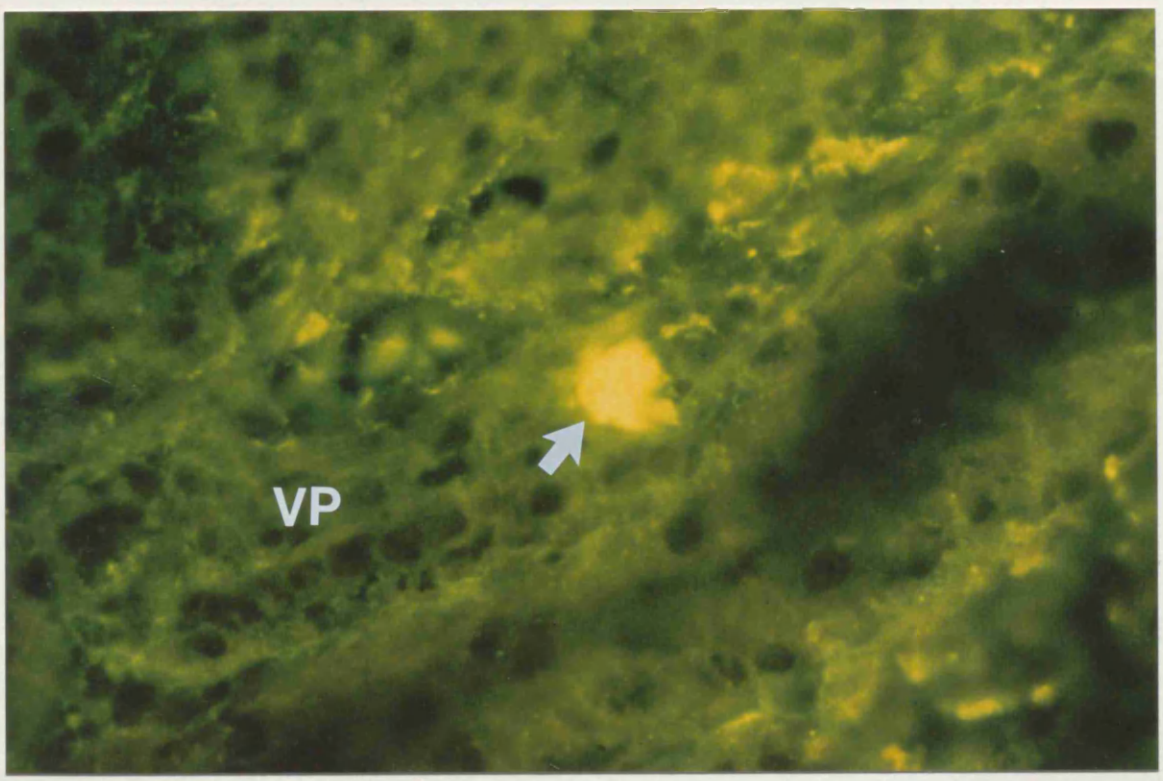


Figure 42. Frozen section of sheep kidney stained for sheep albumin by immunofluorescence technique. Upper: viewed by interference contrast microscopy, the granular peripolar cell is just visible (arrow). Lower: same field viewed by fluorescence nicroscopy; the peripolar cell (arrow) is strongly positive. VP = vascular pole.

(6) EXPERIMENTAL ANIMALS

(a) Experimental alteration in sodium balance

The kidneys of both sodium-loaded and sodium-depleted animals were histologically normal.

(i) Peripolar cells

In the sodium-depleted group, peripolar cells were identified in one of the six animals. The mean PPI was 3.33 (Table 7). The morphology of peripolar cells was similar to those in normal kidneys. In the sodium-loaded group, peripolar cells were identified in two of the six animals. The mean PPI was 1.67 (Table 7). There was no significant difference in PPI between the sodium-depleted and sodium-loaded groups.

(ii) Renin-containing cells

These results are illustrated in Table 7. JGA+ and RCI were significantly elevated in the sodium-depleted group ($p<0.002$ and $p<0.001$, respectively). There was no significant difference in A+. There was correlation between JGA+ and RCI in both groups ($p<0.02$), and between A+ and RCI in the sodium-depleted group only ($p<0.05$). There was no correlation between PPI and any of the renin indices in either group. The sodium-

Table 7. Mean (±SEM) peripolar cell index (PPI) and renin indices for sodium-depleted and sodium-loaded rats. See text for definitions of indices.

	Sodium-depleted n=6	**Sodium-loaded n=6**	
PPI	3.33 (±3.33)	1.67 (±1.05)	N.S.
JGA+	14.1 (±3.0)	3.0 (±0.81)	$p<0.002$
A+	3.75 (±1.1)	1.37 (±0.5)	N.S.
RCI	48.4 (±11.4)	9.8 (±2.9)	$p<0.001$

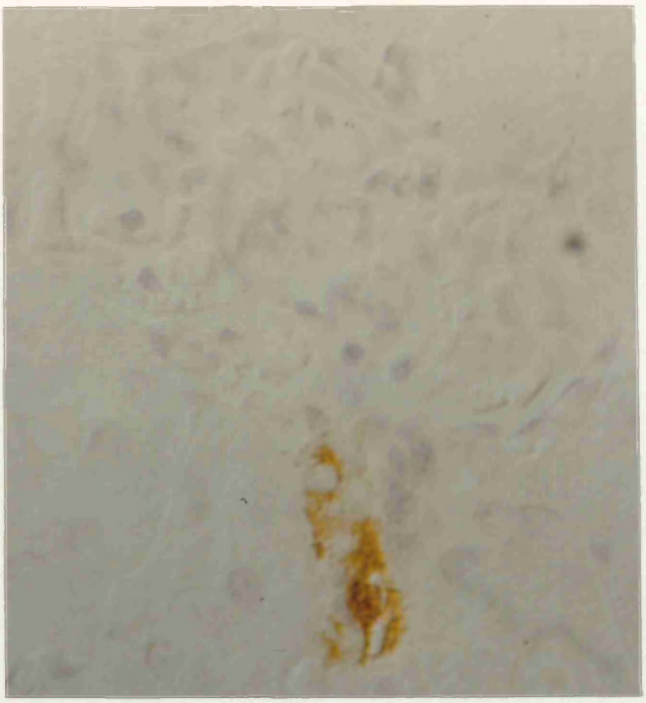


Figure 43. Hyperplasia of the renin-containing cells in a sodium-depleted rat. Immunoperoxidase, interference contrast illumination.

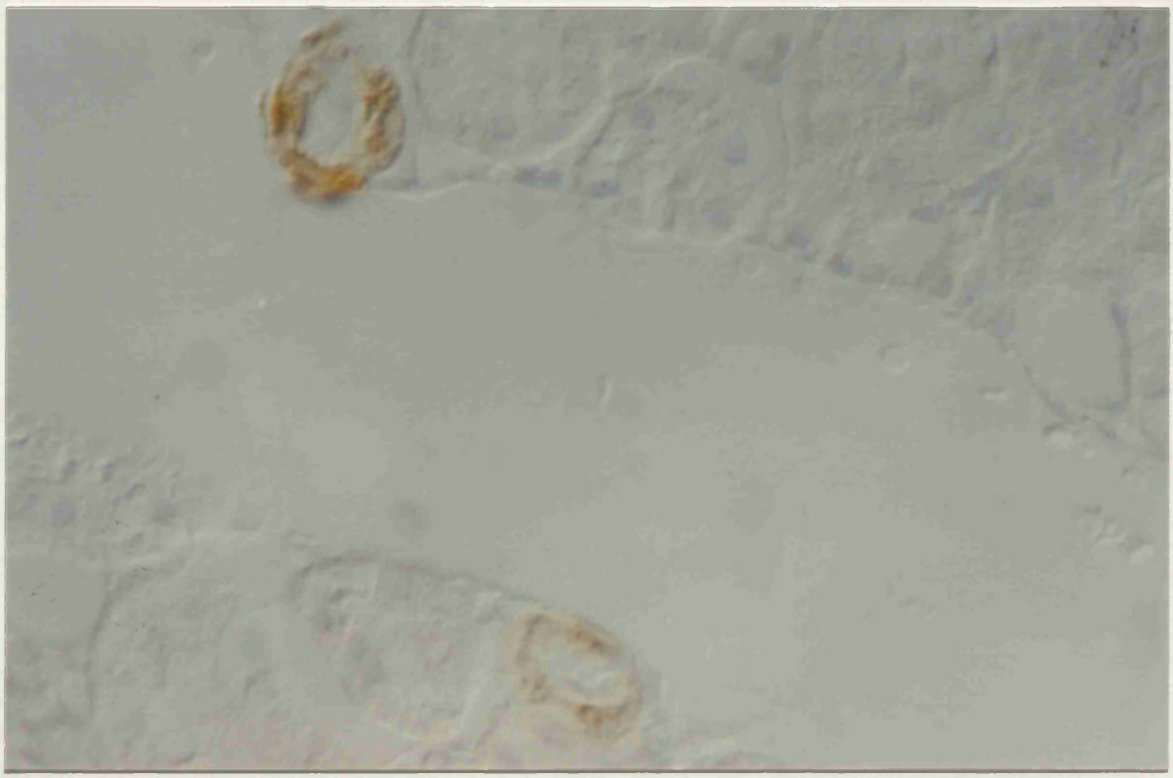


Figure 44. Renin-containing cells in afferent arterioles distal from the glomerulus, in a sodium-depleted rat. Immunoperoxidase, interference contrast illumination.

depleted animal which had an elevated PPI had average renin indices for its group.

In the sodium-depleted group, many JGAs were hyperplastic and there was frequent extension of renin-containing cells into the proximal afferent arteriole (Figures 43 and 44). By contrast, in the sodium-loaded group renin-containing cells were sparse.

(b) Experimental renovascular hypertension

All of the clipped rats became hypertensive. The last recorded systolic blood pressure ranged from 171 to 252 mmHg (mean = 211 mmHg) (Table 8). This compared with 132 to 161 mmHg (mean = 145 mmHg) for the controls. The mean kidney weights were 0.98 g (clipped), 2.02g (unclipped) and 1.635 g (sham-operated) (Table 8).

(i) Peripolar cells

Only one peripolar cell was identified in the sham-operated group (Table 9). No peripolar cells were present in the clipped kidneys, but several were identified in 2 of the 5 unclipped kidneys (Figure 45). The PPI for these kidneys ranged from 0 to 9.1

Table 9. Mean peripolar cell index (PPI) and epithelial cell droplet index (ECDI), for clipped and unclipped rat kidneys in experimental group and sham-operated controls. There are no significant differences between any of the groups for PPI or ECDI.

	EXPERIMENTAL GROUP n=5		CONTROLS n=5
	Clipped	Unclipped	
PPI	0	2.58 (±1.8)	0.62 (±0.62)
ECDI	0	47.1 (±21.4)	0

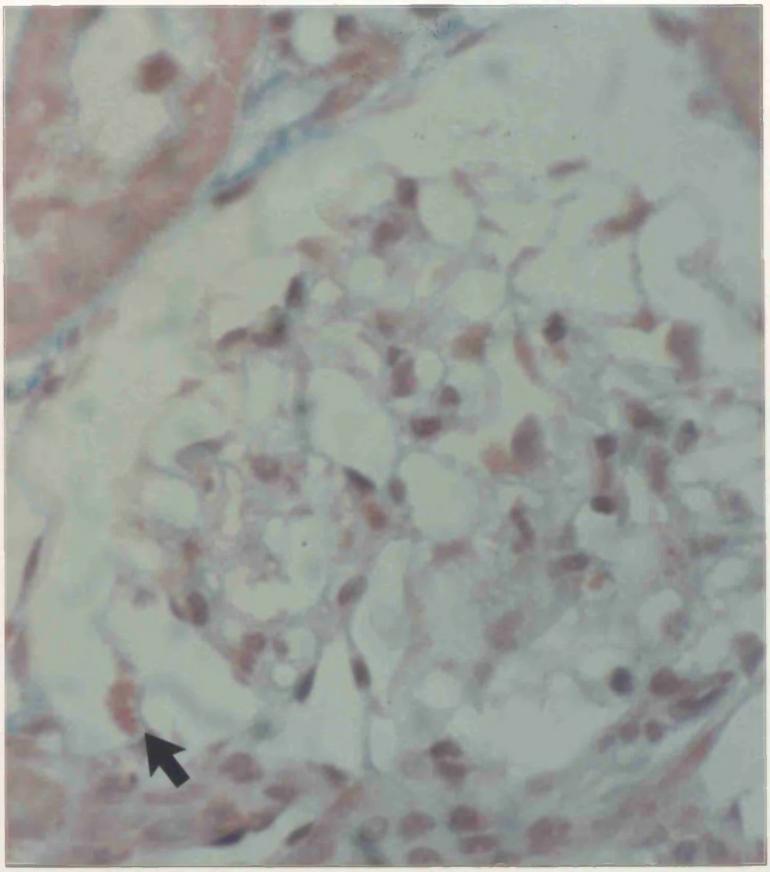


Figure 45. Peripolar cell (arrow) in an unclipped rat kidney in experimental renovascular hypertension. MSB stain.

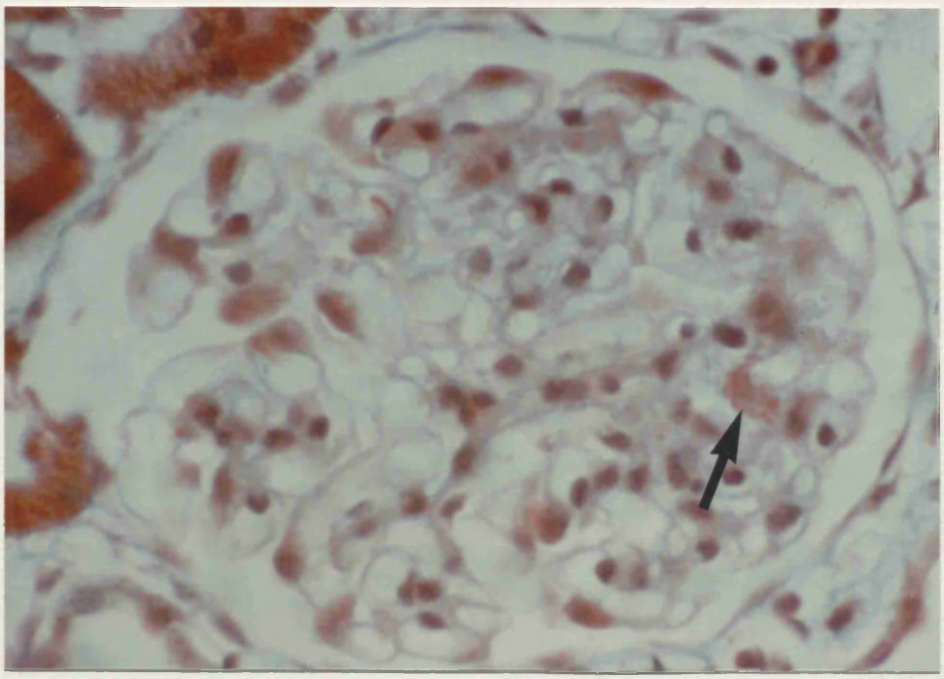


Figure 46. A granulated podocyte (arrow) in an unclipped rat kidney in experimental renovascular hypertension. The vascular pole is not in the plane of section. MSB stain.

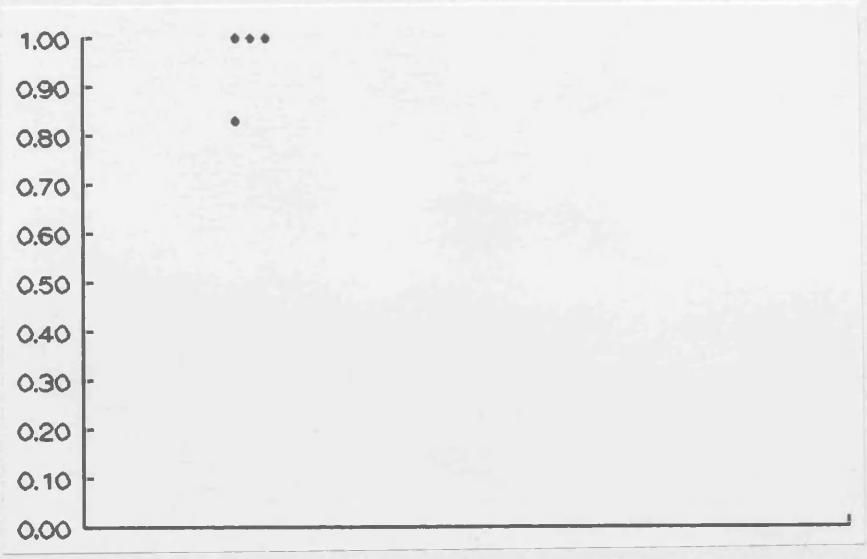


Figure 47. Distribution of granulated podocytes with glomeruli, in the unclipped kidneys of experimental renovascular hypertension in rats. Only a few are plotted, since the vascular pole was rarely in the plane of section. A ratio of 1 represents the edge of the glomerulus, whilst 0 represents the vascular pole. See text for methods of measurements.

(mean = 2.59). There were no significant differences in PPI between any of the groups. There was no correlation between PPI and blood pressure.

(ii) Other granulated glomerular epithelial cells

No granulated glomerular epithelial cells were identified in either the sham-operated group or in the clipped kidneys (Table 9). However, they were present in 3 of the 5 unclipped kidneys (Figure 46). Most granulated epithelial cells were podocytes, although a small proportion were parietal cells. The ECDI ranged from 0 to 104.5 (mean = 47.1) (Table 9). Most granulated podocytes were peripherally situated in the glomerulus (Figure 47). There were no significant differences between any of the groups, and ECDI did not correlate with PPI; however the 2 kidneys which contained peripolar cells also contained ECDs. There was no correlation between ECDI and blood pressure.

(iii) Renin-containing cells

There was hyperplasia of renin-containing cells (RCCs) in the clipped kidney with proximal extension in the afferent arteriole (Figures 48 and 49). All of the renin indices were elevated (Table 10). There was a reduction in numbers of RCCs in the unclipped kidney. All of the renin indices were significantly higher in

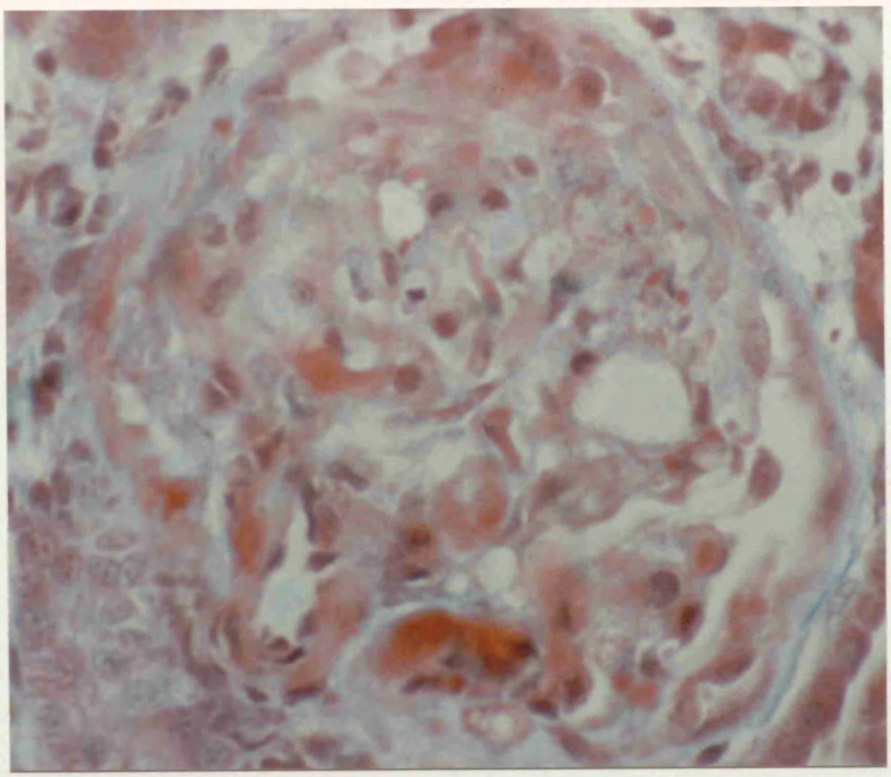


Figure 48. A glomerulus in an unclipped rat kidney, in experimental renovascular hypertension. It shows features of malignant hypertension, such as fibrinoid necrosis, segmental cellular proliferation and cresent formation. MSB stain.

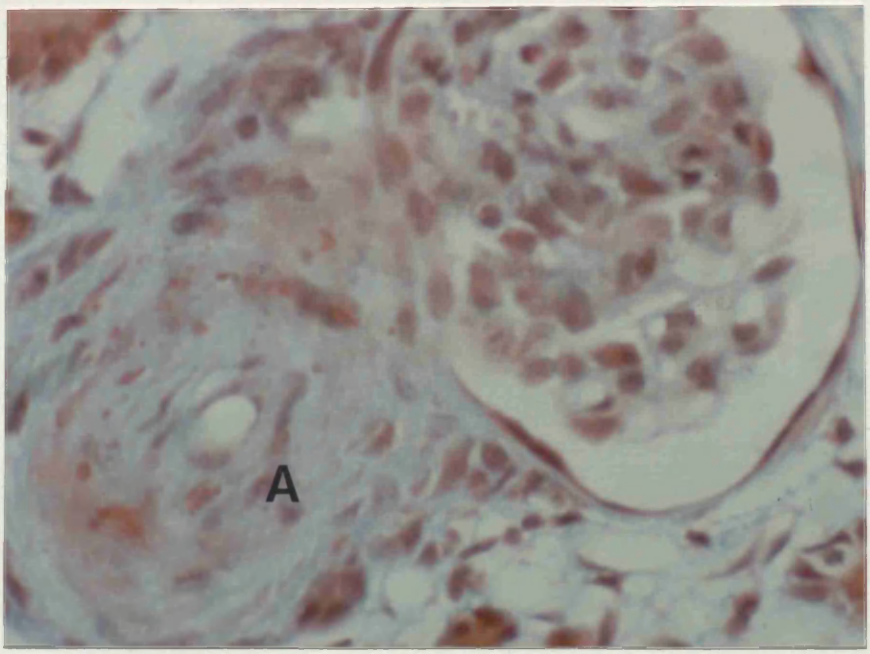


Figure 49. Unclipped rat kidney in experimental renovascular hypertension. There is fibrinoid necrosis and concentric cellular thickening of the afferent arteriole (A). MSB stain.

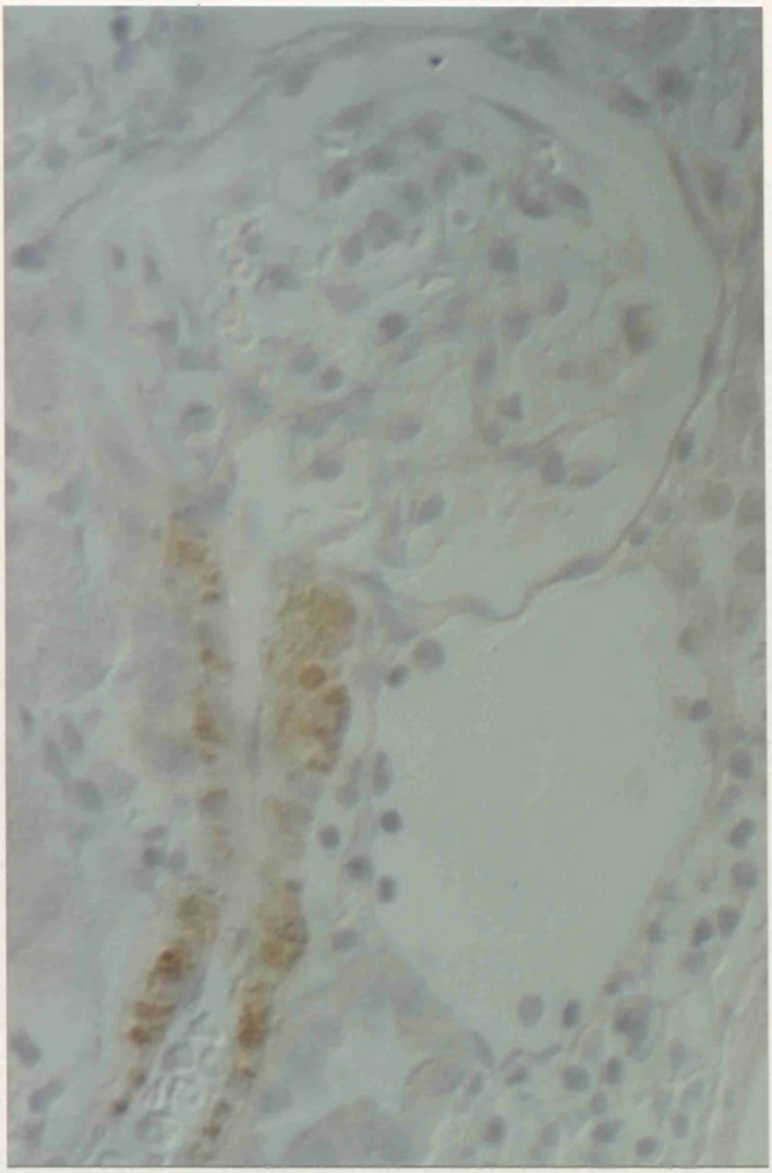


Figure 50. Hyperplasia of the renin-containing cells of the JGA in a clipped rat kidney, in experimental renovascular hypertension. Immunoperoxidase technique, interference contrast illumination.

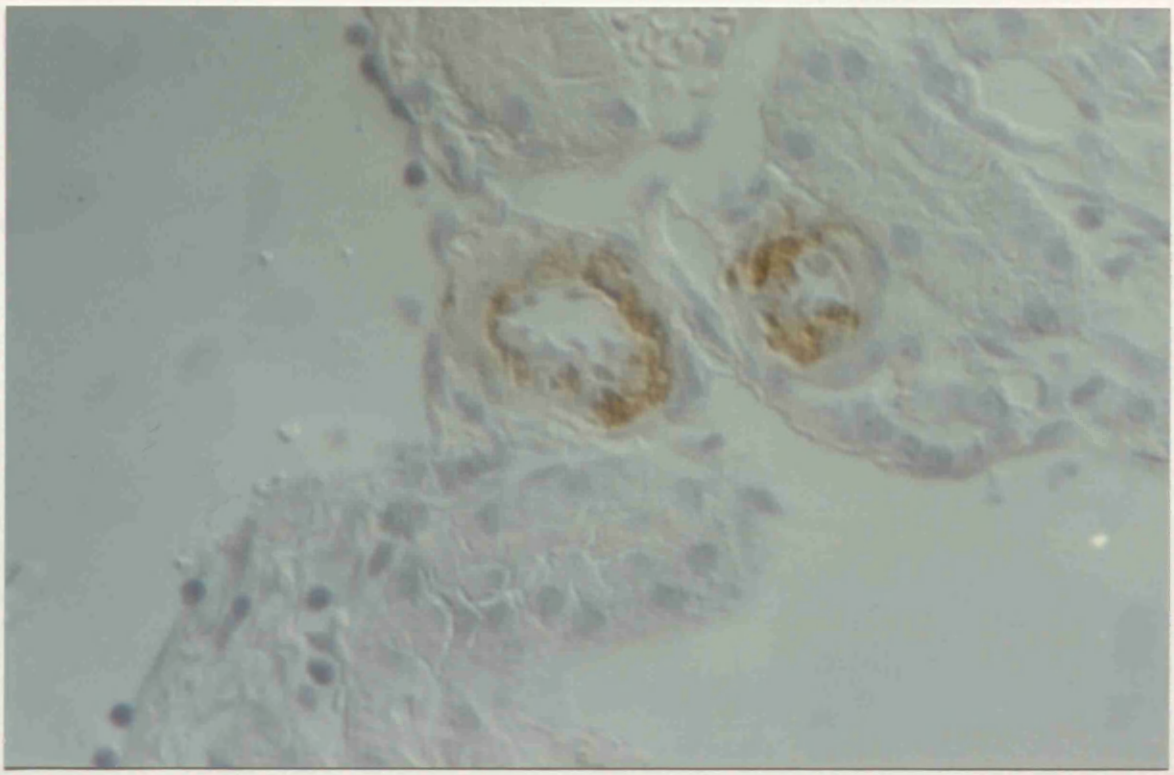


Figure 51. Extension of renin-containing cells into proximal afferent arterioles in the clipped kidney of experimental renovascular hypertension in the rat. Immunoperoxidase technique, interference contrast illumination.

the clipped kidneys compared with the unclipped kidneys ($p<0.001$). JGA+ and RCI were significantly higher in the clipped kidneys compared with the controls ($p<0.02$) and significantly lower in the unclipped kidneys compared with the controls ($p<0.02$). None of the renin indices correlated with PPI or ECDI.

(iv) Assessment of hypertensive damage

The sham-operated kidneys were histologically normal. The clipped kidneys showed glomerular crowding with mild tubular atrophy and interstitial fibrosis. Blood vessels appeared normal. Four of the five unclipped kidneys showed typical histological features of malignant hypertension (Figures 50 and 51). In this group, the percentage of abnormal glomeruli varied from 0 to 13.1% and the percentage of abnormal arteries/arterioles varied from 0 to 10.3%. There was a significant correlation between the percentage of abnormal glomeruli and the percentage of abnormal arteries/arterioles ($p<0.02$), but no correlation between either of these and PPI, ECDI or blood pressure.

(7) PATHOLOGICAL HUMAN KIDNEYS

(a) Addison's disease

(i) Peripolar cells

In untreated Addison's disease, peripolar cells were present in 2 of the 6 kidneys (Figure 52). The PPIs for these kidneys were 4.5 and 13.6. This results in a mean PPI of 3.0 (Table 11). The mean PPI of the control group was 0.72. There was no significant difference in PPI between the Addison's disease patients and their age/sex-matched controls.

In treated Addison's disease, peripolar cells were present in 2 of the 5 kidneys (PPI = 10.0 for both). The mean PPI was 4.0 (Table 11). The mean PPI of the control group was 1.9. There was no significant difference between the treated Addison's disease patients and their controls, nor between the treated and untreated Addison's patients.

(ii) Renin-containing cells

In the control group, RCCs were identified in the afferent glomerular arterioles and rarely in the efferent arterioles (Figure 53). RCCs were occasionaly

Table 11. Mean peripolar cell index (PPI) for patients with Addison's disease and age/sex matched controls. There is no significant difference between each group and its control group. See text for definition of PPI.

	Untreated Addison's n=6		**Treated Addison's n=5**	
	cases	controls	cases	controls
PPI (±SEM)	3.0 (±2.2)	0.72 (±0.72)	4.0 (±2.4)	1.9 (±1.2)

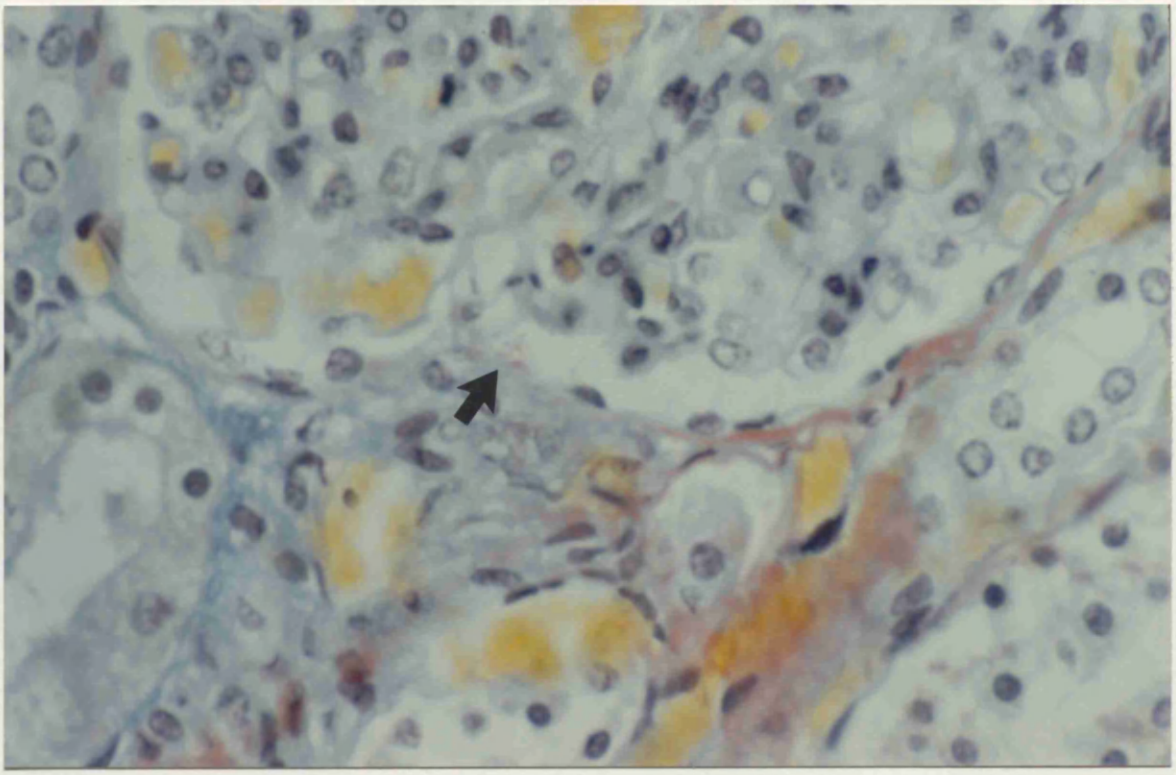


Figure 52. A peripolar cell (arrow) with its red-staining granules, in a case of Addison's disease.

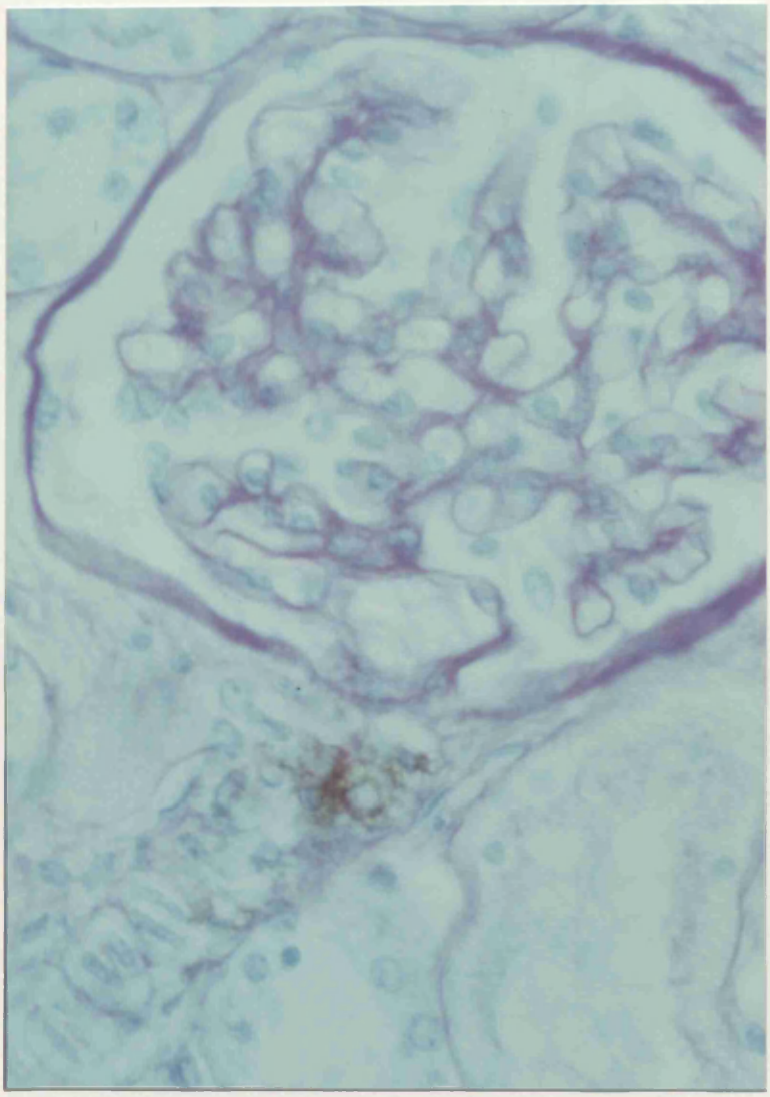


Figure 53. Immunoperoxidase-stained section of normal human kidney using an anti-renin antiserum. Several renin-containing cells are present in the afferent arteriole. PAS counterstain.

Table 12. Mean renin indices for untreated Addison's disease patients and their controls (see text for definitions of indices).

RENIN INDEX	UNTREATED ADDISON'S DISEASE n=6	CONTROLS n=6	
JGA+	30.2 (±4.2)	8.8 (±2.5)	p<0.002
JGA++	38.5 (±9.0)	9.5 (±2.2)	p<0.001
A+	5.8 (±1.5)	1.9 (±0.4)	p<0.013
RCI	160.4 (±24.8)	32.4 (±8.4)	p<0.001

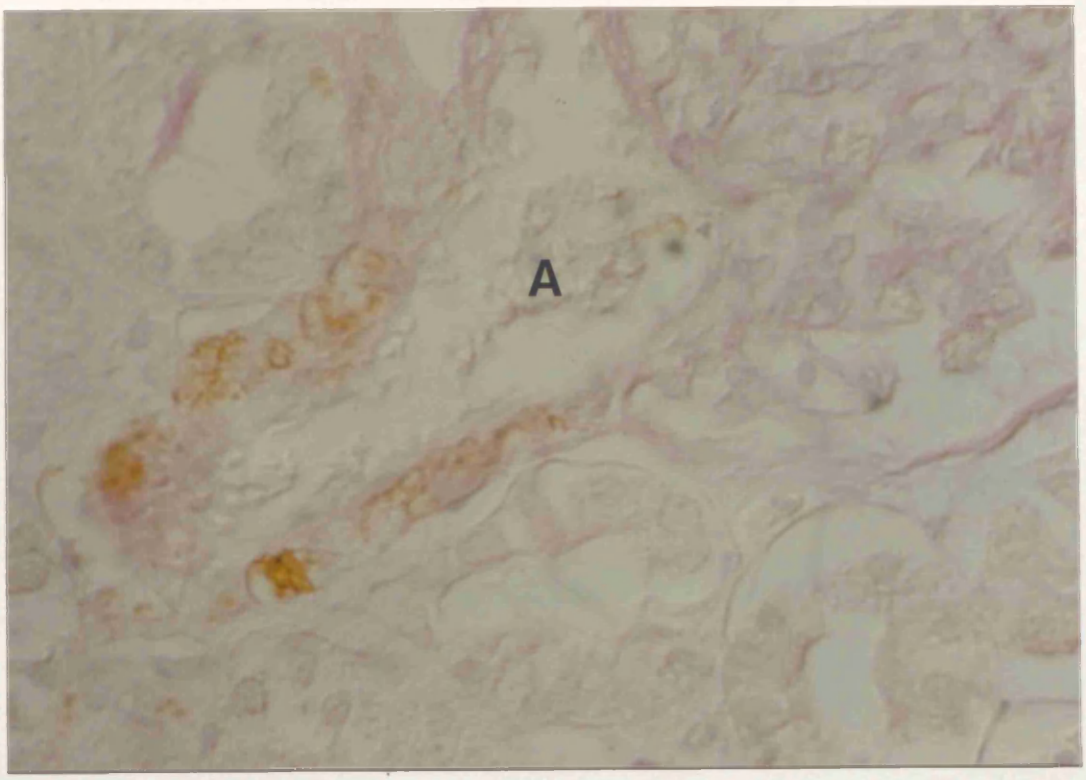


Figure 54. Hyperplasia of juxtaglomerular renin-containing cells in a case of untreated Addison's disease. A = arteriole. Immunoperoxidase technique, PAS counterstain, interference contrast illumination.

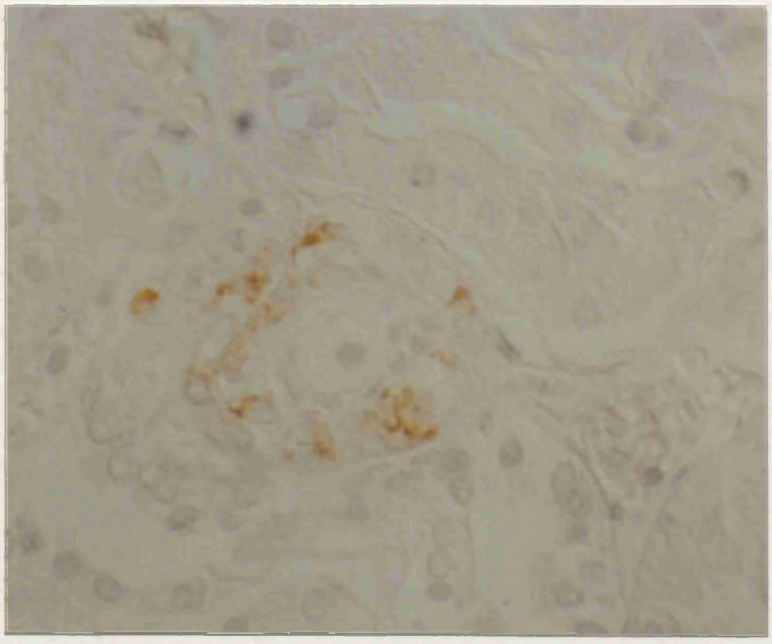


Figure 55. Extension of renin-containing cells into a proximal afferent arteriole, in Addison's disease. Immunoperoxidase technique, PAS counterstain, interference contrast illumination.

Table 13. Mean renin indices for <u>treated</u> Addison's disease patients (see text for definitions of indices). N.S. = not significant.

RENIN INDEX	**ADDISON'S DISEASE n=5**	**CONTROLS n=5**	
JGA+	9.7 (±5.8)	10.0 (±3.3)	N.S.
JGA++	24.8 (±11.0)	5.2 (±2.4)	N.S.
A+	2.5 (±1.3)	2.24 (±1.3)	N.S.
RCI	66.5 (±35.2)	37.9 (±12.1)	N.S.

identified in interlobular arteries. There was no correlation between PPI and any of the renin indices.

In the cases of untreated Addison's disease there was striking hyperplasia of RCCs in the JGA, and also in the walls of proximal afferent arterioles and interlobular arteries (Figures 54 and 55). All of the renin indices were significantly elevated, compared with the controls (Table 12).

Kidneys from patients with treated Addison's disease, who died in Addisonian crisis, contained normal numbers of RCCs. There was no difference in the renin-indices between these patients and age/sex-matched controls (Table 13).

(b) Renal artery stenosis

All of the kidneys showed typical histological features of renal artery stenosis, namely glomerular crowding, tuft contraction, periglomerular fibrosis, interstitial fibrosis, and tubular atrophy. The control group kidneys were histologically normal.

(i) Peripolar cells

Peripolar cells were identified in 6 of the 9 normal kidneys (Table 14, Figure 56). The peripolar

Table 14. Mean peripolar cell index (PPI) and renin indices for renal artery stenosis kidneys and their controls. See text for definitions of indices. N.S. = not significant.

	Renal artery stenosis n=10	**Controls n=9**	
PPI	16.2 (±6.8)	7.6 (±2.4)	N.S.
JGA+	39.2 (±2.4)	11.6 (±1.7)	$p<0.002$
JGA++	43.6 (±7.0)	15.4 (±7.0)	$p<0.02$
A+	5.4 (±1.6)	1.6 (±0.5)	N.S.
RCI	170.0 (±28.0)	32.9 (±6.4)	$p<0.002$

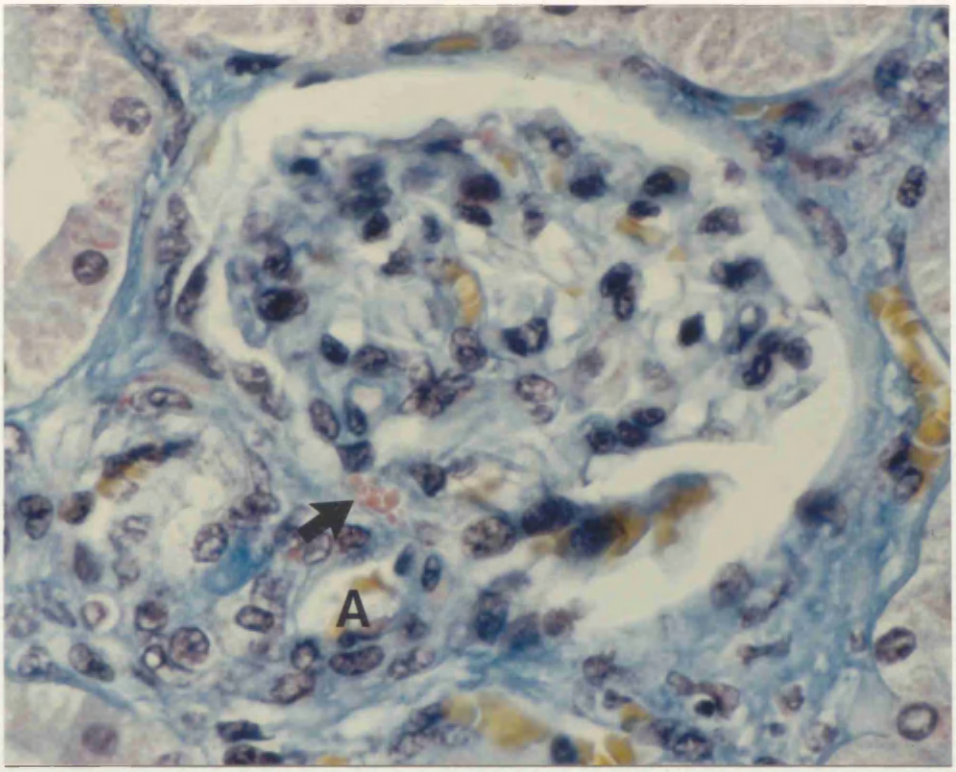


Figure 56. A peripolar cell (arrow) in a shrunken glomerulus in renal artery stenosis. A = arteriole. MSB stain.

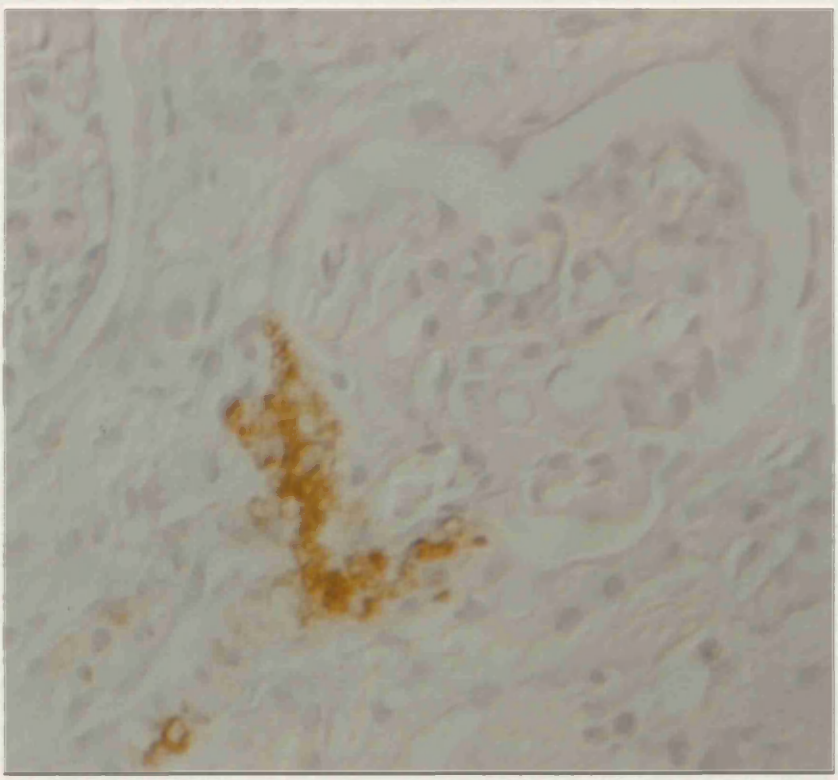


Figure 57. Hyperplasia of renin-containing cells in the JGA in renal artery stenosis. Immunoperoxidase technique, PAS counterstain, interference contrast illumination.

cell index (PPI) varied between 0 and 21 (mean = 7.6). Peripolar cells were present in 7 of the 10 cases of renal artery stenosis. The PPI ranged from 0 to 64 (mean = 16.2). No other glomerular epithelial cells were granulated. There was no significant difference in PPI between the two groups, nor were there any differences in peripolar cell morphology or degree of granulation.

(ii) Renin-containing cells

In the control group, renin-containing cells predominated in the afferent glomerular arterioles but were also present in the efferent arterioles. Occasional renin-containing cells were identified in interlobular arteries. There was no correlation between PPI and any of the renin indices.

In the renal artery stenosis kidneys, there was obvious hyperplasia of the renin-containing cells with frequent extension into the proximal afferent arterioles and interlobular arteries (Figure 57). The renin indices JGA+, JGA++ and RCI were markedly elevated in comparison with the normal kidneys ($p<0.002$, $p<0.02$, $p<0.002$, respectively) (Table 14). The arterial index (A+) was also elevated but this did not achieve statistical significance. There was no correlation between PPI and the renin indices.

(iii) Clinical data

PPI correlated with diastolic blood pressure for normal surgically removed kidneys ($p<0.05$) but not for the cases of renal artery stenosis. Analysis of other clinical parameters and drug therapy was negative. There was no relationship between the renin indices and the clinical data, including plasma renin activity and blood pressure.

(c) Malignant hypertension

Histological features of malignant phase hypertension were present in all of the kidneys. In some, there were acute features such as fibrinoid necrosis of the afferent arterioles sometimes extending into the glomerular tuft, where there was occasional crescent formation (Figure 58). In others there were also features which suggested a chronic phase, such as glomerulosclerosis and concentric fibrocellular thickening of interlobular arteries (Figure 59).

(i) Peripolar cells

Peripolar cells were identified in 7 of the 12 cases (Table 15, Figure 60). The mean PPI was 6.6 (range = 0 - 45). As a group, there were significantly more peripolar cells in these cases of

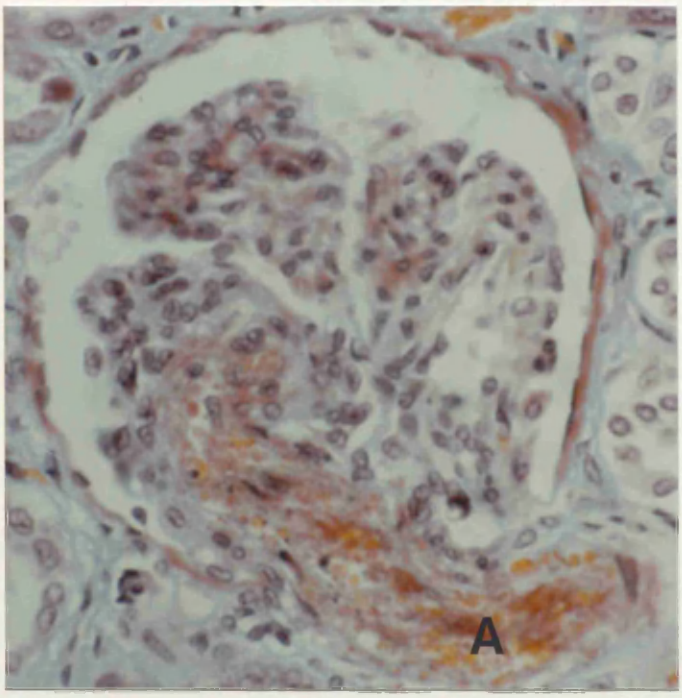


Figure 58. Human malignant hypertension. There is fibrinoid necrosis of the afferent arteriole (A), extending into the glomerular tuft.

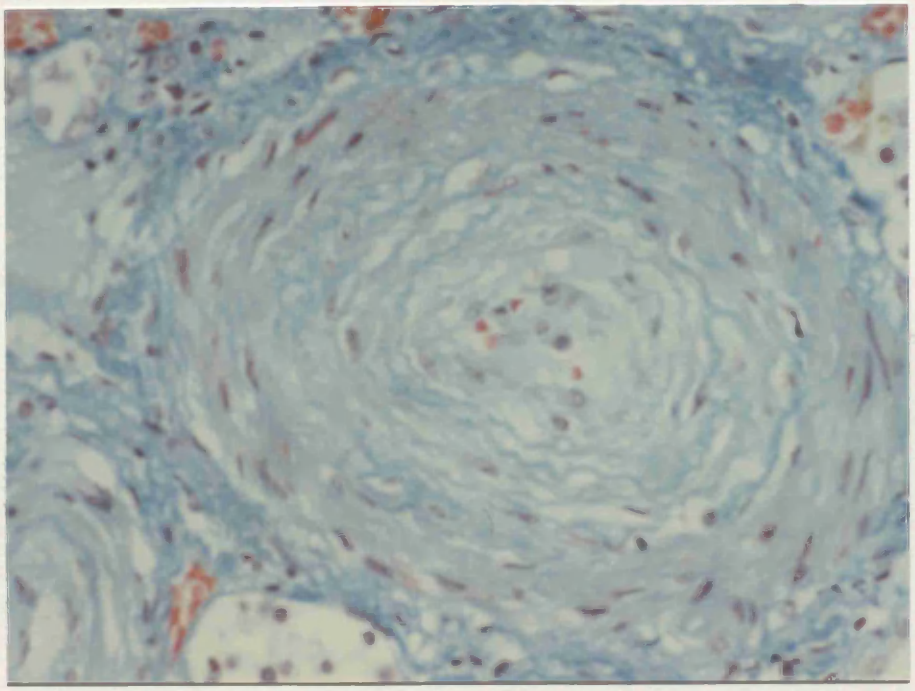


Figure 59. "Onion-skinning" of an interlobular artery in malignant hypertension. MSB stain.

Table 15. Mean Peripolar cell index (PPI), Epithelial cell droplet index (ECDI) and renin indices for patients with malignant hypertension and their controls. See text for definitions of indices. N.S. = not significant, N/A = not applicable.

	Malignant hypertension n=12	**Controls n=9**	
PPI	6.6 (±3.6)	1.25 (±0.65)	p<0.02
ECDI	43.2 (±18.6)	0	N/A
JGA+	14.8 (±4.9)	9.4 (±1.9)	N.S.
JGA++	23.9 (±6.0)	7.3 (±1.7)	p<0.02
A+	3.49 (±0.83)	2.1 (±0.6)	N.S.
RCI	69.3 (±16.9)	35.1 (±6.8)	p<0.05

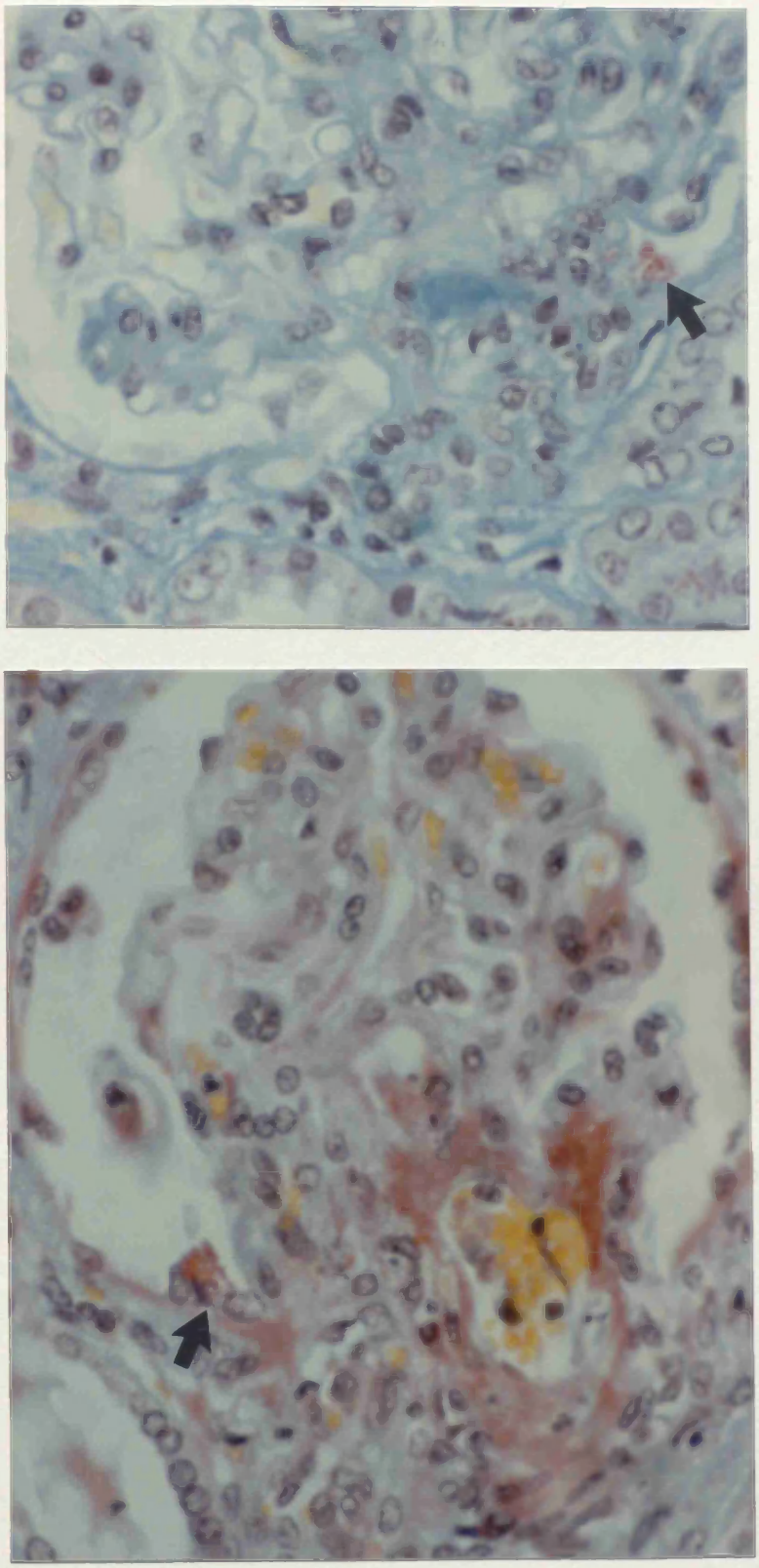


Figure 60. Peripolar cells in malignant hypertension (arrows). In the upper figure the glomerulus is normal, but in the lower figure there is fibrinoid necrosis of the afferent arteriole and glomerular tuft. Both sections are stained with MSB trichrome.

malignant hypertension, when compared with the control group of autopsy kidneys ($p<0.02$). Peripolar cells were identified in both normal and abnormal glomeruli.

(ii) Other granulated glomerular epithelial cells

Granulated glomerular epithelial cells, other than peripolar cells, were identified in 8 of the 12 cases. (Table 15, Figures 61 and 62). The mean ECDI was 43.2, range = 0 - 230. In all kidneys, the majority of granulated glomerular epithelial cells were podocytes. Most of the granulated podocytes were situated at the periphery of the glomerular tuft (Figure 63). ECDI correlated with PPI ($p<0.05$). Granulated glomerular epithelial cells were identified in both normal and abnormal glomeruli.

(iii) Renin-containing cells

There was hyperplasia of RCCs in some of the kidneys (Table 15, Figures 64 and 65). They were often identified within the JGAs of normal glomeruli, but rarely when the glomerular arteriole was necrotic. In some kidneys RCCs were especially prominent in the proximal afferent arterioles and interlobular arteries, even if RCCs in JGAs were relatively sparse. As a group, there was significant elevation of JGA++ ($p<0.02$) and RCI ($p<0.05$) but not JGA+ or A+, when

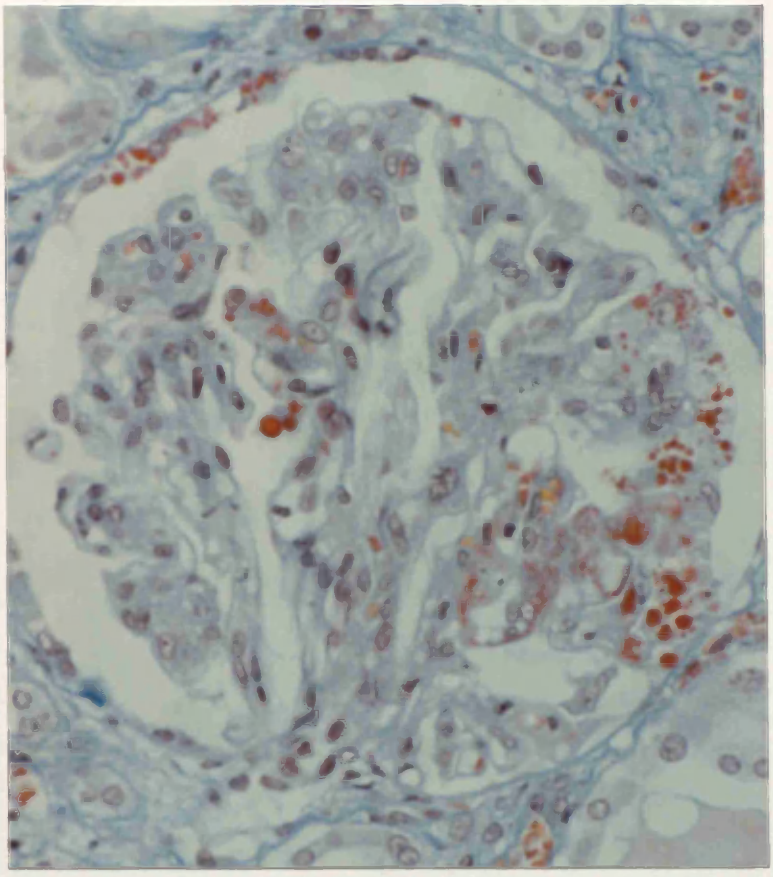


Figure 61. Granulation of visceral and parietal glomerular epithelial cells in malignant hypertension. No peripolar cells are present. There is a small focus of fibrinoid necrosis in the glomerular tuft. MSB stain.

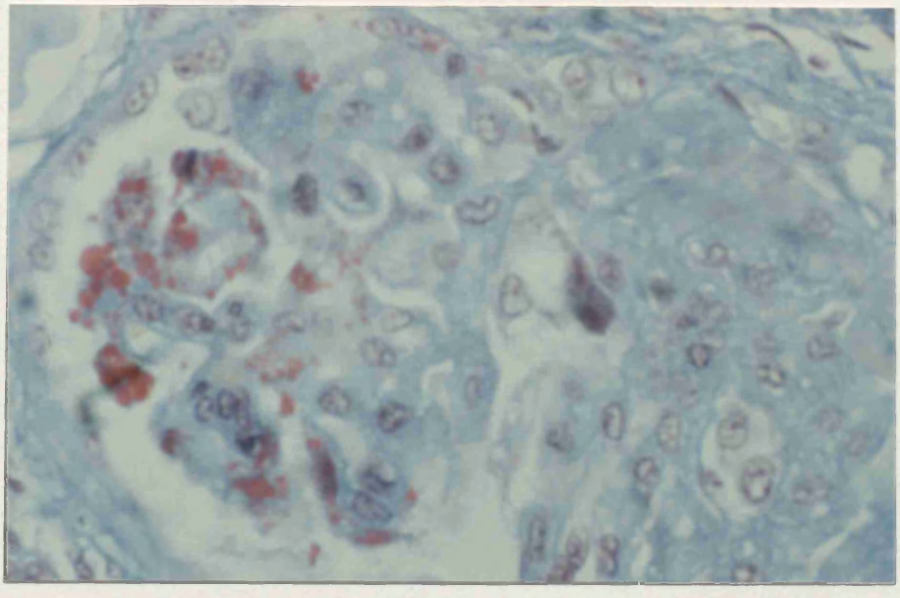


Figure 62. Granulated podocytes in malignant hypertension. A segment of the glomerulus is partially hyalinised. MSB stain.

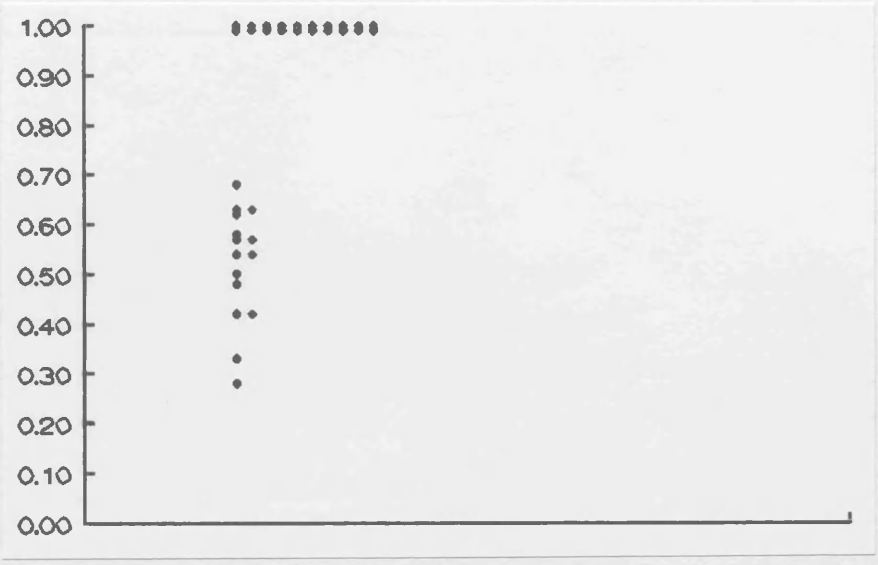


Figure 63. Distribution of granulated podocytes in the glomerulus in malignant hypertension. 1 represents the peripheral edge of the glomerulus and 0 represents the vascular pole. Most granulated podocytes are peripherally situated. See text for methods of measurements.

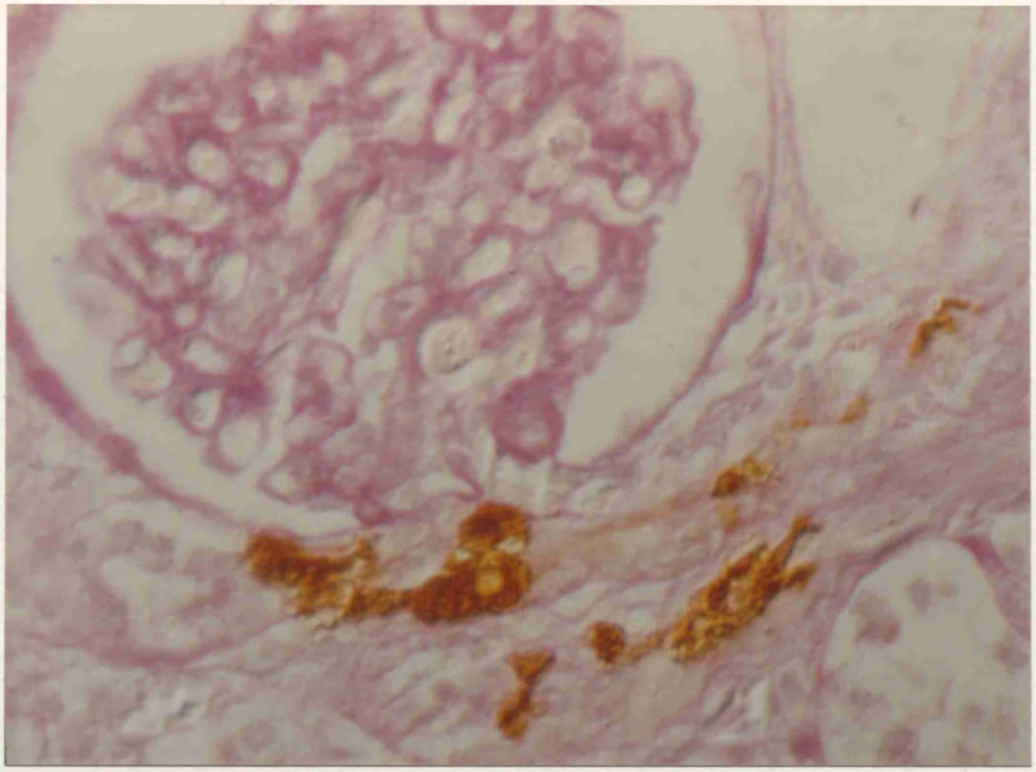


Figure 64. Hyperplasia of juxtaglomerular renin-containing cells in a case of malignant hypertension. Immunoperoxidase technique, PAS counterstain, interference contrast illumination.

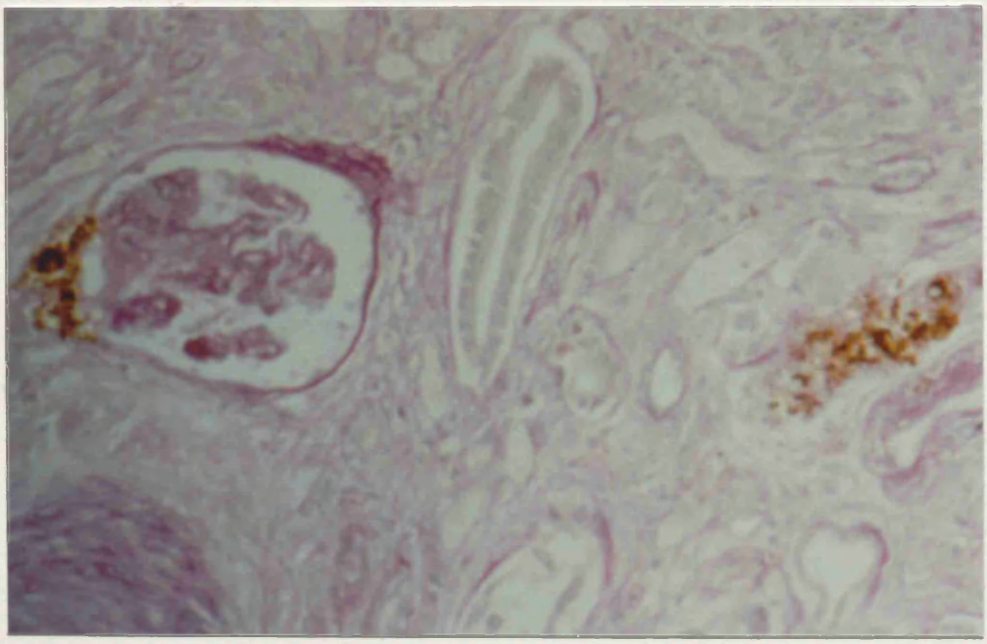


Figure 65. A hyperplastic JGA (left) and renin-containing cells distant from the glomerulus (right) in a case of malignant hypertension. Immunoperoxidase technique, PAS counterstain, interference contrast illumination.

Table 16. Assessment of hypertensive damage in the kidneys of each patient with malignant hypertension. See text for methods of calculation.

Patient	% abnormal glomeruli	% abnormal blood vessels	acute:chronic ratio
1	25.8	40.0	0.18
2	24.7	58.9	1.25
3	25.7	51.4	3.13
4	20.2	21.4	1.50
5	6.1	40.4	0.60
6	11.0	30.2	0.55
7	16.9	35.6	1.94
8	4.5	47.5	1.00
9	13.1	48.9	0.78
10	9.6	45.5	0.38
11	23.3	36.4	0.36
12	15.9	38.5	0.60

compared with the control group (Table 15). There was no significant correlation between PPI or ECDI and any of the renin indices.

(iv) Assessment of hypertensive damage

The results of these assessments are illustrated in Table 16. Peripolar cells and other granulated glomerular epithelial cells were found in both normal and abnormal glomeruli. There was correlation between the percentage of damaged arteries/arterioles and the highest recorded diastolic blood pressure ($p<0.05$), but not the highest recorded systolic blood pressure. There was no correlation between the indices of hypertensive damage and PPI, ECDI or any of the renin indices.

(d) Renal biopsies

(i) Peripolar cells

Peripolar cells were identified in 10 of the 19 diseases (Table 17). The PPI(r) ranged from 0 to 3.9. High values were obtained for mesangioproliferative GN, IgA nephropathy, focal segmental glomerulosclerosis, membranous GN and lupus nephropathy (diffuse and membranous) (Figures 66 and 67). There was a significantly uneven distribution of peripolar cells between the groups ($H = 121.7$, $p<0.001$).

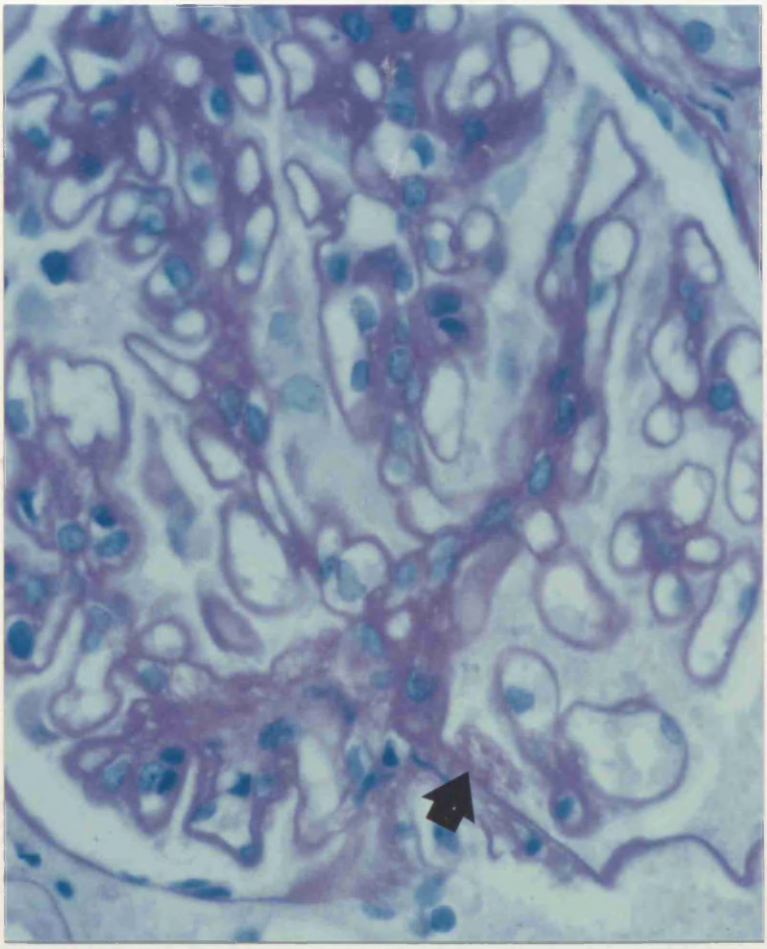


Figure 66. A peripolar cell (arrow) in membranous glomerulonephritis. No other glomerular cells are granulated. PAS stain.

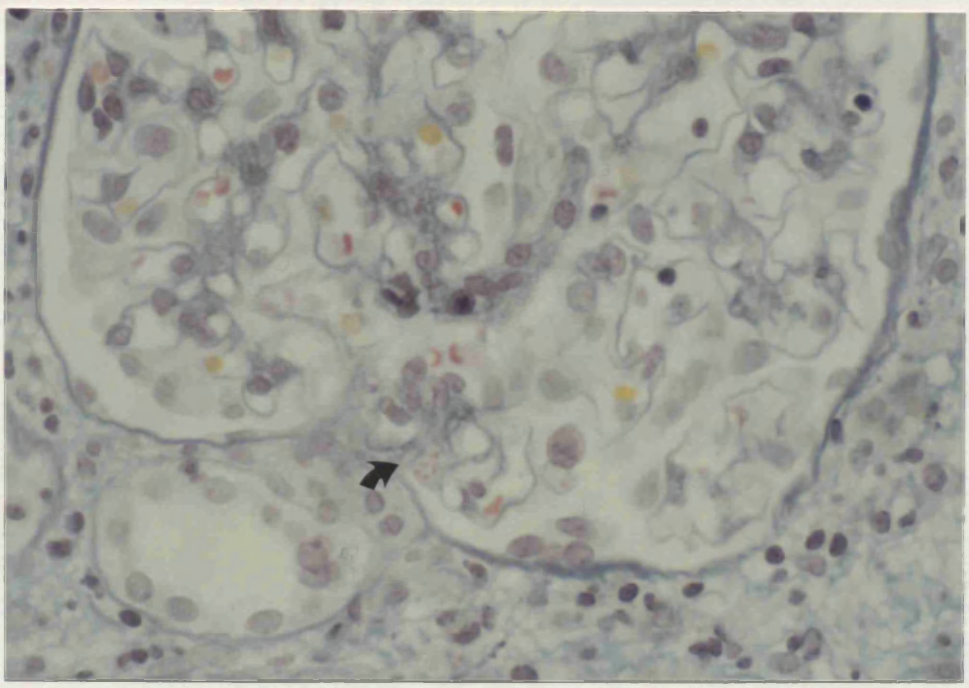


Figure 67. A peripolar cell (arrow) in mesangioproliferative glomerulonephritis. MSB stain.

(ii) Other granulated glomerular epithelial cells

Granulated glomerular epithelial cells, excluding peripolar cells were identified in 15 of the 19 diseases (Table 17). The ECDI(r) ranged from 0 to 24.8. High values were obtained for diffuse lupus nephropathy, focal GN, acute vascular transplant rejection, cresentic GN and mesangioproliferative GN (Figures 68,69,70,71). There was a significantly uneven distribution of these cells between the groups ($H = 32.4$, $p<0.01$). In focal GN, granulated epithelial cells were often related to areas of tuft damage (Figure 69), and in cresentic GN they were often present within crescents (Figure 71).

Distribution of granulated epithelial cells in the glomerulus

Most of the granulated glomerular epithelial cells were podocytes; a minority were parietal cells. Most granulated podocytes were found at the periphery of the glomerular tuft (Figure 72). In many cases, the vascular pole was not in the plane of section, making formal measurement impossible. However, this implies that most granulated podocytes are peripherally situated.

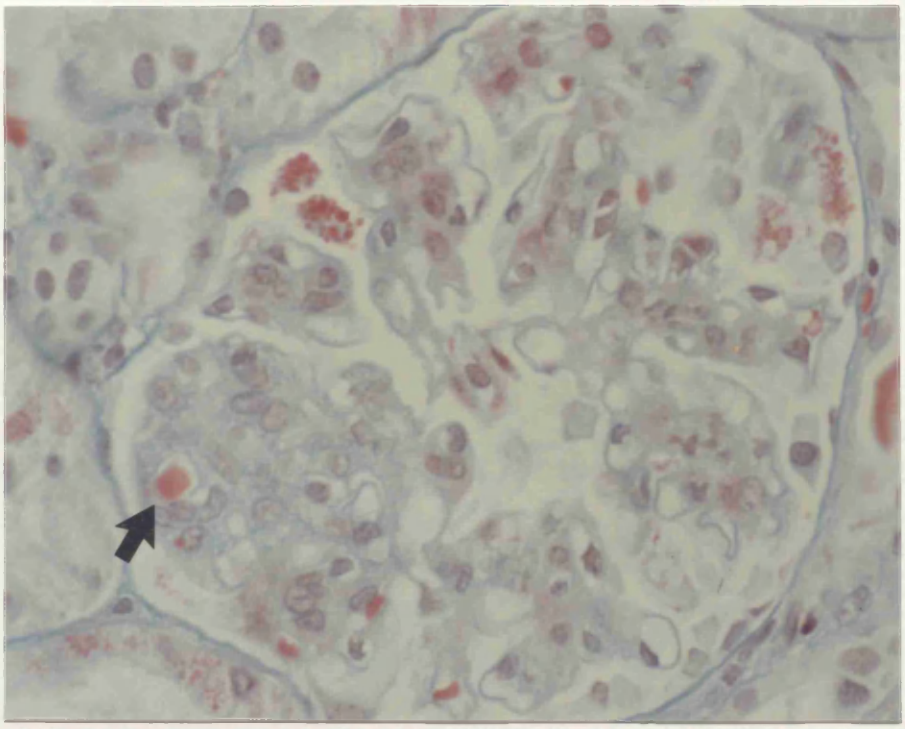


Figure 68. Diffuse lupus nephropathy. Several podocytes are granulated. The vascular pole is not in the plane of section. Note the hyaline thrombus (arrow), and segmentally-accentuated mesangial hypercellularity. MSB stain.

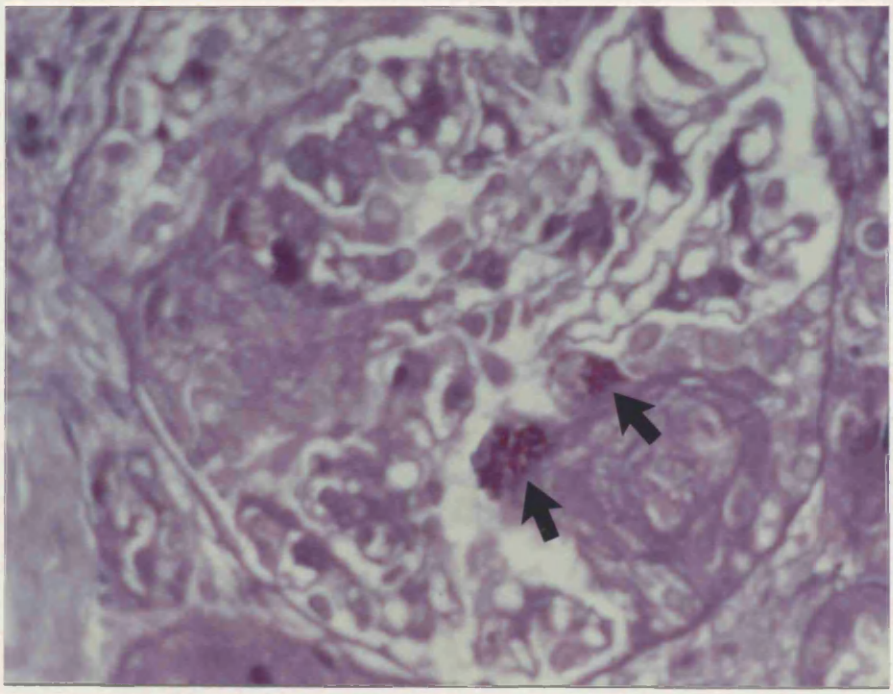


Figure 69. Focal glomerulonephritis. Two granulated podocytes (arrows), are related to an area of tuft damage. Bowie's stain.

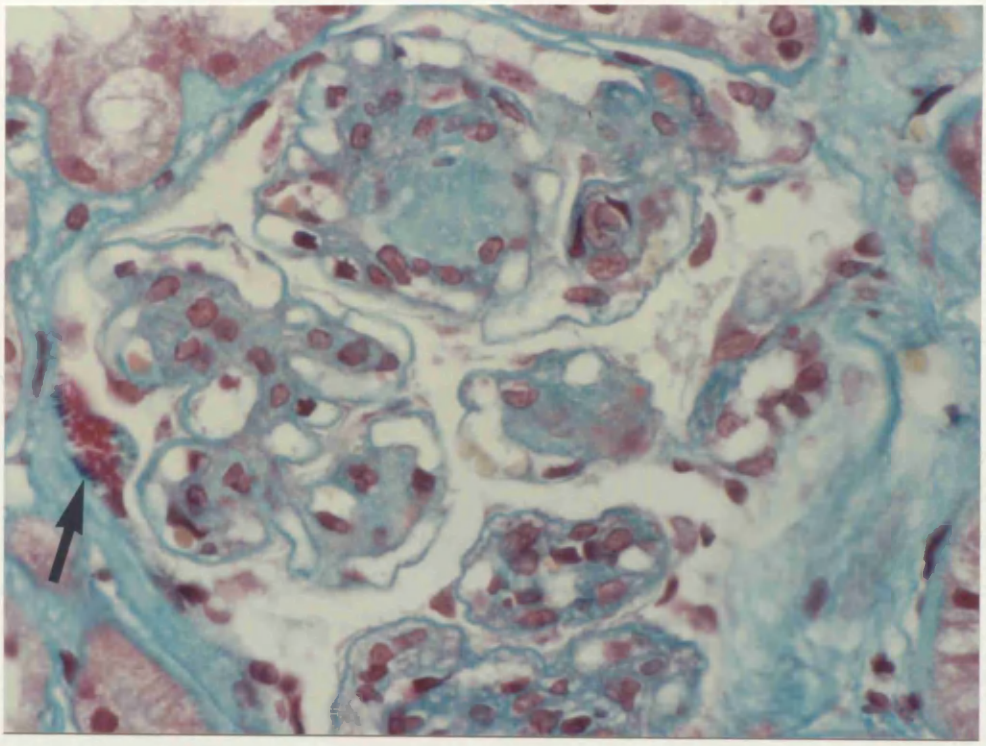


Figure 70. Diabetic glomerulosclerosis. A parietal epithelial cell is granulated (arrow). Masson's trichrome.

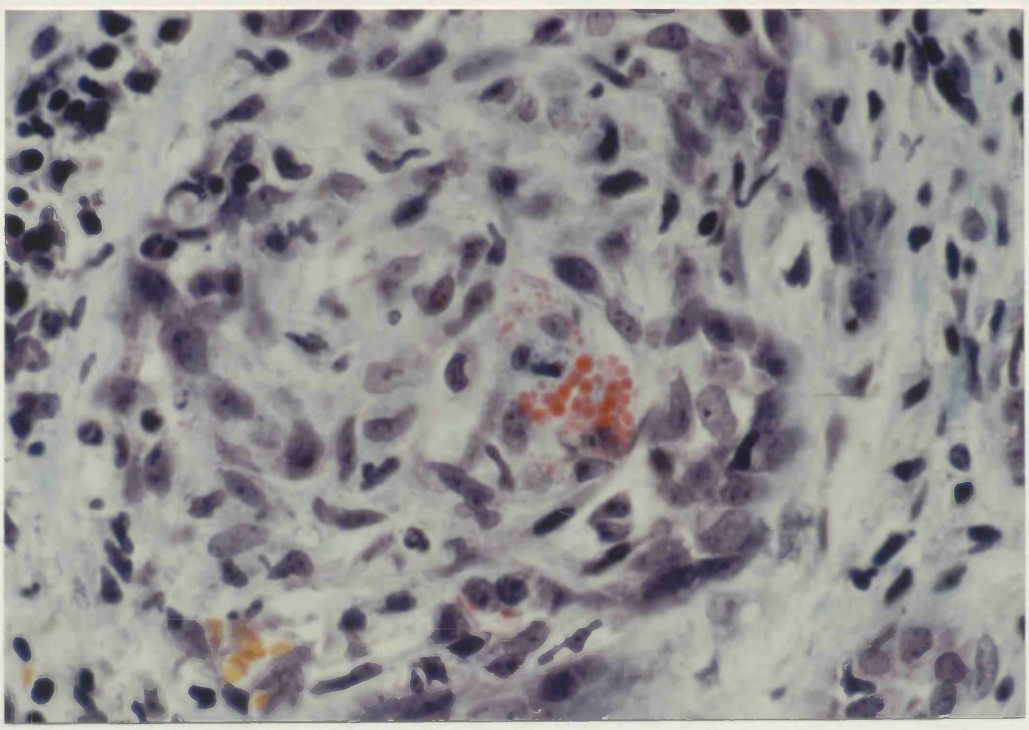


Figure 71. Cresentic glomerulonephritis. Granules are present in at least two of the cells, which compose the cresent. MSB trichrome.

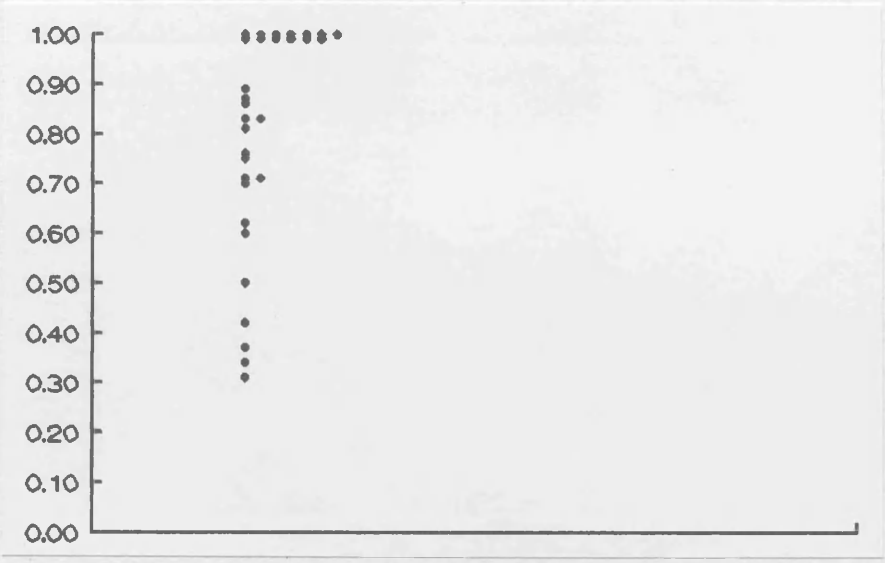


Figure 72. Distribution of granulated podocytes in the glomerulus. 1 represents the peripheral edge of the glomerular tuft, and 0 represents the vascular pole. Most of the cells are situated peripherally. See text for methods of measurement.

(iii) Granulated tubular epithelial cells

Granulated tubular epithelial cells were identified in all but 3 groups (Table 17). The THDI ranged from 1.0 to 3.3, with relatively high values obtained for minimal change GN and amyloidosis (Figure 73). There was a significantly uneven distribution of THDI between the groups ($H = 52.7$, $p<0.001$).

(iv) Comparison of peripolar cells, granulated glomerular epithelial cells and tubular hyaline droplets.

Granules in peripolar cells, other glomerular epithelial cells and tubular epithelial cells, showed similar staining reactions with a variety of stains, namely magenta with PAS, usually red but sometimes blue with MSB trichrome, usually crimson but sometimes green with Masson's trichrome, purple with Bowie's stain and red with phloxine-tartrazine. Peripolar cell granules were generally smaller and more regular in shape than granules in the other cells, although no consistent differences could be identified by light microscopy.

There was a striking difference between numbers of peripolar cells, other granulated glomerular epithelial cells, and tubular hyaline droplets, in the different

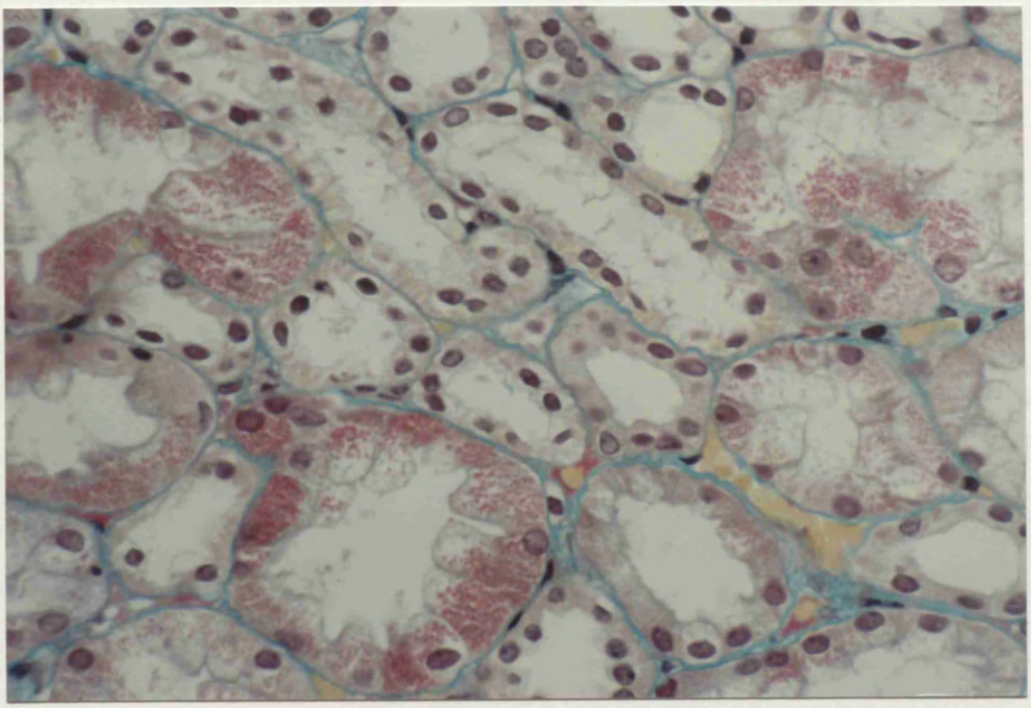


Figure 73. Granululated tubular epithelial cells ("hyaline droplets") in a case of minimal change glomerulonephritis. No glomerular cells were granulated. Masson's trichrome.

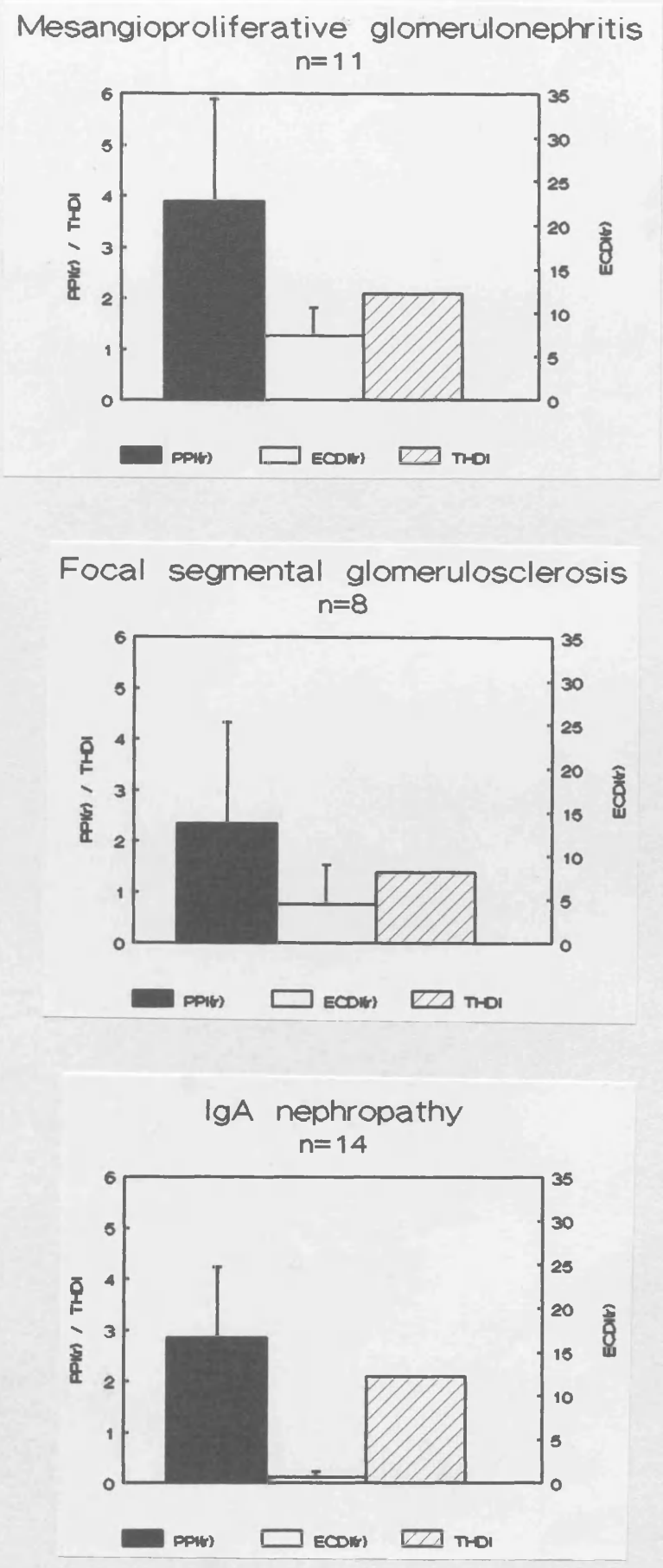


Figure 74. Histograms showing the mean PPI(r), ECDI(r), and THDI for three disease groups.

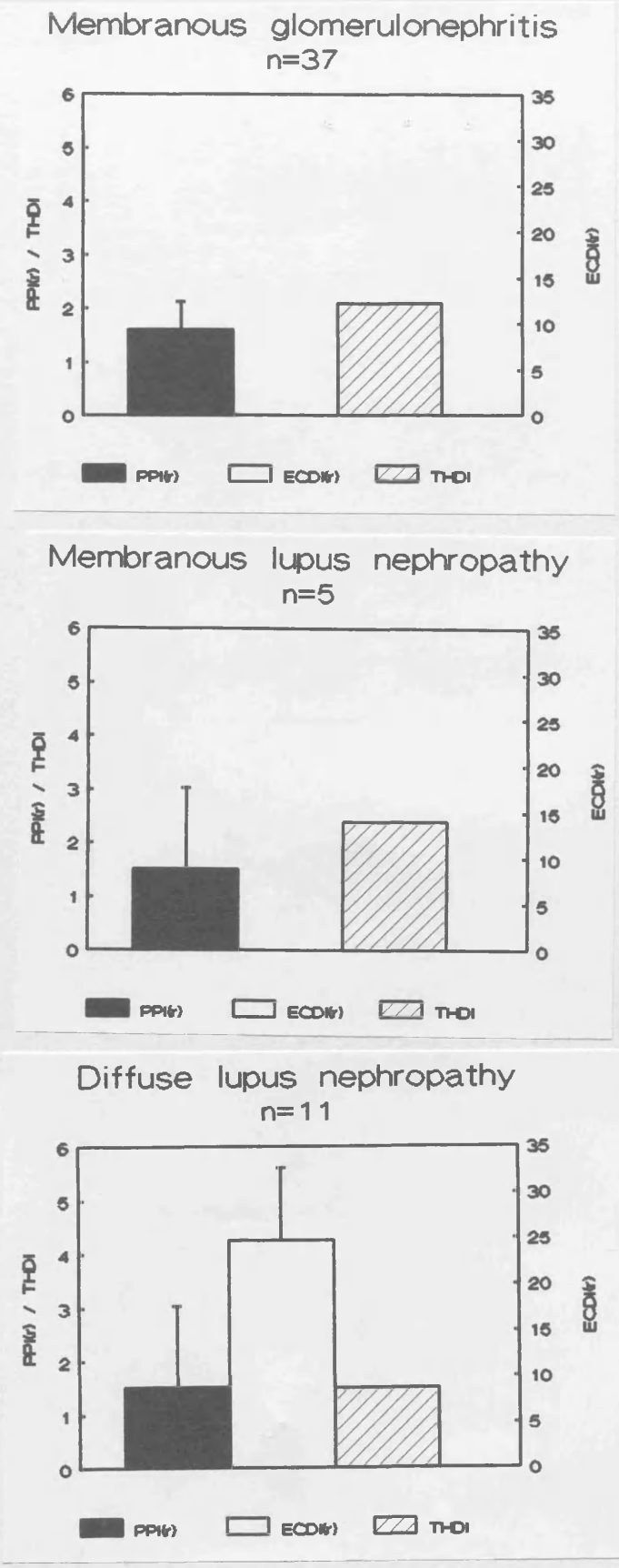
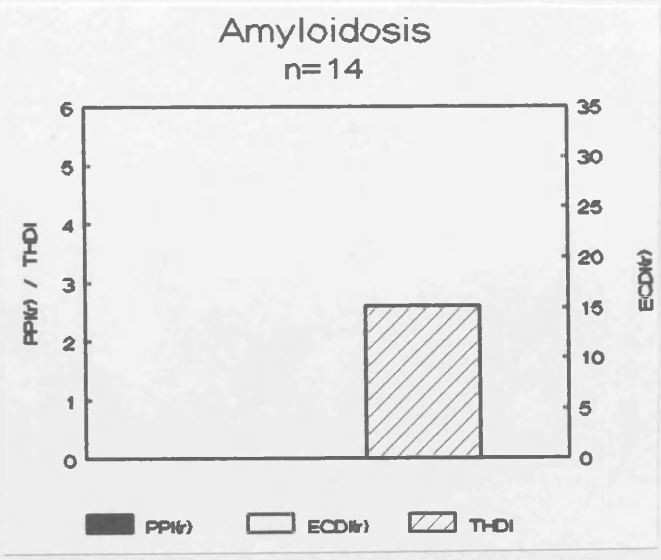


Figure 75. Histograms showing the mean PPI(r), ECDI(r), and THDI for three disease groups. Note the similarity between membranous glomerulonephritis and the membranous form of lupus nephropathy. The diffuse form of lupus nephropathy shows a completely different pattern.



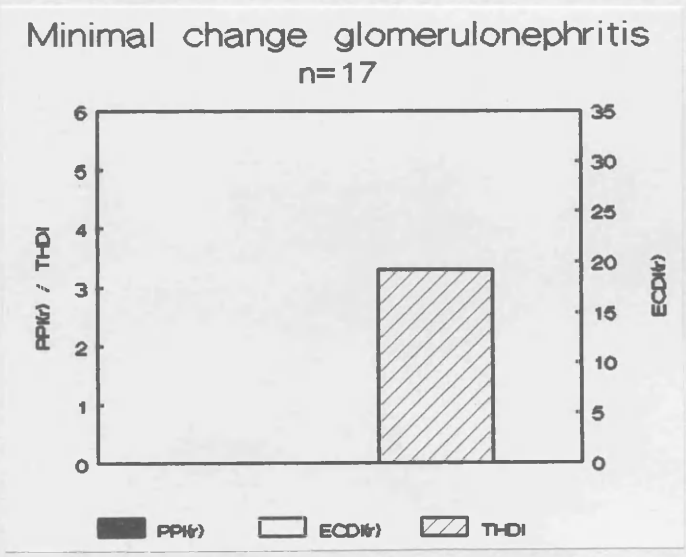


Figure 76. Histograms showing the mean PPI(r), ECDI(r) and THDI for two of the disease groups. In both, there is granulation of the tubular epithelium, but no granulation of glomerular epithelial cells, including peripolar cells.

groups (Figures 74,75,76). In some diseases, notably mesangioproliferative GN and focal segmental glomerulosclerosis, both peripolar cells and other granulated glomerular cells were prominent. However in other diseases, especially membranous GN and IgA nephropathy peripolar cells were prominent, with almost no granulation of other glomerular epithelial cells. By contrast, prominent granulation of glomerular epithelial cells, with few peripolar cells was observed in diffuse lupus nephropathy and focal GN. There was a similarity between membranous GN and membranous lupus nephropathy: in both diseases there was prominent peripolar cells with inconspicuous granulation of other glomerular epithelial cells, however there was a marked difference between these diseases and diffuse lupus nephropathy (prominent granulation of glomerular epithelial cells). Finally, tubular hyaline droplets were most prominent in mimimal change GN and amyloidosis. In both of these diseases there was no granulation of the glomerular epithelium.

(v) Clinical data

The correlation between each index and a variety of clinical variables was calculated and the results are shown in Table 18. There was a positive correlation between urinary protein and PPI(r), but neither of the other indices. PPI(r) and THDI showed a negative

Table 18. Correlations between the Peripolar Cell Index [random] (PPI(r)), Epithelial Cell Droplet Cell Index [random] (ECDI(r)), and Tubular Hyaline Droplet Index (THDI) and clinical variables from each patient. Spearman's rank correlation was used and the rho values are given where the correlation is statistically significant. N.S. = non-significant

	PPI(r)	ECDI(r)	THDI
serum sodium	0.101 p=0.048	-0.181 p=0.017	N.S.
serum potassium	N.S.	N.S.	N.S.
serum chloride	N.S.	N.S.	N.S.
serum bicarbonate	N.S.	N.S.	N.S.
serum calcium	N.S.	N.S.	N.S.
serum urea	N.S.	N.S.	N.S.
serum creatinine	-0.111 p=0.03	N.S.	-0.208 p=0.026
urinary protein	0.129 p=0.012	N.S.	N.S.
creatinine clearance	0.139 p=0.006	N.S.	N.S.
haemoglobin	0.138 p=0.007	N.S.	N.S.
systolic blood pressure	N.S.	N.S.	N.S.
diastolic blood pressure	N.S.	0.221 p=0.047	N.S.

correlation with serum creatinine, and PPI(r) correlated positively with creatinine clearance. Serum sodium correlated positively with PPI(r), but negatively with ECDI(r). ECDI correlated positively with diastolic blood pressure. There was a strong positive correlation between PPI(r) and haemoglobin concentration.

DISCUSSION

(1) MORPHOLOGY OF THE PERIPOLAR CELL

(a) The human peripolar cell

(i) Light microscopy

This is the first morphological description of the human peripolar cell. In common with other species, peripolar cells were situated at the vascular pole of the glomerulus within the reflection of Bowman's capsule (1,2,26,28). Usually only one peripolar cell was found at each vascular pole although on occasion, up to two were identified. The granules showed a considerable range in size: most were small although some granules measured at least 1 μm in diameter. There was no polarity towards any aspect of the cell surface. Some heterogeneity of staining was observed, for example most of the granules stained red with MSB-trichrome, but occasional granules stained blue. Otherwise the staining reactions were similar to those of renin-containing cells. All of these tinctorial stains are relatively non-specific, but it is likely that the peripolar cell granules contain protein; the PAS-positivity suggests the presence of glycoprotein.

By examination of serial sections, I found peripolar cells in 12.4% of glomeruli (range = 3-

27.8%). Generally peripolar cells were sparse in the human kidney and would rarely be found by examining a random histological section. This has subsequently been confirmed by other workers (26).

(ii) Transmission electron microscopy

The ultrastructure of the peripolar cell has been described in several mammals especially sheep (4,30). Amphibians (2), fish (29), and birds (28) have also been studied. This is the first ultrastructural description of the human peripolar cell.

The most striking ultrastructural feature of the peripolar cell was the cytoplasmic granules. The granules measured 1050 nm on average, which is similar to the size of sheep peripolar cells, although the range is larger. The granules were composed of homogeneous material, however some appeared to be undergoing dissolution. This has been described in association with granule exocytosis in sheep ( ) and axolotls ( ). It also accords with the heterogeneity of staining on light microscopy. However no instances of exocytosis were identified; this is perhaps not surprising, since exocytosis is difficult to identify with the electron microscope, even in the most active secretory cells.

The human peripolar cell contained the organelles associated with protein synthesis and secretion, namely rough endoplasmic reticulum and Golgi apparatus, but these were relatively sparse in comparison with adjacent visceral epithelial cells. These cells secrete a variety of proteins *in vitro* such as heparan (74), fibronectin (75), and type IV collagen (76). Microtubules were not identified, in contrast to observations in sheep (4) and axolotl (2) peripolar cells. There was very marked pinocytotic activity at the basal surface of the human peripolar cell, which is not an ultrastructural feature of other species. Another new finding was complex membrane invaginations. The significance of these is uncertain, although similar invaginations have been reported as a feature of exocytosis in juxtaglomerular renin-secreting cells (77). Surface microvilli and cilia have been in sheep peripolar cells (4), but I observed none in human peripolar cells.

(b) <u>The sheep peripolar cell</u>

In contrast to human kidneys, peripolar cells were easily found in sheep kidneys. Like human peripolar cells, they were situated in the reflection of Bowman's capsule at the vascular pole of the glomerulus. Sheep peripolar cells were generally large and packed with cytoplasmic granules. Their anatomy and staining

reactions were similar to those described previously (1,4,26). Having studied serial sections of sheep kidneys I found that when glomeruli were examined in their entirety, between 20 and 100% contained at least one peripolar cell. Up to six peripolar cells were present at a single vascular pole, the median number being two. Gall *et al* reported that in random histological sections, 12% of glomeruli contain peripolar cells and that up to four peripolar cells may be identified in a single glomerulus (26). In general, peripolar cells were more prominent in older sheep. Peripolar cells are prominent in newborn sheep (32), but there has been no study of the effect of age on numbers of peripolar cells in adult sheep. The wide variation in numbers of peripolar cells between different members of the same species remains unexplained.

(c) The rat peripolar cell

The purpose of this study was to identify and study peripolar cells in normal rats, since they are a convenient species in which the response of peripolar cells to various stimuli could be studied. Like human peripolar cells, rat peripolar cells were scanty and difficult to identify. By studying serial sections, I found peripolar cells in only 6% of glomeruli

(range = 0-12.5%), when they were examined in their entirety. Therefore in random histological sections peripolar cells would be rarely identified. This confirms the findings of other workers, who have reported that in random sections less than 1% of rat glomeruli contain peripolar cells (26). By contrast, peripolar cells are prominent in newborn rats, decreasing to adult numbers within 2 days of birth (26). Similar changes occur in peripolar cells in newborn lambs (32).

Recently, Gibson *et al* have studied the vascular pole of the rat glomerulus by scanning electron microscopy (34). They describe a distinctive population of cells which encircle the vascular pole and are distinguishable from podocytes and parietal epithelial cells. It has been suggested that these cells are peripolar cells (34). Such cells were identified in more than 40% of glomeruli, by contrast with the incidence of granulated peripolar cells identified by light microscopy. Whether or not these cells contain granules is not known, however they could represent peripolar cells, which only become granulated in certain situations. This could similarly apply to other species in which granulated peripolar cells are sparse.

(2) THE DISTRIBUTION OF PERIPOLAR CELLS AND RENIN-CONTAINING CELLS IN THE RENAL CORTEX

There are established morphological and functional differences between superficial and juxtamedullary nephrons; for review see (35). The purpose of this section was to study the distribution of peripolar cells in the renal cortex and compare it with that of renin-containing cells.

(a) Peripolar cells

The distribution of peripolar cells in the renal cortex has not been previously studied, although it has been mentioned that they are more prominent in superficial glomeruli in sheep (26). I found peripolar cells to be largely confined to superficial glomeruli and rare in juxtamedullary glomeruli in normal human kidneys. There was a similar distribution in sheep, but this could be explained by the greater number of glomeruli in the superficial cortex. Peripolar cells were too sparse in normal rats to allow conclusions to be drawn.

In renal artery stenosis peripolar cells were mainly located in the superficial renal cortex, except for one kidney, in which there was an even distribution. Interestingly, this kidney had numerous

peripolar cells suggesting that when stimulated, peripolar cells may also be found in juxtamedullary glomeruli. In experimental renovascular hypertension, too few peripolar cells were identified to allow a meaningful plot to be made. In malignant hypertension, peripolar cells were numerous, but were almost always present in superficial glomeruli. There was a similar distribution of glomeruli with granulated epithelial cells, other than peripolar cells.

(b) Renin-containing cells

In normal human kidneys, renin-containing cells (RCCs) were usually located in superficial JGAs and rarely in juxtamedullary JGAs. In rats, there was a similar distribution, but the gradient was not as marked. This has previously been reported using biochemical (36,78,79,80,81,82,83,84), and immunohistochemical techniques (36), in rats (79,81), mice (36,68), rabbits (68,78,80,81,84) and dogs (82) However, other workers have reported an even distribution of RCCs (68,85,86). The gradient could reflect the differences in blood flow in different parts of the cortex, for example juxtamedullary glomeruli are perfused at a greater pressure than superficial glomeruli (35).

Sodium-depleted and sodium-loaded rats had an even

distribution of RCCs. In Addison's disease RCCs were more numerous in superficial glomeruli, however many RCCs were also present in juxtamedullary JGAs. This was more marked in untreated Addison's disease. This confirms some biochemical studies in animals (79,80), although others have reported that the normal gradient is maintained (36,81,85,87). The distribution of RCCs has not been previously studied in Addison's disease.

In renal artery stenosis, the normal gradient of RCCs was maintained, a similar pattern to Addison's disease. In the clipped kidneys of experimental hypertension, the normal gradient was lost and in two cases, reversed. This has previously been reported in renal artery stenosis (71), polyarteritis nodosa (84) and renal artery clipping in rabbits (78). The distribution of RCCs in malignant hypertension was more complex, probably reflecting the heterogeneity of the cases. RCCs were evenly spread throughout the cortex, with reversal of the normal gradient in three of the kidneys. The distribution of RCCs in malignant hypertension has not been previously reported, but resembles that of renal artery stenosis (see above, and 71) and polyarteritis nodosa (84). These changes could therefore reflect the alterations of glomerular haemodynamics, which occur in the renal cortex in malignant hypertension.

In summary, peripolar cells were more often located in superficial glomeruli than juxtamedullary glomeruli, in normal kidneys. This is similar to the distribution of renin-containing cells. Sodium-depletion and a reduction in renal perfusion pressure in rats and humans resulted in recruitment of renin-containing cells in juxtamedullary JGAs. Numbers of peripolar cells in these situations were too small to investigate the possibility that they react in a similar fashion.

(3) IMMUNOHISTOCHEMICAL STUDIES OF THE PERIPOLAR CELL

The first purpose of this investigation was to define immunohistochemical markers which could be used to identify peripolar cells. With scanning electron microscopy, Gibson *et al* have produced evidence for a population of non-granulated peripolar cells, only some of which may become granulated (34,88). Therefore peripolar cell-specific markers could potentially identify these cells by light microscopy. There has been no formal immunohistochemical study of human peripolar cells.

Using a wide range of antibodies I found that a minority of peripolar cells expressed the intermediate filament protein vimentin, but were always negative for cytokeratins (PKK1 and Cam 5.2 antibodies). Podocytes

always expressed vimentin, confirming previous reports (89,90,91). I also found vimentin expression in occasional parietal and tubular epithelial cells. This implies that these cells may be more heterogeneous than is apparent on histological grounds alone. Some human parietal epithelial cells also expressed cytokeratins. This confirms the findings of previous studies (89,90,92). Peripolar cells did not express desmin, the intermediate filament associated with both smooth and striated muscle differentiation. Sheep but not human cells expressed desmin in keeping with the concept that mesangial cells bear some resemblance to vascular smooth muscle cells (93,94). This also confirms previous immunohistochemical studies which have reported desmin-reactivity in the mesangium of various species (95,96) apart from humans (90,91).

Peripolar cells showed no immunoreactivity for the epithelial markers EMA and HMFG. The pattern of staining for other renal cells was in accord with previously published work (91,97,98).

NSE expression has previously been demonstrated in a small minority of sheep peripolar cells, suggesting neuroendocrine differentiation (37). I could not confirm this however, using multiple antibodies which are recognised markers of neuroendocrine differentiation.

The second purpose of this study was to use immunohistochemical techniques to study the composition of peripolar cell granules. This assumes of course, that the granules contain a known protein, to which antisera or monoclonal antibodies are available. If the granules contained a novel hormone, then it would not be detected. Kallikrein has been detected in peripolar cells (39), however these authors have subsequently expressed doubt about the specificity of the antiserum which they used. Other plasma proteins have also been identified in sheep peripolar cells (40). I have demonstrated a variety of plasma proteins in peripolar cell granules in human and sheep kidneys using both immunoperoxidase and immunofluorescence techniques.

Podocytes may take up filtered protein (42,99), although the significance of this is uncertain. Nakajima et al have demonstrated plasma proteins in glomerular epithelial cells, including peripolar cells in a variety of renal diseases (50). Normally, less than 2mg/kg body weight of protein is excreted in the urine in humans every day, and in sheep up to 8mg/kg body weight may be excreted (100). Filtration of plasma proteins by the glomerulus depends not only on molecular weight, but also on the molecular shape and charge (101). Most proteins of a molecular weight of less than 70 000 daltons (such as albumin) are filtered

and then reabsorbed by the proximal tubule. Only small amounts of larger proteins such as IgG and C3 are filtered and are generally not reabsorbed by the tubules (42,101). IgM is not filtered by the glomerulus and is not present in human urine (102). I observed immunohistochemical staining for all plasma proteins except IgM on the brush border of proximal tubules. Similarly, all of the plasma proteins which I looked for were present in peripolar cell granules, apart from IgM. This provides circumstantial evidence that peripolar cells may absorb these proteins from the glomerular filtrate.

In normal sheep kidneys, IgG, IgM, C3 and albumin were localised within the glomerular tuft, with accentuation of staining in the mesangium at the vascular pole. Staining for IgM also extended into the macula densa. The mesangium plays an important role in disposing of macromolecules which are deposited in the glomerulus (93,94). Tracer studies have shown that proteins accumulate in the mesangium and are transported to the vascular pole where they appear to be excreted via the extraglomerular mesangium and macula densa, although the site of egress remains uncertain (94).

In summary, I have been unable to define a specific immunophenotype for peripolar cells and I

could find no evidence of neuroendocrine differentiation. Sheep and human peripolar cells contained several plasma proteins within their cytoplasmic granules. It remains unclear why peripolar cells should absorb proteins and package them into granules, but their presence in normal kidneys at this anatomical site suggests a specialised function.

(4) THE ROLE OF THE PERIPOLAR CELL IN SODIUM METABOLISM

(a) Experimental alteration in sodium balance

The purpose of this study was to investigate the reactions of the peripolar cell to alterations of sodium balance in the rat. Sodium depletion of both normal and pregnant sheep results in no consistent alteration in numbers of peripolar cells (30,31). However exocytosis of granules, which is rarely observed in normal subjects, was prominent only in sodium-depleted animals. These workers have suggested that peripolar cells may play a role in the renal handling of sodium, and in particular that they may secrete a sodium-retaining substance (4,30,31). Morild *et al* demonstrated increased numbers of peripolar cells in chickens, which were acutely sodium-depleted (28). Lastly, prominent exocytosis of peripolar cell granules has been noted in axolotls, which were immersed in distilled water (2).

Peripolar cells were sparse in most sodium-loaded and sodium-depleted animals, and there was no detectable difference in numbers or morphology between the 2 groups. Adequacy of sodium-depletion was demonstrated by hyperplasia of the renin-containing cells (RCCs) in the kidneys. Similarly, RCCs were supressed in the kidneys of sodium-loaded animals. There was a significant difference in the renin indices JGA+ and RCI, but not A+ between the two groups. Increased amounts of renin in sodium-depleted experimental animals has been demonstrated previously by both biochemical (36,79,80,85) and immunohistochemical methods (36).

(b) Addison's disease

In order to study the response of the human peripolar cell to sodium-depletion, I have studied 6 autopsy cases of previously undiagnosed Addison's disease and 5 known cases of Addison's disease, who died durimg Addisonian crisis. Addison's disease is most commonly caused by an auto-immune process which results in deficiency of adrenocortical hormones (103). The lack of aldosterone leads to loss of sodium in the urine, resulting in sodium depletion and hyponatraemia. Consequently, these patients have elevated levels of plasma renin which return to normal after treatment (104). There have been two previous reports of JGA

hyperplasia in Addison's disease (105,106), but this is first study which uses immunohistochemical techniques to localise renin. Peripolar cells have not been previously studied in Addison's disease.

Peripolar cells were sparse in both groups of Addison's patients and in the control group. The mean PPI of the control group was lower than that obtained for normal surgical nephrectomy specimens, which were used as a control group for the renal artery stenosis study. The reason for this is uncertain, although it could be attributed to post-mortem autolysis. There was no difference in PPI between the treated and untreated groups of Addison's disease. Glomerular epithelial cells become granulated in normal foetal rat kidneys, but this may be prevented by maternal adrenalectomy, suggesting that this may be adrenal-dependant (58). However, I have found no evidence that either sodium-depletion and/or adrenal deficiency affects peripolar cells in humans.

I have demonstrated hyperplasia of the renin-containing cells of the kidney in Addison's disease, with significant elevation of all of the semi-quantitative indices which were derived. The values of these were similar to those obtained for renal artery stenosis kidneys (see below). By contrast, the patients who died during Addisonian crisis despite treatment,

demonstrated normal amounts of renin in the kidney. Hence the attempts to compensate for these patients deficiency with salt and exogenous steroids may have been sufficient to suppress renal renin content.

In summary, no difference in numbers of peripolar cells was observed between sodium-depleted and sodium-loaded rats, or in Addison's disease in humans, suggesting that peripolar cells do not have a role in sodium metabolism.

(5) THE PERIPOLAR CELL IN HYPERTENSION

(a) Experimental renovascular hypertension

Peripolar cells form a cuff around the glomerular arterioles and would be ideally situated to respond to alterations in renal perfusion pressure. The aims of this study were to examine the response of the glomerular peripolar cell in experimental renovascular hypertension, in which there is a reduction in renal perfusion pressure, and to investigate whether or not it is part of the JGA. Peripolar cells have not been previously studied in experimental renovascular hypertension.

I have confirmed that granulated peripolar cells are sparse in normal rat kidneys; I found them in only one of the ten control animals. There was no significant difference in numbers of peripolar cells between the control group and the experimental group. Granulated glomerular epithelial cells, other than peripolar cells, are never present in the normal rat kidney (see above). However they were easily identified in three of the five unclipped kidneys in the experimental animals. There was no correlation between PPI and ECDI, although the two unclipped kidneys which contained peripolar cells also contained granulated glomerular epithelial cells. There was no correlation between PPI or ECDI with the renin indices or amount of hypertensive damage.

Granulated glomerular epithelial cells have been described as a feature of human malignant hypertension (59.60,61) and experimental renovascular hypertension (56,57) before, but little attention has been paid to them. Kincaid-Smith found that the number of granulated epithelial cells per glomerulus correlated inversely with the degree of hypertensive damage (57). These workers suggested that the granules could be secretory in nature, although others believe that they are lysosomes which have accumulated urinary protein (56). It has been postulated that lysosomal granules containing urinary proteins would be expected in the

unclipped kidney rather than the clipped kidney, because of its relative hyperperfusion (56). Others have reported similar findings in DOC-salt hypertension, where peripolar cells are unaltered but glomerular epithelial cells become granulated (28).

There was marked hyperplasia of renin-containing cells in the clipped kidneys with suppression in the opposite kidneys. This confirms previous immunohistochemical studies (21,36,69).

In summary, I have found that in experimental renovascular hypertension in rats, numbers of peripolar cells were unaltered in both kidneys. Other glomerular epithelial cells become granulated in the unclipped but not the clipped kidney. Therefore a reduction in renal perfusion pressure, which is a stimulus to renin secretion by the JGA, does not affect numbers of peripolar cells.

(b) Renal artery stenosis

Examination of the control group in this study confirmed that peripolar cells are sparse in normal human kidneys. The range of the peripolar cell index (PPI) was similar range to that recorded in the series of resin-embedded human kidneys. Three kidneys with

renal artery stenosis contained large numbers of granulated peripolar cells, more than twice any of the normal kidneys. There were no clinical or pathological features exclusive to these patients.

Peripolar cells do not contain renin (see below and 28,37). Urinary kallikrein secretion is increased in human and experimental renovascular hypertension (109). Whether or not kallikrein is present in sheep peripolar cells is controversial (37,39). Renal kallikrein mRNA has been localised to the vascular pole of rat glomeruli, but not specifically to peripolar cells (38). Plasma proteins other than kallikrein, have also been identified in sheep peripolar cells (see above and 40). Proteinuria occurs in benign (110) and malignant hypertension (111), and renovascular hypertension (112,113). Granules are present in glomerular epithelial cells in the unclipped kidney of Goldblatt hypertension in rats (see above and 56). However, in this study I found no granulated glomerular cells other than peripolar cells, and the amount of urinary protein did not correlate with numbers of peripolar cells.

I have confirmed previous reports of hyperplasia of renin-containing cells in renal artery stenosis (12,78,114,115). Early studies were limited by the

non-specific methods used to identify renin granules. The development of antisera and monoclonal antibodies to renin has allowed precise immunohistochemical localisation of renin-containing cells. Using these techniques it has been possible to demonstrate hyperplasia of renin-containing in renovascular hypertension (71,116). I found no relationship between numbers of peripolar cells and renin-containing cells in the normal or ischaemic kidneys.

In summary, large numbers of peripolar cells were found in 3 cases of renal artery stenosis whereas there was always hyperplasia of renin-containing cells. Kidneys with renal artery stenosis are heterogenous and the patients received different drug therapies, however these results do not support the hypothesis that peripolar cells respond to alterations in renal perfusion pressure.

(c) Human malignant hypertension

I have examined autopsy kidneys from 12 patients with malignant-phase hypertension. The purpose of this study was two-fold. Firstly, glomerular epithelial cells commonly become granulated in malignant hypertension (59,60,61,111). Peripolar cells have not been previously studied in malignant hypertension and their relationship with other granulated glomerular

epithelial cells is unclear. Secondly, the renin-angiotensin system is stimulated in malignant hypertension (117,118,119,120), and so it should be possible to examine further the relationship between peripolar cells and the juxtaglomerular apparatus.

(i) Peripolar cells

Peripolar cells were found in 7 of the 12 kidneys. In one case, the PPI was markedly increased (= 45.0). As a group, there was a significant increase in PPI compared with the controls. Peripolar cells are normally sparse in human kidneys (see above). Until the nature of peripolar cell granules is better defined, the reason for their prominence in malignant hypertension is speculative. The glomeruli in malignant hypertension are subjected to intense ischaemia, hence peripolar cells could be responding to this by synthesising and releasing a hormone. I have demonstrated increased numbers of peripolar cells in some cases of renal artery stenosis, in which there is also reduced glomerular perfusion, however I could not reproduce similar results in experimental renovascular hypertension in rats (see above).

Alternatively, peripolar cell granules may only contain plasma proteins reabsorbed from the glomerular

filtrate (37,40); patients with malignant hypertension commonly have proteinuria (111).

(ii) Other granulated glomerular epithelial cells.

Glomerular epithelial cells other than peripolar cells become granulated in human malignant hypertension (59,60,61,111) and in several animal models (28,56,57). In DOC-salt hypertension in chickens peripolar cells are unaltered in number, but other glomerular epithelial cells acquire granules (28). Traditionally these granules are believed to contain plasma proteins reabsorbed from the glomerular filtrate (28,56), however the exact route of entry of plasma proteins into these cells and the mechanism remains unclear.

(iii) The relationship between peripolar cells and other granulated glomerular epithelial cells.

Peripolar cell granules were histologically indistinguishable from granules in other glomerular epithelial cells. Most granulated glomerular epithelial cells were peripherally situated in the glomerular tuft. The explanation for this is uncertain, however it appears that there is no "gradient" of granulation of glomerular epithelial cells, maximal at the vascular

pole. There was a positive correlation between numbers of peripolar cells and other granulated glomerular epithelial cells, hence both cell types may have a similar function and react in a similar manner in malignant hypertension. However, normal human kidneys contain peripolar cells but other glomerular epithelial cells are non-granulated.

(iv) Renin-containing cells

There have been several studies of the morphology of the JGA in malignant hypertension (117,118,119). All demonstrated hyperplasia and/or increased secretory activity of the renin-containing cells. This accords with the elevated plasma renin concentration in these patients (70,120). There has been no previous immunohistochemical study of the renin-containing cells in kidneys affected by malignant hypertension. I found hyperplasia of renin-containing cells in some, but not all cases. There was significant elevation of the renin indices JGA++ and RCI but not JGA+ or A+, compared with the control group. Therefore the increase in the total number of renin-containing cells, is probably due to hyperplasia of renin-positive JGAs since the proportion of renin-positive JGAs (represented by JGA+) was unaltered. This would perhaps be expected, since renin)containing cells were almost never present in

necrotic arterioles, whilst surviving JGAs were hyperplastic.

(v) The relationship between peripolar cells, other granulated glomerular epithelial cells and the renin-angiotensin system.

There was no correlation between PPI or ECDI and any of the renin indices. If both peripolar cell and other epithelial cell granules contain predominantly plasma proteins, it could be suggested that locally generated angiotensin II plays a role in inducing proteinuria (121), however it seems likely that its contribution is minor compared to the extensive ischaemic damage which occurs in the glomeruli. Alternatively, these cells could have an endocrine function which opposes the actions of the renin-angiotensin system, although in the absence of an identified hormone this is speculative.

In summary, peripolar cells and other granulated glomerular epithelial cells were frequently identified in the kidneys of patients with malignant hypertension. There was a positive correlation between numbers of these cells and their granules were morphologically indistinguishable. Renin-containing cells were prominent in many cases, but there was no correlation with numbers of peripolar cells or other granulated

glomerular cells. Whilst both peripolar cells and other glomerular epithelial cells could have a secretory function, it seems more likely that the granules in both cell types represent accumulation of proteins filtered by the abnormal glomeruli in malignant hypertension.

(6) THE PERIPOLAR CELL IN HUMAN RENAL DISEASE

This is the first formal study of peripolar cells in a range of human diseases. Peripolar cells have previously been described in human pre-eclampsia (62,63) and ovine toxaemia of pregnancy (123). When individual human glomeruli are examined in theirentirety by serial sectioning, 12.4% on average containat least one peripolar cell (see above). This means that peripolar cells are rarely identified in random histological sections. Assuming that the vascular pole measures 50 μm in diameter, it may be calculated that a peripolar cell would be identified in approximately 1in every 85 glomeruli. This represents a PPI(r) of1.12. I was unable to obtain a control group for this biopsy series, since normal human kidneys are generally not biopsied. Despite this, I have identified several human diseases in which peripolar cells are prominent: mesangioproliferative GN, IgA nephropathy, focal segmental glomerulosclerosis, membranous GN and both diffuse and membranous lupus nephropathy. Unlike

granulated glomerular and tubular epithelial cells, there was a positive correlation with the amount of urinary protein. There were also positive correlations with sodium and haemoglobin concentrations and creatinine clearance.

Other glomerular epithelial cells may also become granulated, although not in normal human kidneys. they have been reported to be most prominent in malignant hypertension (59,60,61) and pre-eclamptic toxaemia of preganancy (62,63). In addition, they are found in several animal models of hypertension (28,56,57) and experimental proteinuria (42,43). There has been no detailed study of these cells in human disease. I found them to be most prominent in diffuse lupus nephropathy, focal GN, acute vascular transplant rejection, cresentic GN and mesangioproliferative GN.

Most workers have interpreted the formation of granules in glomerular epithelial cells as being due to absorption of plasma proteins from the glomerular filtrate (28,42,43,50,56,57) and plasma proteins have been identified by immunohistochemistry (43,45,50). In experimental proteinuria, the prominence of these granulated cells usually (42,43), but not always (124), correlates with the amount of urinary protein. In these patients, there was no correlation with the amount of urinary protein. There was however a positive

correlation with diastolic blood pressure and anegative correlation with sodium concentration. Granules have been observed in the tubular epithelium for many years, and there is good evidence that they are lysosomes, which contain protein reabsorbed from the tubular lumen (60). These granules, which are often referred to as "hyaline droplets" contain a variety of lysosomal enzymes (48), and accumulate tracer materials (60). They are usually associated with severe proteinuria, although there has been no formal study of different renal diseases, nor any attempt to correlate them with amounts of urinary protein. Tubular hyaline droplets were most prominent in minimal change GN, amyloidosis and membranous GN. There was no correlation with the amount of urinary protein.

I could detect no consistent morphological differences between the granules in each cell type, using tinctorial stains and light microscopy. In a study of pre-eclampsia, peripolar cell granules could not be distinguished from granules in other glomerular epithelial cells (63). In the definitive account of pre-eclampsia, Sheehan and Lynch found that glomerular epithelial cells acquired granules, and that this occured most commonly at the vascular pole (62). Recently, differences have been demonstrated in the distribution of plasma proteins within individual granules of glomerular epithelial cells, but not

tubular epithelial cells (50). These workers also found that glomerular epithelial cells did not acquire granules in minimal change GN, and interpreted the granules as a reflection of damage to the glomerular basement membrane (50).

The most important finding in this study is that peripolar cells, other granulated glomerular epithelial and granulated tubular epithelial cells appear to react differently in different diseases. If the diseases are divided into two groups on the basis of whether they are primarily immune-complex mediated or not, then PPI(r) and ECDI(r) are greater in the immune-complex mediated group, although in neither is this statistically significant (Table 19). THDI is similar in the two groups. This implies that the tubular hyaline droplets represent non-specific reabsorption of urinary protein, whereas the granulated glomerular cells, including peripolar cells may represent a more specific response to glomerular basement membrane damage.

In addition, peripolar cells appear to react differently from other glomerular epithelial cells. For example, in membranous GN all granulated glomerular epithelial cells were peripolar cells. This is also well illustrated in lupus nephropathy: in diffuse lupus nephropathy granulated glomerular epithelial cells were

Table 19. Mean PPI(r), ECDI(r) and THDI for diseases grouped, where possible into those which are immune-complex mediated and those which are not. Cresentic GN, vascular transplant rejection and polyarteritis nodosa are difficult to classify and have not been included.

Immune-complex mediated	Non immune-complex mediated
Mesangioproliferative GN	Focal segmental glomerulosclerosis
IgA nephropathy	Diabetes mellitus
Membranous GN	Minimal change GN
Lupus nephropathy	Amyloidosis
Focal GN	Interstitial nephritis
Acute diffuse GN	Wegener's syndrome
Membranoproliferative GN	
Henoch Schonlein purpura	

	Immune-complex mediated	Non immune-complex mediated
Mean PPI(r)	1.32	0.52
Mean ECDI(r)	6.28	2.55
Mean THDI	1.80	1.78

very prominent, but peripolar cells less so. However in the membranous form of lupus nephropathy, peripolar cells were numerous but other granulated glomerular epithelial cells were absent, resembling the pattern ofidiopathic membranous GN. This could reflect the different distribution of immune complexes in the glomerulus in these diseases.

In summary, I have studied peripolar cells, other glomerular epithelial cells and granulated tubular epithelial cells in 242 renal biopsies. There were striking differences in numbers of each cell type in different diseases. It is likely that the granules in tubular epithelial cells represent non-specificr eabsorption of urinary protein, but this seems lesslikely for glomerular epithelial cells, including peripolar cells. Peripolar cells were especially prominent in only certain renal diseases, suggesting a specific response to certain patterns of immune-complex deposition.

(7) THE RELATIONSHIP BETWEEN PERIPOLAR CELLS AND THE RENIN-ANGIOTENSIN SYSTEM

When the glomerular peripolar cell was first described it was suggested that it could be part of the juxtaglomerular apparatus (JGA) (1). This suggestion was based on the observation that it was situated at the glomerular vascular pole, close to the renin-secreting cells of the JGA. Subsequently, it was suggested that sodium-depletion is a stimulus to exocytosis of peripolar cell granules, raising the possibility of functional interaction between peripolar cells and the renin-angiotensin system (4,30,31).

I have studied the morphology of peripolar cells in normal human, rat and sheep kidneys and have confirmed that there is a close anatomical relationship between peripolar cells and the renin-containing cells of the JGA. By electron microscopy, human peripolar cells are often separated from the renin-containing cells only by the basement membrane of Bowman's capsule. Renin is secreted into the renal interstitium close to the vascular pole, and angiotensin II is generated locally (19,20,21,22). Therefore peripolar cells are ideally situated to be influenced by these important humoral agents; indeed the peripolar cell secretory product could also affect the renin-containing cells of the JGA, although exocytosis of

granules has only been observed from the luminal surface.

Using an immunoperoxidase technique, I have shown that the human peripolar cell does not contain renin. The antiserum which was used has been shown previously to reliably localise renin in human kidneys (8,10,124,125). In addition, the adjacent renin-containing cells of the JGA provided a positive control in the same tissue section. Others have subsequently failed to demonstrate renin in sheep (37) and chicken (28) peripolar cells. Peripolar cells may contain kallikrein, but this is controversial (see above); there are many interactions between the renin-angiotensin system and the renal kallikrein-kinin cascade (110).

I have investigated the possibility of a functional relationship between peripolar cells and the renin-containing cells of the JGA, by quantifying both cell types in normal kidneys, and in experimental and disease states in which the renin-angiotensin system is stimulated. I found no correlation between numbers of peripolar cells and renin-containing cells in normal human kidneys and rat kidneys. Sodium depletion is one of the main stimuli to renin secretion (15). I identified hyperplasia of renin-containing cells in the kidneys of rats and of patients with Addison's

disease, who are known to be sodium-depleted; however peripolar cells were unaltered in both. A decrease in renal perfusion pressure is the other main stimulus to renin secretion by the JGA (15). I investigated two situations where renal perfusion pressure is reduced: the two-kidney one-clip model of hypertension in rats and human renal artery stenosis. In both instances there was hyperplasia of the renin-containing cells, but peripolar cells were unaltered.

In summary, peripolar cells do not respond to the major stimuli of renin-secretion, in experimental animals and in human disease. Therefore I have produced no evidence other than anatomical site, that the peripolar cell is part of the JGA.

(8) FINAL CONCLUSIONS

I have identified peripolar cells in human kidneys and studied their morphology by light microscopy and transmission electron microscopy. They are situated at the glomerular vascular pole, close to the renin-containing cells of the juxtaglomerular apparatus (JGA). Peripolar cells are sparse in random histological sections of human kidneys, but are prominent in sheep.

I have produced no evidence that peripolar cells are part of the JGA, other than their anatomical site. Peripolar cells do not contain renin. I have investigated the response of the peripolar cell to patho-physiological stimuli which are known to affect the renin-containing cells of the JGA. Sodium-depletion (in experimental animals or Addison's disease) or a reduction in renal perfusion pressure (experimental renovascular hypertension or renal artery stenosis) resulted in no consistent alteration in peripolar cells, although there was hyperplasia of renin-containing cells. In malignant hypertension, peripolar cells were prominent and other glomerular epithelial cells acquired granules. Peripolar cells were also prominent in several human renal diseases, notably membranous glomerulonephritis, mesangioproliferative glomerulonephritis, IgA nephropathy and focal segmental

glomerulosclerosis. Other granulated glomerular epithelial cells were prominent in different diseases. It would be interesting to investigate the relationship between peripolar cells and other granulated glomerular epithelial cells in experimental models of glomerulonephritis.

Peripolar cells may be secretory cells, although a secretory product has not yet been identified, and so this must remain speculative. Alternatively, peripolar cell granules may contain only plasma proteins, which they absorb from either the glomerular filtrate or the glomerular basement mebrane. However, the presence of peripolar cells in normal kidneys in a unique and important anatomical site suggests that they have a specialised function, which merits further investigation.

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