THE PERIPOLAR CELL: A NEW COMPONENT OF THE JUXTAGLOMERULAR APPARATUS?

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THESIS SUBMITTED
FOR THE DEGREE OF MSc
TO THE FACULTY OF MEDICINE,
UNIVERSITY OF GLASGOW
APRIL 1992

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ACKNOWLEDGEMENTS.

I am grateful to Professor R.N.M. MacSween and in particular Professor W.R. Lee, for allowing me to use the facilities of their departments. The image analyser was supplied by a grant from the Scottish Home and Health Department.

Thanks also go to my supervisors Dr. G.B.M. Lindop and Dr. I.A.R. More for their invaluable advice, guidance and encouragement and to the rest of the "Peripolar Institute", in particular Dr. D. S. Gardiner, for helpful advice and discussion.

I am indebted to Mr. T.T. Downie and the staff of the Pathology Dept. for their technical assistance and friendship throughout.

Last, but not least, may I thank my wife, Marion whose help and support I could not have done without.

DECLARATION.

I declare that I carried out the work in this project with the following exceptions:

1) The animal experiments were carried out by Dr. C. Kenyon, in the MRC Blood pressure Unit, Western Infirmary, Glasgow,

2) The processing of tissue for light microscopy was performed by the technical staff of the Pathology Dept., Western Infirmary, Glasgow,

3) The serial sectioning and staining for light microscopy was carried out by Mr. R. Muirhead. The sections were examined by Dr. D. S. Gardiner,

4) Tissue for transmission electron microscopy examination were processed by the electron microscopy unit of the Pathology Dept., Western Infirmary, Glasgow. The ultrasectioning and examination were carried out by Mr. T. T. Downie.

SUMMARY.

The peripolar cell is a glomerular epithelial cell at the vascular pole, lying between the parietal epithelium and the podocytes of the tuft. Morphological evidence indicates that the peripolar cell could be a secretory cell. The secretory product is unknown. Experimental evidence has suggested that the peripolar cell may be a previously unrecognised component of the juxtaglomerular apparatus.

The aim of this project was to examine whether or not the peripolar cell responds to stimuli which activate or suppress the juxtaglomerular apparatus. Light microscopy, which relies on the presence of granules to identify peripolar cells, and scanning electron microscopy, which relies on surface characteristics, were used to count peripolar cells in the rat kidney.

Light microscopy showed that 4% of glomeruli had granulated peripolar cells, however scanning electron microscopy showed peripolar cells in 55%. Sectioning of peripolar cells identified by scanning electron microscopy confirmed an absence of granules. This shows that non-granulated peripolar cells exist and that scanning electron microscopy is the best method for identifying and counting peripolar cells.

There was no change in the number, size or granulation of peripolar cells in both experimental renal artery constriction and alterations in sodium intake. The peripolar cell does not respond to the major stimuli of the juxtaglomerular apparatus and its functon remains unknown.

1. INTRODUCTION.

In 1979, Ryan *et al.* described a previously unidentified cell in the sheep glomerulus. These cells are situated on the basement membrane within Bowman's capsule and, since they encircle the vascular pole, they were called peripolar cells. Peripolar cells have been since identified in a wide range of species (Hanner and Ryan, 1980; Gall *et al* , 1986; Lacy and Reale, 1989; Mbassa, 1989) including humans (Gardiner and Lindop, 1985; Gardiner*et al* , 1986).

The peripolar cell has a close relationship with the juxtaglomerular apparatus (JGA) being only separated from it by the basement membrane of Bowman's capsule (Gardiner *et al*, 1986). An increase in peripolar cell numbers (Morild *et al*, 1988) and in their secretory activity in sodium depleted animals (Hill *et al*,1983, 1984) led to the suggestion that the peripolar cell was a previously unrecognised part of the JGA and that its secretory product could influence tubular function (Ryan *et al*, 1979, 1982; Hill *et al*, 1983).

1.1 Structure of the Juxtaglomerular Apparatus.

Traditionally the JGA comprises (Figure 1.1):

1) The renin-secreting cells of the afferent and efferent glomerular arterioles,

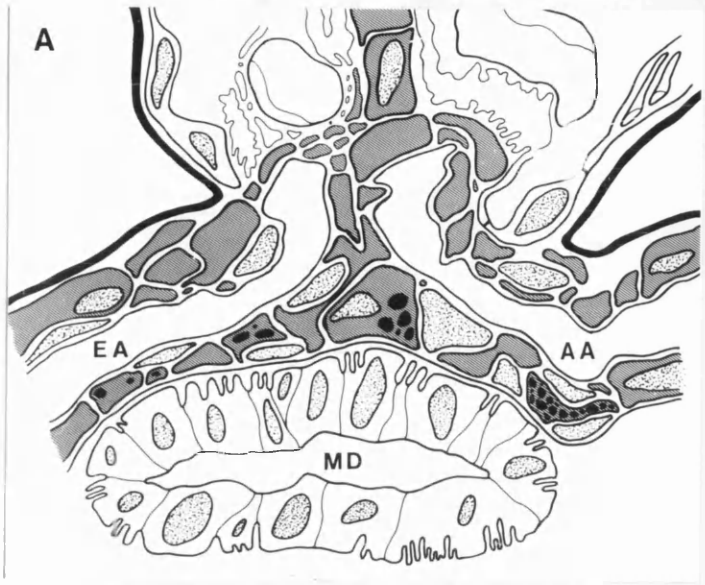
2) The macula densa, a sensor for electrolyte concentration in the distal tubule,

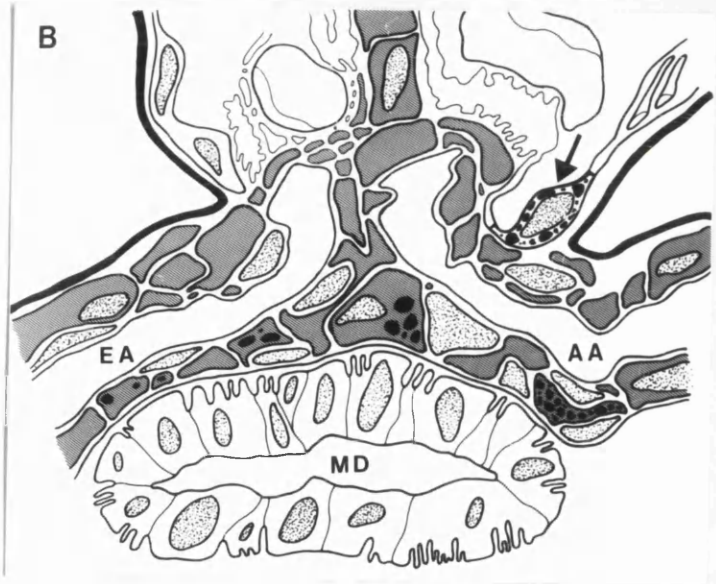
3) The extraglomerular mesangium.

The JGA lies at the vascular pole of the glomerulus. The extraglomerular mesangium is located between the macula

Figure 1.1 (a) Diagram of the juxtaglomerular apparatus before the discovery of the peripolar cell. The myoepithelioid cells contain renin granules, in the walls of the arterioles. AA = afferent arteriole, EA = efferent arterioles, MD = macula densa.

(b) Juxtaglomerular apparatus showing the position of the peripolar cell at the vascular pole within the Bowman's capsule (↓).


A
EA
AA
MD


B
EA
AA
MD

densa and Bowman's capsule at the point of entry and exit of the afferent and efferent arteriole (Barajas and Latta, 1963). The complex is pyramidal or conical in shape, the macula densa being the base and the afferent and the efferent arterioles the sides. At the apex the extraglomerular mesangium is continuous with the mesangial cells of the glomerular tuft (intraglomerular mesangium) both being embedded in a well developed intercellular matrix of basement membrane-like material (Cantin, 1983).

1.2 Function of the JGA.

The JGA maintains blood pressure, controls extracellular fluid volume and glomerular filtration rate (Jackson *et al*, 1985). This is achieved by the production of the enzyme renin, which cleaves a globulin substrate - angiotensinogen, to form the decapeptide angiotensin (ANG) I. A further enzyme, angiotensin converting enzyme, cleaves two amino acids from ANG I to form ANG II, a potent vasoconstrictor and stimulator of aldosterone production.

Cleavage of ANG II by aminopeptidases results in the formation of a heptapeptide - ANG III, which also stimulates aldosterone release from the adrenal gland, but it has a much weaker direct pressor effect than ANG II (Kotchen and Roy, 1983).

All components of the renin-angiotensin system are present in the kidney (Mendelsohn, 1982; Jackson *et al*, 1985; Lindop and Lever, 1986; Inagami *et al*, 1990). ANG II has been found in the granules of myoepithelioid cells and may be

generated intracellularly (Taugner *et al*, 1984). Also, following renin secretion ANG II is generated within the kidney (Mendelsohn, 1982; Taugner *et al*, 1984; Lindop and Lever, 1986). Locally produced ANG II may therefore alter renal haemodynamics and regulate glomerular filtration within individual nephrons (Mendelsohn, 1982).

1.3 Factors controlling renin-secretion.

Renin release is controlled by many agents; for review see Davis and Freeman (1976). However, there are three main stimuli; variations in renal arterial perfusion pressure, delivery of sodium chloride to the macula densa and activity of the sympathetic nerves.

1. The epithelioid cells of glomerular arterioles act as stretch receptors and change their rate of renin secretion as the arteriolar wall alters its degree of stretch in response to variations in the perfusion pressure (Tobian *et al* , 1959; Blaine *et al*, 1971).

The signal perceived by the renal vascular receptor is not fully understood. However, several stimuli influence the receptor (Vander, 1967) and these are: 1) changes in the diameter of the renal afferent arteriole, 2) changes in the transmural pressure gradient, 3) renal sympathetic nerve activity which alters renal arteriole tone, 4) intrinsic myogenic factors and 5) alterations in elastic elements of the vessel wall.

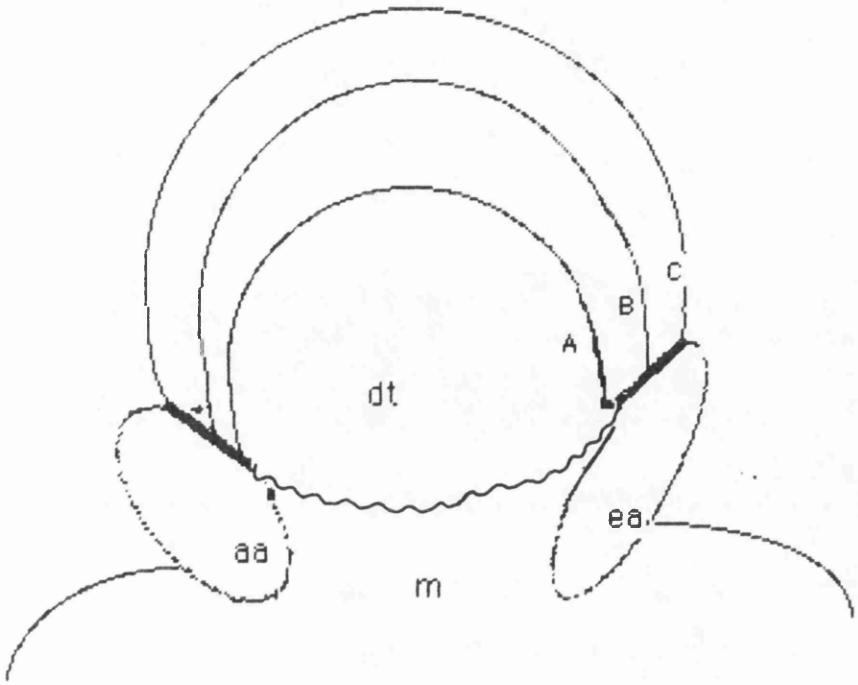
Like smooth muscle cells, myoepithelioid cells respond to stretch by depolarisation (Fray, 1976). With

increased stretch there is increased permeability of the cells to ions and inhibition of renin release. Increased intracellular calcium levels produce contraction in smooth muscle cells and inhibition of renin release by epithelioid cells; decreased cellular levels produce relaxation of smooth muscle and stimulation of renin release by the epithelioid cells (Fray, 1976).

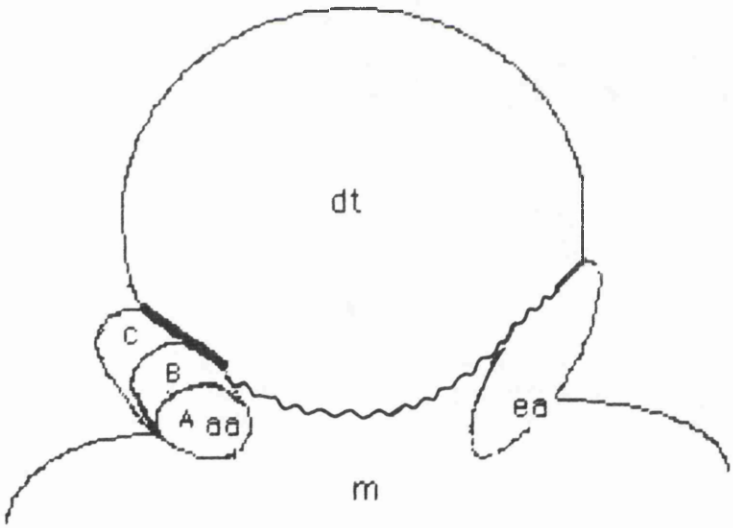
2. Renin release is inversely related to the rate of sodium chloride delivery to the macula densa (Vander and Miller, 1964; Churchill *et al* , 1978). How the macula densa is stimulated and the signal to the myoepithelioid cells is unknown. Renin release is supressed by loading with sodium chloride. sodium bromide and choline chloride, suggesting that the chloride ion is at least as important in the regulation of renin release (Kirchner *et al* , 1978)

A model put forward by Barajas (1971) explains the control of renin secretion by both the baroreceptor and the macula densa. Using 3-dimensional reconstruction of the JGA, Barajas noted a variation in areas of contact between the macula densa and the afferent and efferent arterioles. He suggested that less contact between the macula densa and the granular cells would lead to increased renin secretion and more contact to less secretion. Therefore, a smaller sodium load results in decreases in the volume in the tubule, decreased contact with granular cells and increased renin secretion. Large loads have the opposite effect. Similarly in the baroreceptor theory, a large intra-arteriolar volume would increase the contact and decrease renin secretion, whereas small intra-arteriolar volume has the opposite effect (Figure

Figure 1.2 A simplified schematic representation of the proposed function of the juxtaglomerular apparatus. The contact between the distal tubule (dt), the mesangial region (m) and the efferent arteriole (ea) which is interpreted as permanent is represented by wavy lines, whereas the reversible type of contact is represented by heavy lines.
(A) As the distal tubule expands (lines B and C) the area of "reversible" contact with the arterioles increases.
(B) Representation of the changes in contact between the distal tubule and the afferent arterioles resulting from changes in the volume of the afferent arteriole. (Barajas L (1971) Science 172 485)



A



B

1.2).

3. The renal sympathetic nerves end on the myoepithelioid cells and the smooth muscle cells of the afferent arteriole. Renal nerve stimulation and an increase in circulating noradrenaline increase renin release, via a B-adrenergic receptor mechanism (Vander,1965; Vander, 1967).

In addition to the three main mechanisms many other humoral factors, such as vasopressin, prostaglandins, calcium ion and ANG II (reviewed by Davis and Freeman, 1976), also affect renin release from the JGA.

The Peripolar Cell

Peripolar cells can be identified at the vascular pole in sections stained by trichrome stains such as Lendrum's Martius Scarlet Blue method. Peripolar cells are distinguished by the presence of cytoplasmic granules. By electron microscopy, the granules are large membrane bound secretory granules (0.4-2μm), mostly round in shape but varying in size and containing homogeneous electron dense material (Ryan *et al*, 1979; Gardiner*et al*, 1986; Kelly *et al*, 1990).

In sheep, peripolar cells are present in most glomeruli (Kelly *et al*, 1990), and easily counted by light microscopy. However, the sheep is not a suitable experimental animal. Granulated peripolar cells are scanty in the rat (Gall *et al*, 1986; Downie *et al*, 1991) and human kidney (Gardiner and Lindop, 1985). Scanning electron microscopy (SEM) shows

peripolar cells in 50-60% of rat glomeruli (Gibson *et al*, 1989). Non-granulated peripolar cells may therefore exist and only under appropriate conditions store their secretory product and become granulated (Gardiner and Lindop, 1985; Gibson *et al*, 1989).

The function of the peripolar cell is unknown, but it is ideally situated to release factors directly into the Bowman's space, perhaps triggered by electrolyte changes, by variations in the afferent or efferent arteriolar calibre, or in response to diffusable mediators released from other components of the JGA complex (Ryan *et al*, 1979; Gibson *et al*, 1989).

Aims.

The aim of this study was to test the hypothesis that the peripolar cell is part of the JGA by:

1) applying a new technique of scanning electron microscopy and microdissection to study the peripolar cell in the rat kidney, then

2) assessing how the peripolar cell responds to experimental stimuli which activate and suppress the JGA.

2. MATERIALS AND METHODS.

2.1 Animals.

Young adult male Sprague-Dawley rats (180-200gms) were used throughout. They were killed by subcutaneous injection of barbiturate (0.1ml/100g). The abdominal aorta was cannulated just proximal to its bifurcation and the inferior vena cava was cut. The aorta was clamped above the kidneys but below the liver. The kidneys were then perfused, first with heparinised saline until they blanched, then with 2% glutaraldehyde in Millonig's buffer (pH 7.4) for 10 minutes (Gibson *et al*, 1989). The kidneys were weighed, sectioned perpendicular to their long axis and further fixed by immersion for 24 hours in 2% glutaraldehyde.

2.2 Experimental stimulation and suppression of the JGA.

A) Renal artery constriction.

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 0.2mm) was applied to the left renal artery, close to the aorta. The right kidney was untouched. After the procedure, the animals were allowed free access to drinking water and were fed a standard rat diet (41B Oxoid). Four control animals were sham-operated: the arteries were exposed but left untouched. After 8 weeks, blood pressure was measured immediately prior to killing, using an automated tail cuff method.

B) Sodium loading/depletion.

Twelve animals were given a low sodium diet (Special Diet Services Ltd). They were divided into 2 groups: 6 rats were given 2% sodium chloride as drinking water, to produce a high sodium intake, while the second group (low sodium) had free access to tap water. At the end of four weeks the animals were killed.

2.3 Microscopy.

A) Light microscopy.

A block of kidney tissue from each animal was embedded in "Transmit" resin and fifty serial 3μm sections were stained with toluidine blue. Twenty glomeruli from each animal were selected from all areas of the renal cortex such that the superficial and deep zones were represented equally. Their vascular poles were examined in their entirety by following them through the serial sections and noting whether they contained granulated peripolar cells. A peripolar cell index for light microscopy, (PPI[LM]), was derived by expressing the number of glomeruli in which at least one granulated peripolar cell was identified as a percentage of the total number of glomeruli examined in each kidney. The position of each granulated peripolar cell within the renal cortex was mapped, with the aid of an eyepiece graticule, by measuring in high power (X40) microscope fields the distances from the corticomedullary junction to the peripolar cell and from the corticomedullary junction to the most superficial glomerulus in the same straight line (Figure 2.1). These two distances were expressed as a ratio; therefore, a ratio of one represents the outermost glomerulus and zero the innermost glomerulus. The

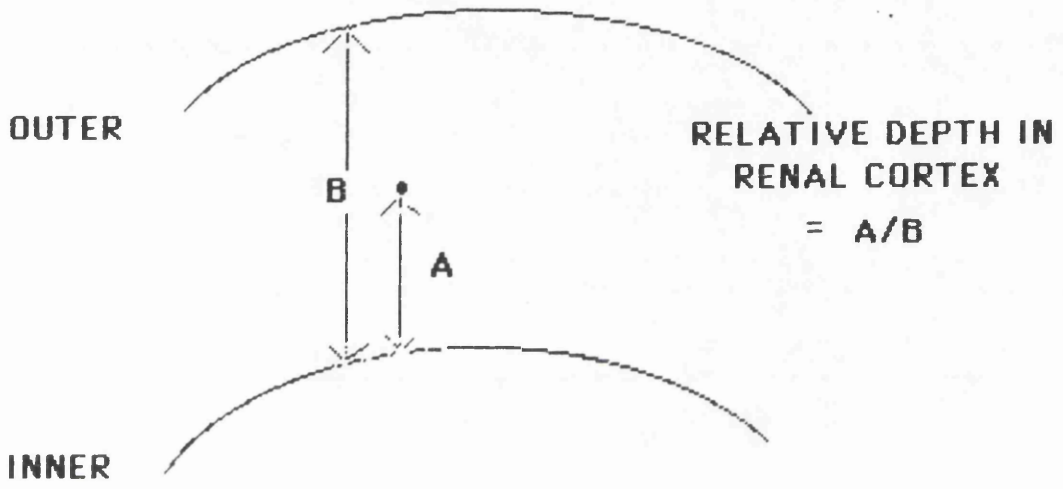


FIGURE 2.1 Calculation of relative depth of glomeruli in renal cortex. A = distance of glomeruli from medullary cortical junction, B = full depth of cortex.

deepest glomerulus represented the corticomedullary junction.

Immunocytochemistry.

To identify renin-secreting cells, we used an immunoperoxidase technique and affinity purified FAb fragments prepared from rabbit anti-mouse renin antiserum. The antiserum, a gift from Professor K. Poulsen (Copenhagen), cross-reacts with rat renin and has been extensively used for immunohistochemistry (Taugner *et al*, 1979). To accentuate the staining positivity for image analysis the immunohistochemical technique was modified: the staining with the secondary and tertiary antibodies was repeated and the nuclear counterstain was omitted. The area of renin staining was measured, the number of glomeruli counted, and the results were expressed as an area of renin positivity per 100 glomeruli.

B) Scanning electron microscopy.

After fixation, 1mm slices of kidney were cut on a vibratome and washed in Sorensen' s buffer. The slices were then osmicated for 1 hour in 1% Osmium tetroxide, dehydrated through graded alcohols and critical point dried using carbon dioxide. The kidney slices were mounted on metal stubs using conductive paint and glomerular tufts were removed by microdissection using a fine ophthalmic scalpel and a stereo-dissecting microscope. The tufts were collected and placed on metal stubs covered with double sided tape. Both the tissue slices and the dissected tufts were coated with gold in a Polaron sputter coater and examined with a Jeol scanning electron microscope (JSM 6400).

Bowman's capsules were searched for the presence of a vascular pole, which in turn was examined for peripolar cells. Twenty vascular poles were examined in each animal, 10 from the superficial cortex and 10 from the deep cortex. A peripolar cell index (PPI[BC]) was derived by expressing the number of vascular poles in which at least one peripolar cell was identified as a percentage of the total number of vascular poles examined.

On the glomerular tufts, twenty vascular poles were also examined and peripolar cells counted. A PPI[tufts] was calculated as above. The total number of peripolar cells found on the tufts and in the Bowman's capsules were added together and the mean number of peripolar cells per glomerulus was then calculated for each group.

Low power scanning electron micrographs were used to map the relative position of each peripolar cell within the renal cortex by measuring the distance from the peripolar cell to the corticomedullary junction and the distance from the corticomedullary junction to the most superficial glomerulus in the same straight line (Figure 2.1). The measurements were expressed as a ratio as for light microscopy.

C) Transmission electron microscopy

Ten Bowman's capsules previously examined by SEM and known to contain peripolar cells were dissected from the kidney slices. The small tissue blocks were washed in acetone, cleared in propylene oxide and embedded in Araldite resin. Six were serially sectioned and the 1μm sections were stained with toluidine blue and examined by light microscopy to identify granulated peripolar cells. The other 4 glomeruli were

sectioned until light microscopy of 1um sections indicated that the plane of section was approaching the vascular pole. Then, ninety nanometre serial ultrasections were then cut until the entire vascular pole had been sectioned. The ultrasections were stained with uranyl acetate and lead citrate and examined on a Philips CM10 electron microscope.

2.4 Statistics.

Results were analysed using the Mann Whitney U test. Spearmans' rank correlation coefficient was used to examine the relationship between the renin containing cells and the number of peripolar cells.

3. RESULTS.

3.1 Normal rat kidney.

(1) Peripolar cell.

Anatomy

Light Microscopy.

Granulated peripolar cells were identified by their cytoplasmic granules and by their position at the vascular pole (Figure 3.1). They were small, rounded or elongated cells, with scanty cytoplasmic granules.

The serial sections of the six Bowman's capsules containing peripolar cells previously identified by SEM showed no granulated peripolar cells, although granular myoepithelioid cells were identified. These were always extraglomerular and easily distinguishable from peripolar cells.

Scanning electron microscopy.

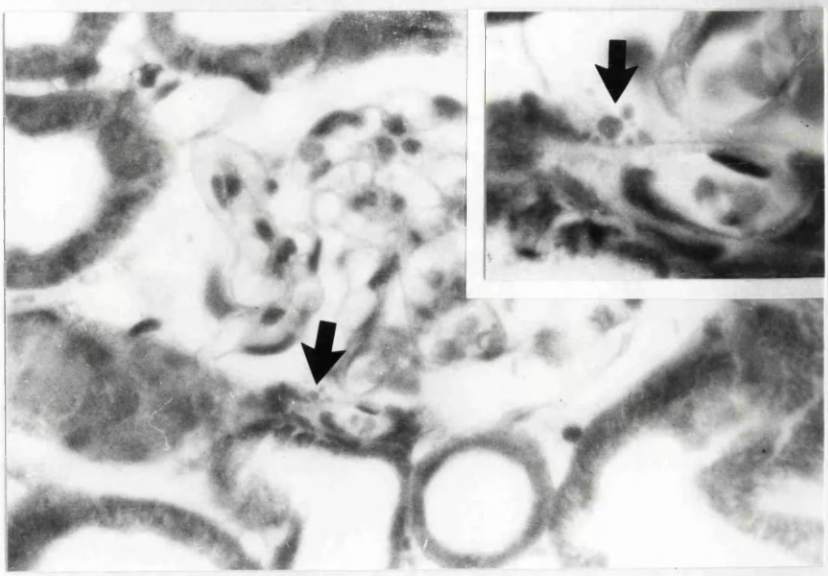
Peripolar cells (Figure 3.2) were found in all kidneys at the vascular poles of glomeruli, both within Bowman's capsule (Figures 3.2 and 3.3) and on the tufts (Figure 3.4). Usually one or two, but occasionally up to four peripolar cells were found at a single vascular pole. They were identified by their location and by their surface morphology. They lay between the parietal cells and podocytes but were distinguishable from both. Peripolar cells were associated with both afferent and efferent arterioles (Figure 3.1); some were situated between the two vessels sending processes around both.

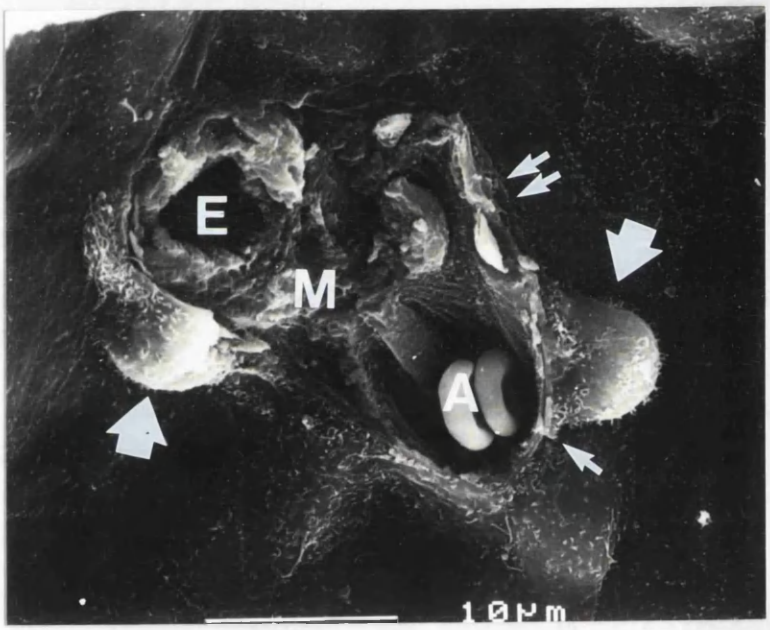
On the glomerular tufts it was not always possible to see how far the processes of the peripolar cells extended due to encroachment of the podocytes; in other cases, processes and

Figure 3.1 A light micrograph of a vascular pole of glomerulus showing a granulated peripolar cell (arrow). Insert shows large granules, which vary in size. MSB X400, Insert x1000

Figure 3.2 A scanning electron micrograph of a vascular pole consisting of 1 large afferent (A) and 1 small efferent (E) arteriole connected by mesangial region (M). A peripolar cell (arrow) is associated with each arteriole. Note: One peripolar cell associated with afferent arteriole has a broken process (small arrow) and a process encircling the arteriole (double arrow). The peripolar cell associated with the efferent arteriole has a process that fuses with the parietal epithelium. X4600

Figure 3.3 Vascular pole with 3 peripolar cells (arrows). X3000




E
M
A
10μm


10μm

cell bodies could be seen encircling the arterioles. In some cases podocytes themselves sent processes around the arterioles of the vascular pole, but pedicels were always identified on the processes.

Peripolar cells were dendritic. The cell body (4-6μm in diameter) had a smooth surface with a variable number of microvilli (Figure 3.5). The cell bodies varied in shape but the majority were spindle-shaped (Figures 3.5 and 3.6). Usually two tapering processes extended from the cell body in opposite directions (Figure 3.6). The cell processes, and when spindle-shaped, the cell body itself encircled the arterioles (Figure 3.5). The cell processes varied in length; the longest encircling the full circumference of the arteriole. Some processes flattened out and merged with the parietal epithelium (Figure 3.1), while others were broken, presumably at the time of tuft removal. Pedicels or branching processes were never observed.

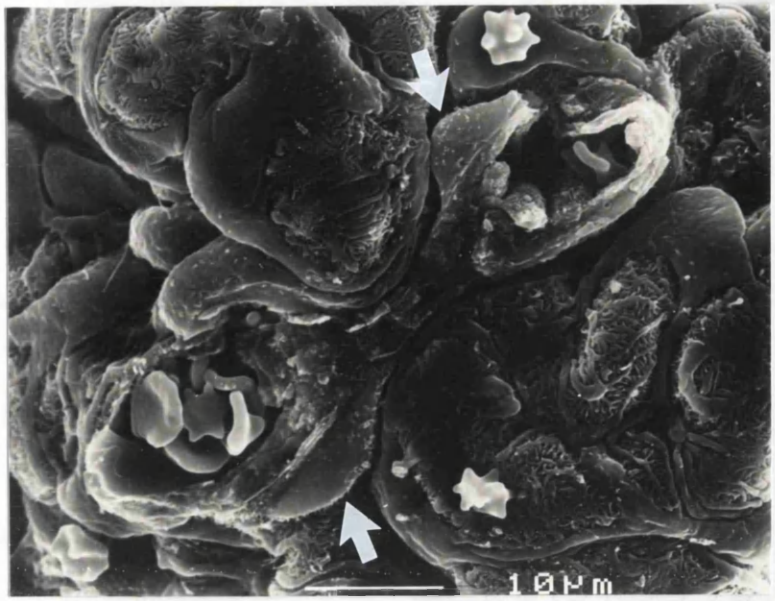
Transmission electron microscopy.

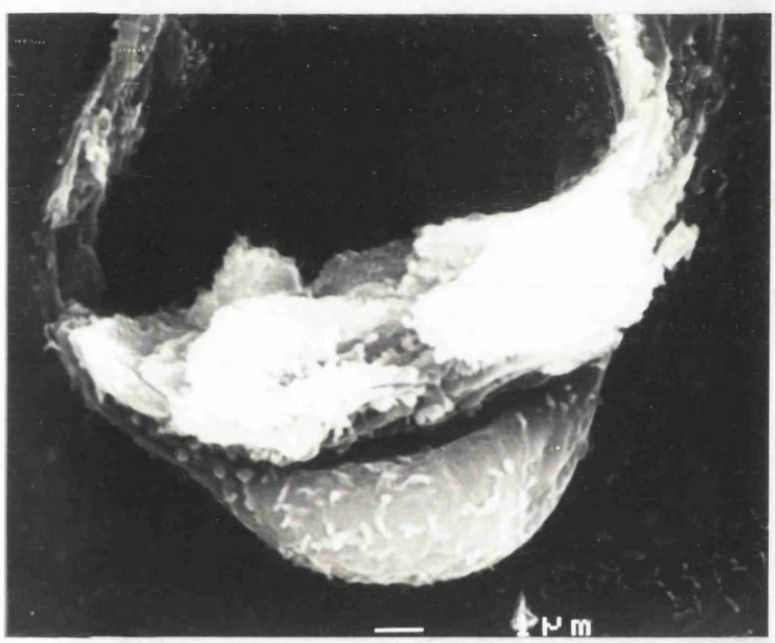
Peripolar cells rested only partly on the basement membrane of Bowman's capsule; both cell processes and parts of cell bodies overlay squamous parietal cells (Figure 3.7). The nuclei had a thick rim of heterochromatin. The cell body cytoplasm contained mitochondria, small amounts of rough endoplasmic reticulum, Golgi apparatus, polyribosomes and occasional lipid droplets. No secretory granules, dense lysosomes or multivesicular bodies were identified on serial ultrasectioning and no nerve terminals were identified on the basal aspect of the cells.

Figure 3. 4 A vascular pole on the glomerular tuft, showing both arterioles with a peripolar cell (arrow) associated with each. x3000

Figure 3.5 A dendritic peripolar cell with the cell body encircling an arteriole and sending processes in opposite directions. x12000

Figure 3.6 A peripolar cell with processes (arrows) extending in opposite directions. x10000


10μm


1μm

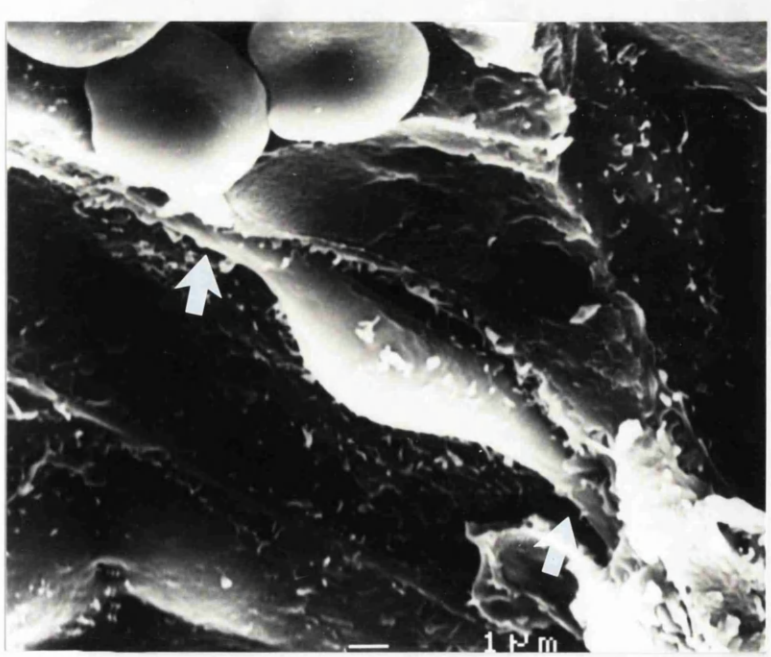
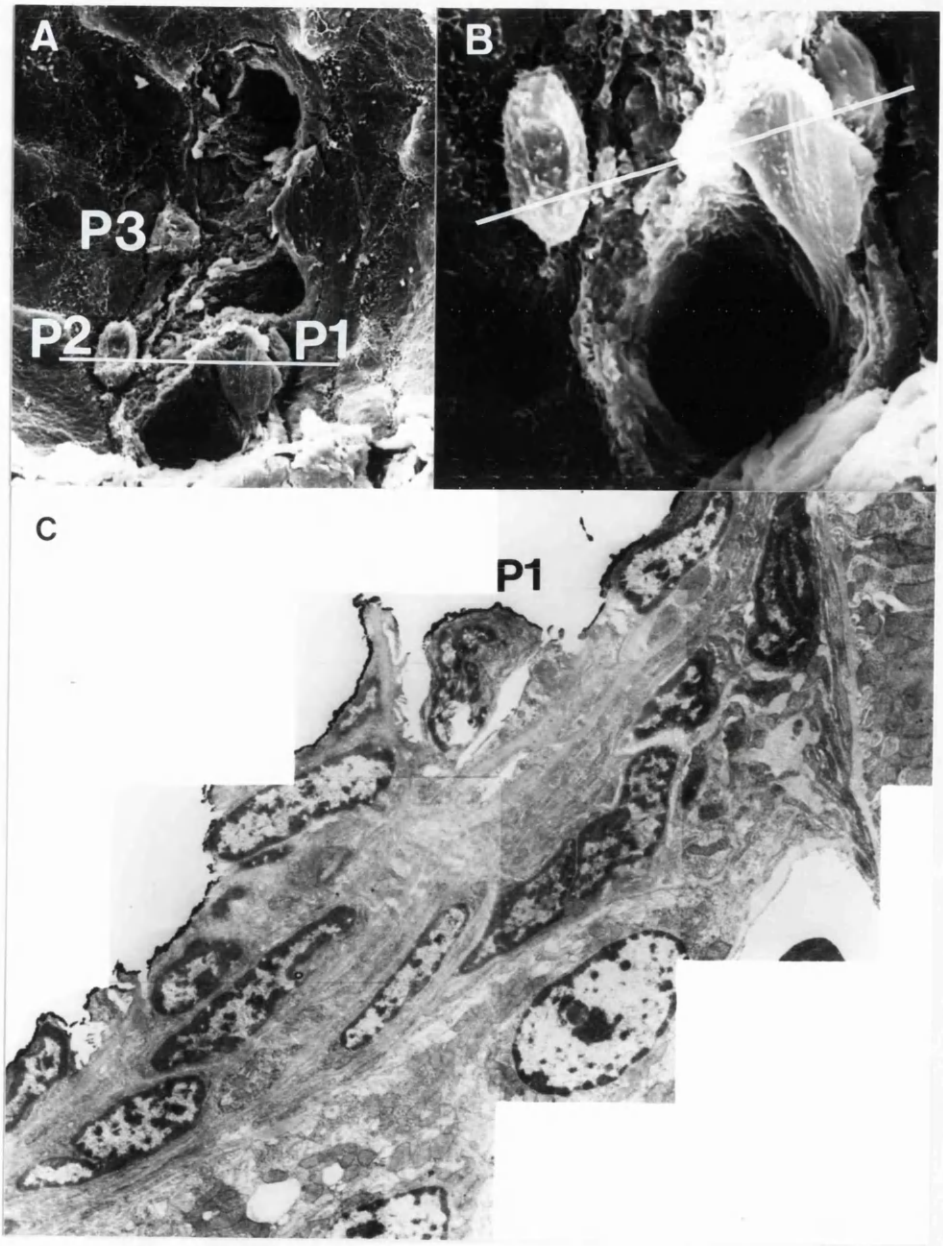

1μm

Figure 3.7 (a) A vascular pole with 3 lumina. Three peripolar cells can be identified P1, P2 and P3. Line across vascular pole shows approximate line of sectioning. X3000

(b) High power view of area with line in (a). X7000

(c) Montage of transmission electron micrographs showing area of vascular pole depicted by line in (a) and (b), including one of the peripolar cells P1. X6000


A
P3
P2
P1
B
C
P1

Peripolar cell numbers.

Light microscopy.

No more than one granulated peripolar cell was identified at any vascular pole. The mean (±SEM) PPI[LM] was 3.9% ± 2.3 (range 0 - 14.3). No granulated peripolar cells were found in three of the six kidneys. Granules were never identified in either parietal or podocytic glomerular epithelial cells.

Scanning electron microscopy.

Peripolar cells were found in more than half of the vascular poles in Bowman's capsule (mean PPI[BC] = 55% ± 4.65) compared with a fifth of the vascular poles on the tufts (PPI[tufts] = 21.2% ± 4.59). The mean number of peripolar cells per glomerulus was 0.9 ± 0.11.

Distribution of peripolar cells within the renal cortex.

Glomeruli with peripolar cells were randomly distributed throughout the renal cortex (Figure 3.8).

(2) Glomerular morphology.

Vascular pole.

The vascular pole consisted of two (Figure 3.2) or sometimes three vessels. The extra lumen was always an efferent vessel. The arterioles varied in diameter, with the larger afferent arteriole being up to 15μm in diameter and the smaller efferent arteriole up to 10μm in diameter. The arterioles were separated by the broken mesangial region. The

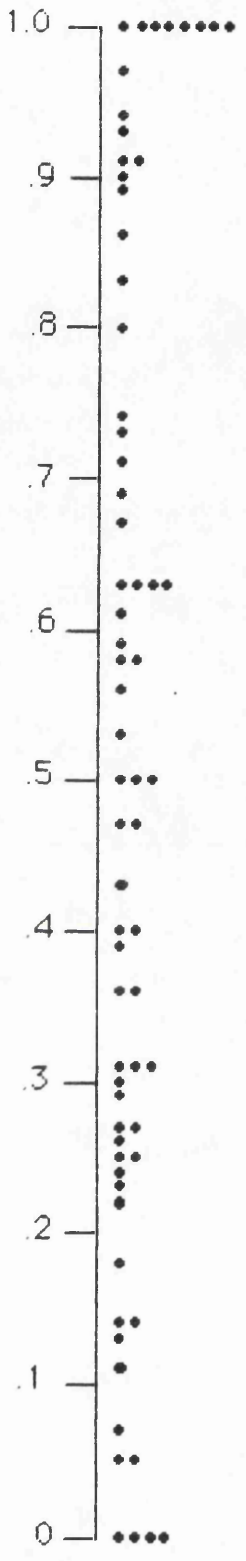


Figure 3.8 Distribution of all glomeruli with peripolar cells in the normal kidneys (n=6). 0 = cortico-medullary junction, 1.0 = outer cortex.

distance between the afferent and efferent arterioles varied from 5um to 25um. In Bowman's capsule the cells surrounding the arterioles were parietal epithelial cells, podocytes or peripolar cells: on the tuft only podocytes and peripolar cells could be identified.

Parietal epithelium.

Bowman's capsule was lined by polygonal squamous epithelial cells with single cilia and a variable covering of short microvilli which predominated at the cell border. Some parietal cells adjacent to the arterioles were elongated and partially surrounded the vascular pole.

Occasionally, dendritic cells with branching processes and interdigitating pedicels - parietal podocytes-were found lining Bowman's capsule (Figure 3.9). These cells completely covered Bowman's capsule. When the glomerular tuft was identified, it was usually shrunken.

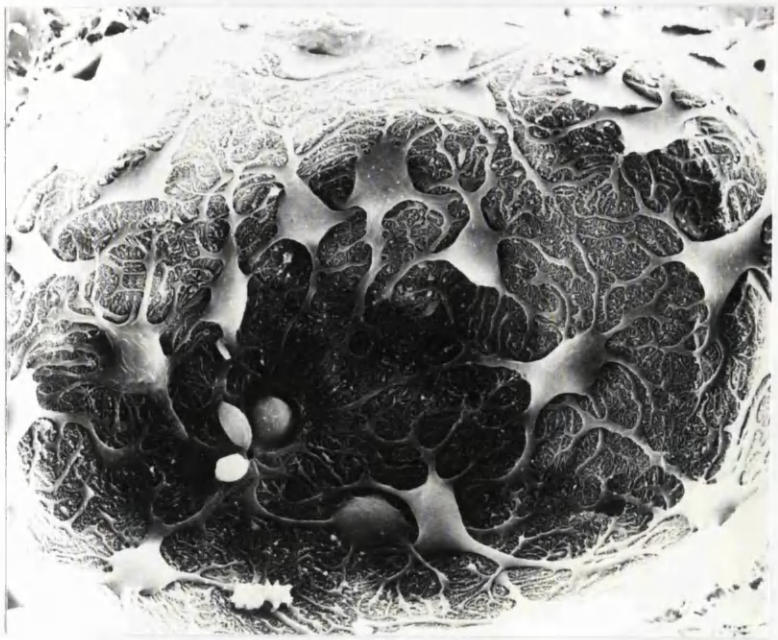
Glomerular tufts.

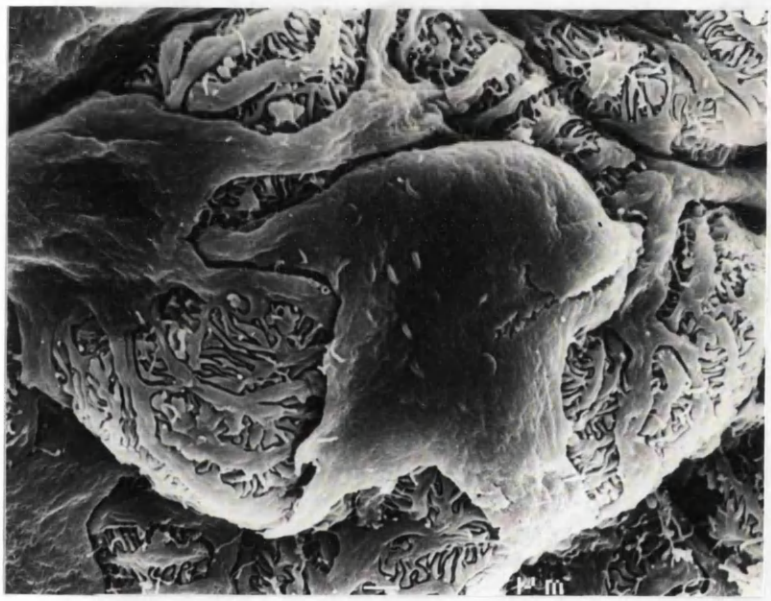
The capillaries were covered with podocytes which had a ruffled cell body, and occasionally, a variable number of microvilli. Their branching processes terminated in interdigitating pedicels (Figure 3.10).

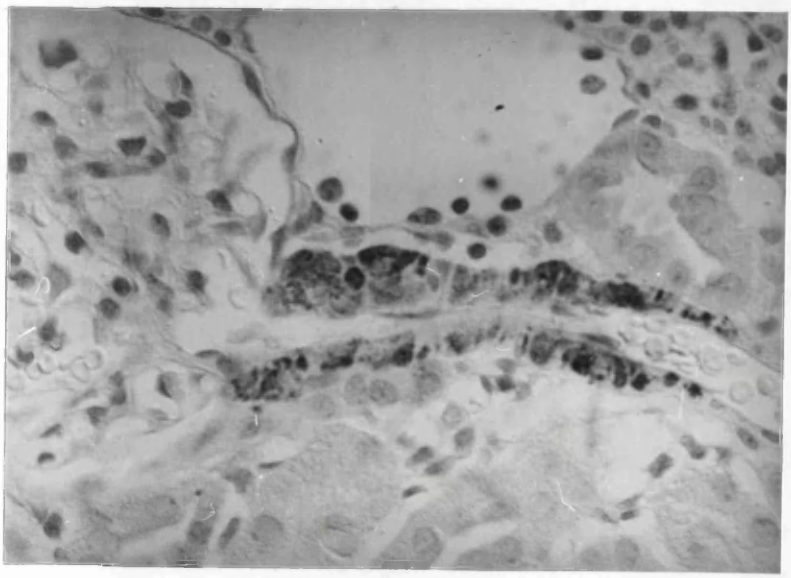
Figure 3.9 Parietal podocytes lining Bowman's capsule. X3500

Figure 3.10 Typical appearance of podocytes found on the glomerular tuft. X1000

Figure 3.11 Increase in renin-containing cells due to renal artery constriction. Renin PAP X400







3.2 Experimental stimulation and suppresion of the JGA.

A) Renal artery constriction.

(1) Blood pressure

All clipped animals were hypertensive. The mean (±SEM) systolic blood pressure was 211± 15 mmHg, compared with 146± 6 mmHg for the sham-operated controls. The mean (±SEM) kidney weights were 1.53± 0.13g (clipped), 2.02± 0.13g (unclipped) and 1.63± 0.09g (sham-operated).

(2) Renin immunohistochemistry.

The clip on the left renal artery caused hyperplasia of the renin secreting cells with extension along glomerular arterioles (Figure 3.11), while in the unclipped kidneys there were fewer renin-secreting cells. The area occupied by renin-secreting cells and their number were significantly higher in the clipped left kidney compared to the sham-operated kidneys and the unclipped right kidney, and significantly lower in the unclipped right kidney compared to the sham-operated kidneys (Table 1).

(3) Peripolar cell.

Light microscopy.

Only one peripolar cell was identified in the sham-operated group. No peripolar cells were present in the clipped kidneys, but some were identified in 2 of the 5 unclipped kidneys (Table 1). The mean (±SEM) PPI[LM] for these kidneys was 2.6± 1.8. There was no difference in PPI[LM] between any of the groups. PPI[LM] did not correlate with the

TABLE 1

Mean peripolar cell indices (PPI) , mean number of peripolar cells per glomerulus and mean renin areas and cell numbers for the clipped and unclipped kidneys in the experimental group and the sham-operated group. Superscript letters indicate where there are significant differences between compared groups. Significant differences between paired indices are shown by matching superscript letters

	EXPERIMENTAL GROUP			
	CLIPPED	UNCLIPPED	CONTROL	STATISTICAL
	n=5	n=5	n=8	SIGNIFICANCE
PPI[LM]	0	2.58 (± 1.8)	0.62 (±0.62)	NS
PPI[BC]	56.4 (±5.82)	53.6 (±5.99)	53.4 (±6.64)	NS
PPI[tuft]	13.4^{a} (±3.43)	30.8^{a} (±4.59)	17.6 (±3.55)	$^{a}0.004$
No./GLOM	0.95 (±0.14)	1.08 (±0.12)	1.08 (±0.15)	NS
RENIN AREA	1.82^{bc} (±0.45)	0.04^{bd} (±0.01)	0.58^{cd} (±0.14)	$^{bcd}0.001$
RCI	94.7^{bc} (±40.2)	0.84^{bd} (±0.84)	21.2^{cd} (±4.9)	$^{bcd}0.001$

PPI=Peripolar Cell Index

LM=Light Microscopy

BC=Bowman's Capsule

RCI= Renin Cell Index

No./GLOM = Number of Peripolar Cells per Glomeruluus

number of renin containing cells or blood pressure.

Scanning electron microscopy.

PPI[tufts] of the unclipped right kidneys was significantly higher than the clipped left kidneys (Table 1). There were no other differences between the clipped, unclipped kidneys and controls (Table 1). There was no correlation between peripolar cell numbers and the area of immunoreactive renin staining.

Distribution of peripolar cells within the renal cortex.

Glomeruli with peripolar cells were randomly distributed throughout the renal cortex in all experimental groups (Figure 3.12).

(4) Glomerular Morphology.

The sham-operated kidneys were histologically normal. The clipped kidneys showed tubular atrophy with glomerular crowding. Some glomeruli in three of the unclipped kidneys contained granulated podocytes. The blood vessels appeared tortuous but intrinsically normal. Four of the five unclipped kidneys showed histological features of malignant hypertension.

By scanning electron microscopy the parietal epithelium was usually normal, but occasional glomeruli contained parietal podocytes. Adjacent to the vascular pole occasional parietal epithelial cells had a bossellated surface appearance suggesting the presence of intracellular granules (Figure 3.13).

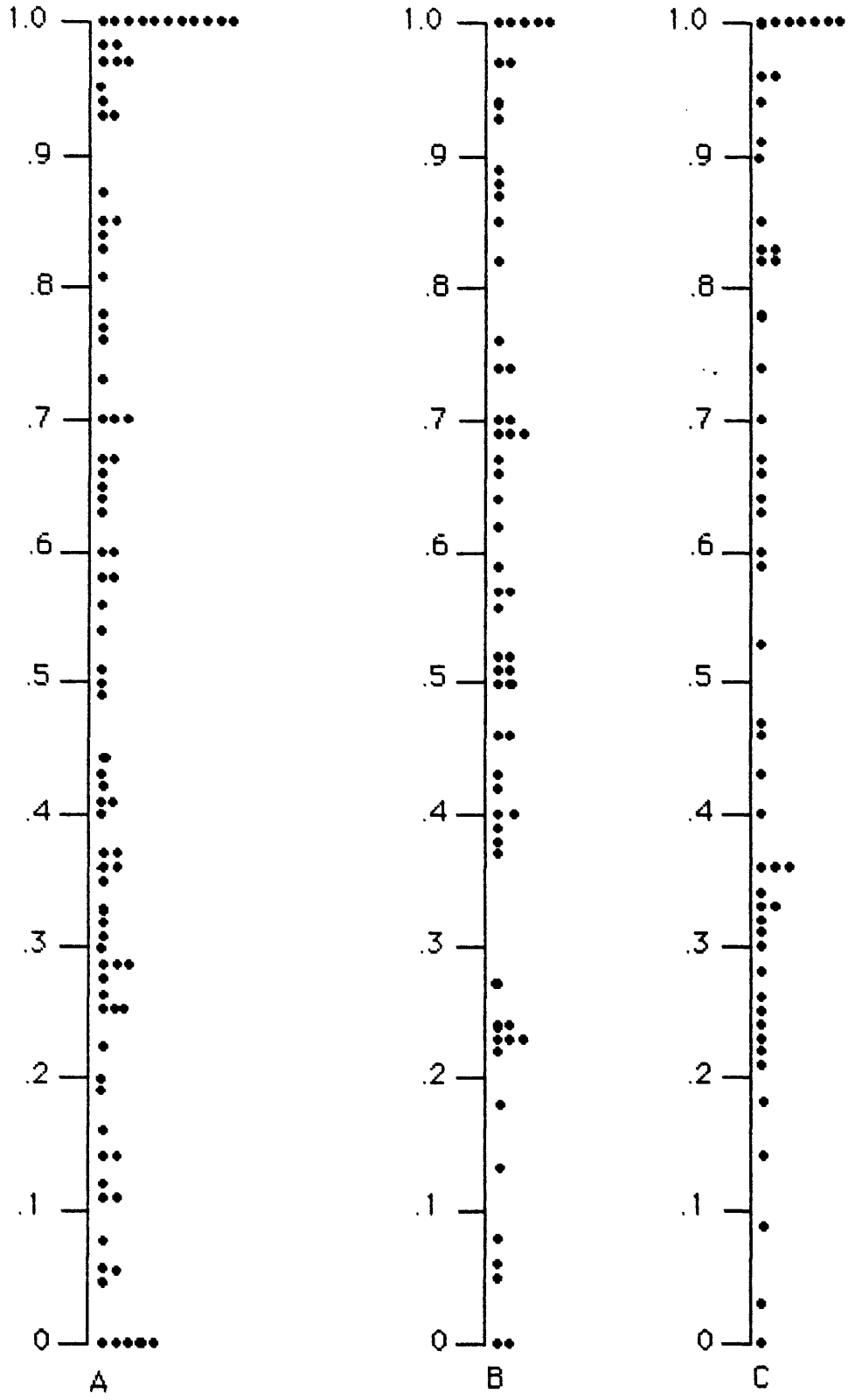


Figure 3.12 Distribution of all glomeruli with peripolar cells for the (A) sham-operated (n=8), (B) clipped left (n=5) and (C) unclipped right (n=5) animals. 0=corticomedullary junction, 1.0 =outer cortex.

Minor changes were found in the glomerular tufts. Bossellations were found in podocytes in both clipped and unclipped kidneys (Figure 3.14). In addition, occasional podocytes had more microvilli, some microblebs, collapsed vacuoles and occasionally loss of pedicels (Figures 3.14 and 3.15).

B) Sodium loading/depletion.

(1) Renin immunohistochemistry.

There was hyperplasia of renin containing cells in the low sodium animals (Figure 3.16) while, in the high sodium animals there was a reduction in the number of renin-secreting cells. In the low sodium animals the area occupied by renin-secreting cells and their number were significantly higher than in the high sodium animals (Table 2).

(2) Peripolar cell.

Light microscopy.

In the low sodium group, granulated peripolar cells were identified in one of the six animals; the mean PPI[LM] of the group was 3.33 (Table 2). In the high sodium group, granulated peripolar cells were identified in two of the six animals; the mean PPI[LM] was 1.67 (Table 2). There was no significant difference between the low sodium and the high sodium groups.

Scanning electron microscopy.

There was no significant difference in any of the

TABLE 2.

Mean peripolar cell indices (PPI), mean number of peripolar cells per glomerulus and the mean renin area for sodium depleted and sodium loaded groups.

	SODIUM DEPLETED n=6	SODIUM LOADED n=6	STATISTICAL SIGNIFICANCE
PPI[LM]	3.33 (±3.33)	1.67 (±1.05)	N.S.
PPI[BC]	63.3 (±7.09)	74.7 (±1.6)	N.S.
PPI[tufts]	26.2 (±4.01)	34.8 (±4.2)	N.S.
No./GLOM	1.34 (±0.12)	1.58 (±0.09)	N.S.
RENIN AREA	0.75 (±0.22)	0.09 (±0.03)	$p<0.001$
RCI	48.4 (±11.4)	9.8 (±2.9)	$p<0.001$

PPI=Peripolar Cell Index

LM=Light Microscopy

BC=Bowman's Capsule

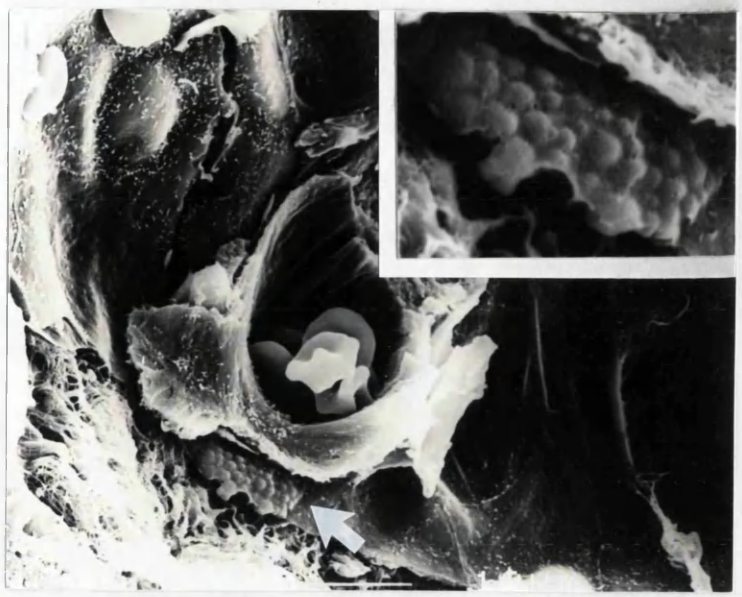
RCI= Renin Cell Index

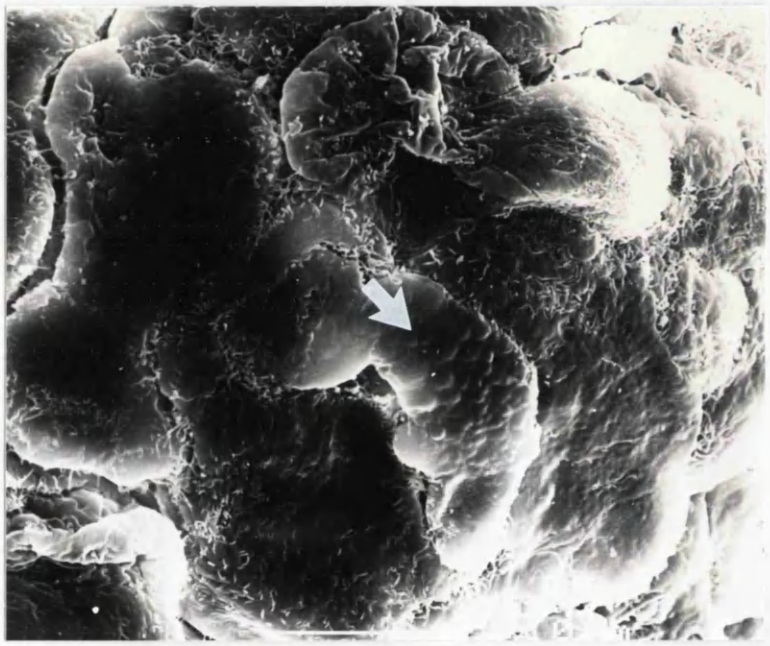
No./GLOM = number of peripolar cells per glomerulus

Figure 3.13 Granulated parietal cell lying adjacent to the afferent arteriole X3600. Insert. High power showing bossellated surface. X5000

Figure 3.14 Podocytes exhibiting bossellations (arrow) and loss of foot processes. X5000

Figure 3.15 Podocytes showing a variable number of microvilli and loss of foot processes. X5000






1 µm

Figure 3.16 Increase in renin-containing cells in sodium depletion. Renin PAP, interference microscopy. X400

peripolar indices between the two groups (Table 2). There was no correlation between the number of peripolar cells and the area of immunoreactive renin staining or the number of renin-secreting cells.

Distribution of peripolar cells in the renal cortex.

In both groups glomeruli with peripolar cells were randomly distributed throughout the renal cortex (Figure 3.17).

(3) Glomerular Morphology.

The kidneys of both sodium-loaded and sodium-depleted animals were histologically normal. On SEM examination, occasional Bowman's capsules had parietal podocytes and rarely granulated parietal epithelial cells were identified.

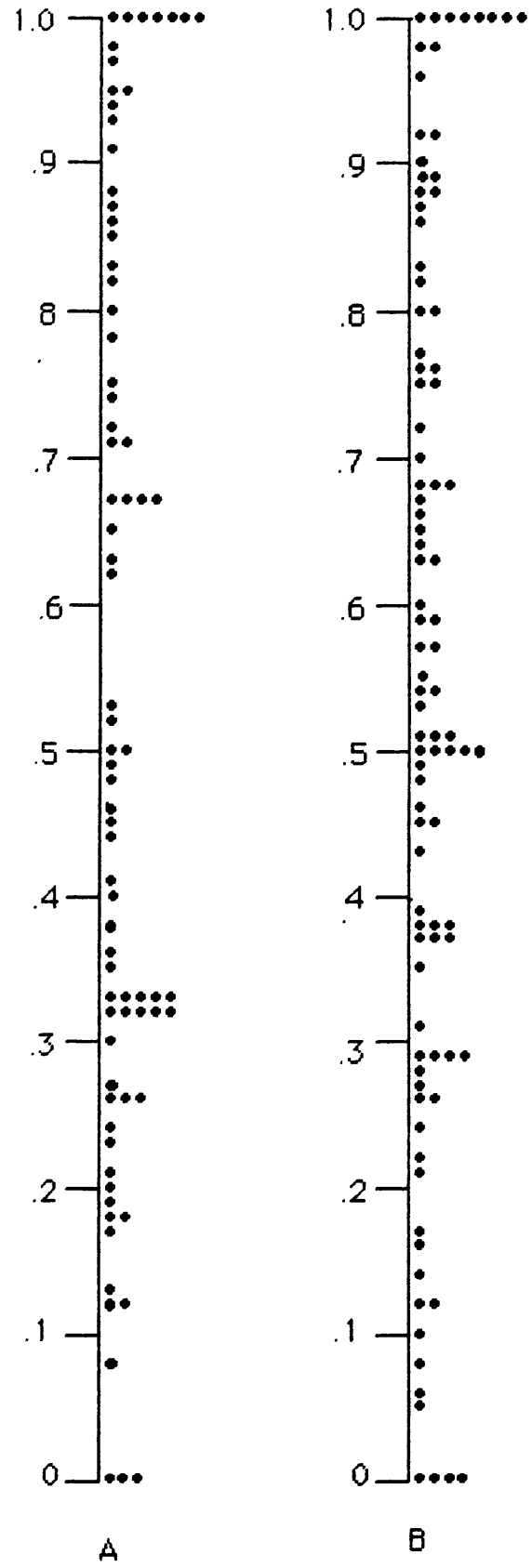


Figure 3.17 Distribution of all glomeruli with peripolar cells in (A) sodium depleted (n=6) and (B) sodium loaded (n=6) animals. 0 = corticomedullary junction, 1.0 = outer cortex.

4 DISCUSSION.

The anatomy of glomerular epithelial cells has been well documented (Burke, 1976; Andrews, 1979; Jones, 1979). However, only recently has the removal of the glomerular tufts allowed SEM examination of the cells surrounding the vascular pole. This technique allowed the peripolar cell to be distinguished as a specific cell type in the kidney (Gibson *et al*,1989; Kelly *et al* ,1990).

Removal of glomerular tufts has been achieved previously (Casellas 1986). The technique employed was to roll a stick covered in adhesive tape over the surface of a section of kidney; the glomerular tufts adhere and can be examined by SEM. The advantages of our technique are that less damage is done to the tissue, and removing individual glomerular tufts can allow a comparison of the Bowman's capsule with the particular tuft removed from it.

4.1 Normal Kidney.

(1) Peripolar cell.

Light microscopy.

The peripolar cell is a granulated epithelial cell situated within the glomerulus in the reflection of Bowman's capsule between the podocytic and parietal epithelium (Ryan *et al*,1979). In sheep, peripolar cells are large and the granules closely packed (Ryan *et al*, 1979). In comparison, rat peripolar cells are small with scanty cytoplasmic granules. Also, as in man (Gardiner and Lindop, 1985) and mice (Gall *et al*, 1986) granulated peripolar cells in the rat, are sparse and difficult to find.

Scanning electron microscopy.

Gibson *et al* (1989) used scanning electron microscopy (SEM) to examine the surface morphology of the rat peripolar cell for the first time. This work provided the first corroboration that the peripolar cell is a distinct cell type. The location and morphological appearance were the criteria for the identification of peripolar cells. In this study, the peripolar cells were similar in appearance to those described by Gibson *et al* (1989). In sheep almost all the peripolar cells are granulated and by SEM they are globular in shape, occasionally elongated, with a bossellated cell surface (Kelly *et al*, 1990). In the rat, peripolar cell granules are scanty and SEM is unable to confirm their presence or absence. The difference in cell size and granulation between the sheep and rat cannot be explained.

In some species peripolar cells are globular, in other species dendritic, and some species have a mixture of the two types (Gibson *et al*, -unpublished results). In this study, some parietal cells were elongated and encircled the vascular pole, some peripolar cells processes fused with the parietal epithelium and some peripolar cells resembled podocytes. In the rat transitional forms may indicate that parietal cells may transform in to podocytes or podocytes to parietal cells, with the peripolar cell being the intermediate stage. The peripolar cell is situated at a junction between two other epithelial cell types and there may be a gradual change of differentiation from one cell type to the other. The appearance of the peripolar cells and the presence of both parietal podocytes and proximal

tubular-like cells both in humans (Gibson *et al*, 1992), mice (Carpino *et al*, 1976) and rat (Crabtree 1941), could indicate that either the parietal epithelium is pluripotent or that cell migration may occur. As all the components of the glomerulus are of mesodermal origin a transformation is possible (Morita *et al*, 1973). These observations could also suggest that the border-line between the parietal epithelium and the tubular epithelium and between the parietal epithelium and the podocytes may be dynamic in nature.

In contrast to previous reports (Gibson *et al*, 1989; Kelly *et al*, 1990), in this study peripolar cells were found on glomerular tufts. The numbers present on the tufts varied and this suggests that examination of the tufts is also required to obtain true peripolar cell numbers. However, one of the drawbacks of the technique of microdissection is the lack of orientation of the glomerular tufts when removed; the vascular pole is not always visible. To obtain the true number of peripolar cells at one vascular pole requires examination of both the vascular pole in Bowman's capsule and on the tuft removed from that Bowman's capsule. In this study the mean number per glomerulus was calculated from the total number of cells on the tufts and in the Bowman's capsule. Photographing the blocks before removal of the glomerular tufts and then after removal by placing the tufts in numbered rows with their position marked on the photograph will tell which tufts have been removed successfully. Therefore, a visible vascular pole on the Bowmans capsule and on the glomerular tuft will give a true total of peripolar cells for each vascular pole.

Transmission electron microscopy.

We searched for granules by sectioning individual peripolar cells previously identified by SEM. A variety of tissues have been subsequently examined by TEM following critical point drying (Wickham and Worthen, 1973; Gessinger *et al*, 1978, 1979). Previous processing and examination by SEM placed limitations on the assessment of the fine detail of cell structure. However, the cytoplasmic organelles could still be distinguished, and secretory granules would have been obvious. No granules were found proving that non-granulated peripolar cells exist; indeed in the rat, most peripolar cells are probably not granulated. It has been suggested that non-granulated peripolar cells exist and only under appropriate conditions would they become granulated (Gardiner *et al*, 1986; Gibson *et al* , 1989).

The morphological evidence of differentiation did not suggest an absorptive function - microvilli were sparse and no dense lysosomes were seen. There was also no strong evidence of a secretory function - there was little RER, the Golgi apparatus was not large and no granules and no nerve terminals were identified. The nuclear morphology and the small number of organelles revealed by TEM suggest that the peripolar cells were inactive.

Peripolar cell numbers.

The number of peripolar cells varies between species and even between animals. They are prominent and numerous in sheep but are present in smaller numbers in almost all other mammals studied (Gall *et al*, 1986; Mbassa,1989) including man

(Gardiner and Lindop, 1985). Peripolar cells have also been identified in chickens (Morild, 1988), amphibians (Hanner and Ryan, 1980) and in elasmobranch fish (Lacy and Reale, 1989). Light microscopy showed that a minority of rat glomeruli contain granulated peripolar cells (maximum PPI = 14%). This confirms the observation of Gall *et al* (1986) who examined random histological sections of rat kidney and showed that granulated peripolar cells were scanty. A more accurate assessment of peripolar cell numbers was obtained by using serial sections. Non-granulated peripolar cells would be unidentifiable by LM. SEM shows peripolar cells in the majority (approximately 55%) of glomeruli in the rat. This is close to the proportion found by Gibson *et al* (1989) using the same technique. Scanning electron microscopy is clearly the best way to identify and count peripolar cells.

Distribution of peripolar cells in the renal cortex.

It has been reported that in the rat (Gibson *et al*, 1989) and in sheep (Ryan *et al*, 1979) there are more peripolar cells in the outer cortex of the kidney. However, both LM and SEM showed no clear zonal distribution of peripolar cells within the sheep renal cortex once the uneven distribution of the glomeruli had been taken into account - the outer renal cortex contains more glomeruli than the inner cortex (Kelly *et al*, 1990). This study confirms that finding.

(2) Glomerular morphology.

Vascular pole.

In general, one arteriole was larger than the other. Vascular casts have shown that, except for some juxtamedullary glomeruli, the afferent arteriole has a larger diameter than the proximal end of the efferent arteriole (Evan and Dail, 1977). Hence, the larger lumen was assumed to be the afferent arteriole and the smaller the efferent arteriole. When 3 lumina were present one large lumen was associated with 2 small lumina and it was hence regarded as 1 afferent arteriole and 2 smaller efferent arterioles (Gibson *et al*, 1989). This interpretation is also supported by vascular cast studies; efferent arterioles run a very short course before dividing to form the peritubular capillary plexus, close to the parent glomerulus (Evan and Dail, 1977). Alternatively, the two vessels may represent the converging glomerular capillaries about to form a single efferent arteriole (Nopanitaya, 1980).

Parietal epithelium.

The parietal epithelial layer of Bowman's capsule has attracted little attention and is thought to have a passive role within the nephron. It consists of squamous epithelial cells with a single cilium and with microvilli demarcating the cell borders (Andrews, 1979). The presence of parietal podocytes lining Bowman's capsule has been noted in individual cases of haemolytic-uraemic syndrome and experimental polycystic disease in rabbits (Wilson, 1977; Ojeda and Garcia-Porrero, 1981; Ros *et al*, 1987). In this study they were occasionally

found in individual glomeruli in both normal and the experimental groups. The presence of a shrunken tuft within the Bowman's capsule may also indicate a glomerular lesion. Gibson *et al* (1992) showed that parietal podocytes were present around the vascular pole of essentially normal kidneys in humans. The significance of this observation is currently being assessed.

Parietal podocytes may allow leakage of fluid from the urinary space into the surrounding interstitial tissue (Wilson, 1977) in particular into the interstitium of the JGA (Rosivall, 1990). Fluid flow from the urinary space into the interstitium of the JGA would provide a rapid feedback path to the JGA (Rosivall and Taugner, 1986) which would have a more immediate effect than signals of tubular origin in which there would be a greater delay (Rosivall and Taugner, 1986). The above proposal was suggested for the parietal podocytes which covered the glomerular stalk. Gibson *et al* (1992) have reported the presence of parietal podocytes in "normal" human kidney, covering up to 20% of the area of Bowman's capsule. Fluid flow in these instances would not only be into the JGA but also into the interstitium of the renal cortex surrounding the glomerulus. This could have considerable functional importance. In this study occasional glomeruli had the whole of the Bowman's capsule covered with parietal podocytes. The tufts were always abnormal. There were so few that this would have little functional importance, but it may mean that the appearance of podocytes on Bowman's capsule is due to an abnormality of glomerular tufts.

Glomerular morphology.

My findings in normal glomerular tufts were similar to previous studies (Arakawa and Tokunaga, 1972; Skaaring and Kjaergarrd, 1974; Jones, 1979; Andrews, 1988). Each podocyte on the tuft consists of a centrally nucleated cell body from which major processes arise. These go on to branch further to form secondary and tertiary processes. Arising from the processes, usually at right angles, are the finger-like pedicels.

4.2 Experimental stimulation and suppression of the JGA.

The aims of this study were to compare the response of the peripolar cell with that of the renin-secreting cells of the JGA. The peripolar cell is ideally situated to respond to alterations in the calibre of the renal arterioles. A renal artery clip would be expected to narrow these in the clipped kidney and the increased perfusion pressure should dilate those in the unclipped kidney.

A) Renal artery constriction.

Changes in arterial pressure can be achieved by using Goldblatt's 2-kidney, 1-clip model. Using this model we have successfuly produced hypertension and hyperplasia of the renin-secreting cells in the clipped kidney and a fall in their number in the unclipped kidney. This confirms previous reports (Taugner *et al*, 1982; Helmchen and Kneissler; Fisher, 1961; Brown *et al*, 1966).

1) Peripolar cell.

Peripolar cells have not previously been examined in experimental renovascular hypertension. Both SEM and LM showed no significant difference in their number and size between the control group and the experimental group, and application of a unilateral renal artery clip did not affect the number in the contralateral kidney. The numbers of peripolar cells did not correlate with the renin containing cells. This implies that in this situation, the peripolar cells do not respond to the same factors which stimulate or suppress the renin secreting cells of the juxtaglomerular apparatus.

2) Glomerular morphology.

The pathological changes found in renovascular hypertension by light microscopy have been well documented, especially in man where hypertension due to stenosis of one renal artery, the opposite renal artery remaining intact, is analogous (reviewed by Heptinstall, 1992). Light microscopy of the clipped kidney shows tubular atrophy without glomerular damage. However, severe vascular and glomerular changes occur in the opposite kidney which is exposed to elevated blood pressure; the clipped (or stenosed) kidney is protected. The damage in the unclipped (nonischaemic) kidney is attributed to the high blood pressure, while the changes in the clipped kidney may be due either to ischaemia or to humoral factors (Szokol *et al*, 1979). Both renin and ANG II may induce proteinuria (Buhrer *et al*, 1977; Montolu *et al*, 1979; Eiger *et al*, 1982).

The glomerular tuft in experimental and pathological conditions has a limited variety of reponses to injury or

abnormal states (Arakawa and Tokunaga, 1972; Skaaring and Kjaergarrd, 1974; Jones, 1979; Andrews, 1988). The responses (increased microvilli, microblebs, loss of foot processes and uptake of protein) have been regarded as an attempt by the glomerulus to try to minimise protein loss (Arakawa and Tokunaga, 1972; Andrews, 1977). Scanning electron microscopy of the two kidney 1 clip model has not been previously reported.

Occasionally, both podocytes and parietal cells, at the vascular pole have a bossellated appearance and therefore probably contain granules. By LM, because of their location and the presence of granules, they would be called peripolar cells. Therefore, SEM can discriminate between peripolar cells and other glomerular epithelial cells.

B) Sodium loading/depletion.

This study confirms the effect of modifying sodium chloride intake on the JGA. An increase in sodium chloride delivery to the macula densa switches off renin production, resulting in a decrease in the number of renin secreting cells, while a reduction in sodium chloride causes an increase in renin production, with an increase in the number of renin secreting cells (De Rouffignac *et al*, 1974; Hill *et al*, 1983).

1) Peripolar cell.

Exocytotic release of granular material from peripolar cells into the urinary space has been found only in animals acutely depleted of sodium (Hanner and Ryan, 1980; Hill *et al*, 1983; Hill *et al*, 1984). This was interpreted as indicating

increased activity. The number of peripolar cells increases in acute sodium depletion in chickens (Morild *et al*, 1988) but not in sodium depleted normal and pregnant sheep (Hill *et al*, 1983; Hill *et al*, 1984). We chose a chronic model of sodium manipulation and examined peripolar cells by SEM to assess any change in cell size or number. In this study there was no significant difference in peripolar cell numbers or distribution between low sodium and high sodium animals when examined by both LM and SEM. Also the number of peripolar cells did not correlate with the response of the renin-containing cells to dietary sodium manipulation.

4.3 Function of the peripolar cell.

It has been proposed that the peripolar cell was a previously unrecognised part of the JGA. The main reasons proposed for this are 1) they increase in number in sodium and water depletion (Morild *et al*, 1988), 2) granule exocytosis into the urinary space has been seen in sodium depleted animals (Hill *et al*, 1983) and 3) the peripolar cell appears prominent in species with a small number of myoepithelioid cells (Ryan *et al*, 1979; Trahair and Ryan, 1988). It was further claimed that the peripolar cell was the source of a secretory factor, that caused proximal tubular sodium reabsorption (Ryan *et al*, 1979; Hill *et al*, 1983), yet to be identified. The peripolar cell does not contain immunoreactive renin (Gardiner and Lindop, 1985; Trahair *et al*, 1989). In this study the peripolar cell did not respond to the major stimuli for renin production, suggesting that the peripolar cell is not part of the JGA.

Plasma proteins such as albumin, immunoglobulins and fibrinogen have been found in the granules of the peripolar cells by means of immunocytochemistry (Trahair and Ryan, 1988; Nakajima *et al*, 1989; Gardiner and Lindop, 1991) suggesting that peripolar cells absorb protein from the glomerular filtrate (Trahair *et al*, 1989; Trahair and Ryan, 1988; Nakajima *et al*, 1989). Granules occur both in podocytes and in parietal epithelial cells in various pathological conditions (Dick and Kutz, 1968; Szokol *et al* 1979; Kincaid-Smith *et al*, 1984). In particular, in toxaemia of pregnancy granulation of glomerular epithelium predominates at the vascular pole (Sheehan and Lynch, 1973). Also, in the neonate, granulated cells occur at the vascular pole of the glomerulus, diminishing in number as the animals mature (Eguchi, 1974; Dhiab al naimi and Bearn, 1981; Alcorn *et al*, 1984; Gall *et al*, 1986). This has been attributed to the fact that serum protein levels rise rapidly in the immediate post-natal period resulting in proteinuria (Trahair and Ryan, 1988). There is no morphological difference between peripolar cell granules and the granules found in podocytes in disease (Morild *et al*, 1988; Nakajima *et al*, 1989). With specialised fixation, the ultrastructural appearance of the granules in both cells display an electron dense inner core containing immunoreactive fibrinogen and a less dense peripheral zone positive for IgM (Nakajima *et al*, 1989). The reason why epithelial cells in this peripolar position should be specialised for the uptake of filtered plasma proteins is not clear.

Finally, absorption of protein does not exclude a secretory function. Cathepsins and other lysosomal enzymes

are found in the renin-containing granules of the myoepithelioid cells (Taugner *et al*, 1985a; Taugner *et al*, 1985b). Renin secreting cells also absorb macromolecules and store them in their granules suggesting that they may in fact be modified lysosomes (Taugner *et al*, 1985a; Taugner *et al*, 1985b).

4.4 Conclusions.

In summary,

1) This study has confirmed that the peripolar cell is a morphologically distinct cell surrounding the vascular pole between the parietal epithelium and the glomerular podocytes.

2) The morphological evidence of differentiation did not suggest an absorptive or a secretory function. The nuclear morphology and the small number of organelles revealed by TEM suggest that the peripolar cells were inactive.

3) Despite previous reports to the contrary we found peripolar cells on isolated glomerular tufts; therefore it is essential that the glomerular tufts are also examined to obtain a true assessment of peripolar cell numbers.

4) Experiments which increased and decreased renin-containing cells showed no correlation between the numbers of peripolar cells and numbers of renin-secreting cells, suggesting that the peripolar cell is not part of the JGA. An experimental model which produces variation in the number of peripolar cells would help to elucidate their function and the relationship between the granulated or non-granulated forms.

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