Virchows Archiv A Pathol Anat Histopathol (1988) 412:471–477

Virchows Archiv A
Pathological Anatomy and Histopathology

Peripolar cells in the avian kidney

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Summary. Four white leghorn chickens were injected with furosemide (20 mg per kg body weight) three times at 12 h intervals and the kidneys fixed by perfusion after 36 h. Five chickens were injected with DOCA (desoxycortone trimethylacetate, 75 mg per kg body weight) three times at 12 day intervals and the kidneys fixed by perfusion after 36 days. Serial sections from the kidneys of these two groups of birds were made and the number of peripolar cells recorded. These recordings were compared with the number of peripolar cells in four normal, untreated chickens. A significant increase in the number of peripolar cells was recorded in the furosemide treated group. No significant change was seen in the DOCA treated group. However, in the DOCA-group heavily granulated podocytes were found. No distinct morphological difference was found between the granules in the podocytes and the granules in the peripolar cells. A possible lysosomal nature of the peripolar cell granules is discussed.

Key words: Peripolar cells – Furosemide – DOCA – Lysosomes – Chickens

Introduction

The juxtaglomerular apparatus (JGA) is composed of the afferent and efferent arterioles with the granulated epithelioid cells, the macula densa, and the Goormaghtigh or lacis cells. Recently a morphologically different cell type, the peripolar cell, has been detected in the glomeruli of sheep (Ryan et al. 1979). The peripolar cells are located at the junction between the podocytes of the glomerular capillaries and the epithelial lining of Bowman's capsule, encircling the hilar region of the glomerular tuft. Later these cells have been described in many mammalian species (Ryan et al. 1982; Gall et al. 1986) and in amphibians (Hanner and Ryan 1980). Functionally, the peripolar cells have been considered to be a part of the juxtaglomerular apparatus (JGA) but the precise nature of the cells has not been identified (Gardiner and Lindop 1985). It is uncertain whether or not peripolar cells contain renin or some substance that could influence the tubular resorptive function (Ryan et al. 1979). However, renin has never been demonstrated in the granules of the peripolar cells.

Recently it has been found that an antibody against rat urinary kallikrein reacted positively with sheep peripolar cells (Gall et al. 1984). This finding has led to the suggestion that the peripolar cells in some way may influence the renin secretion by the kallikrein-kinin system.

Peripolar cells have not previously been described in birds. In studies on serial semithin sections we were able to demonstrate peripolar cells as a regular finding in the normal chickens. In addition we investigated the cells in sodium depleted and hypovolaemic chickens after furosemide treatment and in salt loaded hypertensive chickens after DOCA administration.

Materials and methods

One year old female chickens were used. They were bought as one day old chickens and kept in our department, singly in cages. They were fed a standard diet which contained 0.19% NaCl, 1.20% Ca, 0.65% P and 29% protein. The birds had free access to water and food during the experimental period. After recording blood pressure, weight and erythrocyte volume fraction (EVF), 4 birds were made hypovolaemic with furosemide which was injected in three doses of 20 mg per kg body weight (Lasix Vet. Hoechst 50 mg/ml) into the pectoral muscle. Weight, blood pressure and EVF were again recorded at the end of the experiment. The results from similar measurements

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have been published earlier (Morild et al. 1987). After anesthesia with equithesin combined with diazepam (Christensen et al. 1987), the kidneys were fixed by perfusion from the heart (Kjærheim 1969) with 4% neutral formaldehyde or 2% glutaraldehyde in phosphate buffer 36 h after the first injection with furosemide.

After embedding the tissue in plexiglass, serial semithin sections were cut from the kidney tissue with a Reichert Rotocut microtome. The sections were stained with toluidine blue. From the 4 furosemide treated birds 7–8 glomeruli were randomly chosen from the cortical, and the same number from the deeper juxtamedullary tissue. The criteria for selection were that the glomeruli could clearly be identified as belonging either to the smaller reptilian type or the larger juxtamedullar mammalian type. In this manner 30 reptilian and 30 mammalian type glomeruli were examined. Each glomerulus was followed on the serial sections throughout the whole glomerular diameter. This enabled us to record all glomerular cells with light microscopic visible granules in their cytoplasm. Further, the serial sections made an exact localization of these cells within the glomerulus possible. The peripolar cells were identified at the glomerular hilus where the parietal sheet of Bowman's capsule continued into the podocytes of the glomerular capillaries. Care was taken only to consider cells as peripolar if they were located within a distance of two cells on each side of the imaginary line of division between the parietal and the visceral sheet of Bowman's capsule. This restriction was made after podocytes with intracytoplasmic granules had been found within the glomeruli. All peripolar cells found in each glomerulus were counted and as few were found, each cell could be followed separately on the serial sections and the exact number present recorded. The peripolar cells were easily separated from the granulated juxtaglomerular cells and contained light microscopically identifiable toluidine blue positive granules of varying size.

The same number ($n=30$) of reptilian and mammalian type glomeruli were also examined on serial sections from the kidneys of 4 normal, untreated chickens and the number of peripolar cells were registered.

Five birds were injected in the pectoral muscle with DOCA (desoxycortone trimethylacetate in microcrystalline suspension, 25 mg/ml, Percorten M, CIBA-Geigy Limited, Basle, Switzerland) in doses of 75 mg per kg body weight. The pre-experimental weight, blood pressure, EVF and electrolytes were registered. The injections with DOCA were made with 12 days interval, and each bird received three injections. The birds also received drinking water containing 1% NaCl. They were followed closely with respect to the blood electrolytes. These measurements were made with an autoanalyszer (Technicon SMAC, Terrytown, NY, USA) in the same manner as in the normal and furosemide-treated birds. The perfusions were made 12 days after the last injection with DOCA (i.e. 36 days after the first injection). The method of anesthesia and method of fixation by perfusion were the same as for the chickens treated with furosemide. The blood pressure measurements in all birds included in this study were made with Doppler ultrasound equipment. A plastic cuff connected to a sphygmomanometer was placed on the leg and the ultrasound probe was placed in the region of the plantar arcuate artery. In this way the pulse was registered as an acoustic signal and it was possible to register the blood pressure in the regular manner. After fixation by perfusion kidney tissue was embedded in plastic and in paraffin. Both semithin and regular paraffin sections were made in serials. The semithin sections were stained with toluidine blue and the paraffin sections were stained with MSB trichrome (Lendrum et al. 1962). From the five birds, 6 glomeruli of each type were investigated and the peripolar cells counted in the same way as in the furosemide trial. No attempt was made to count the podocytes with granules in their cytoplasm.

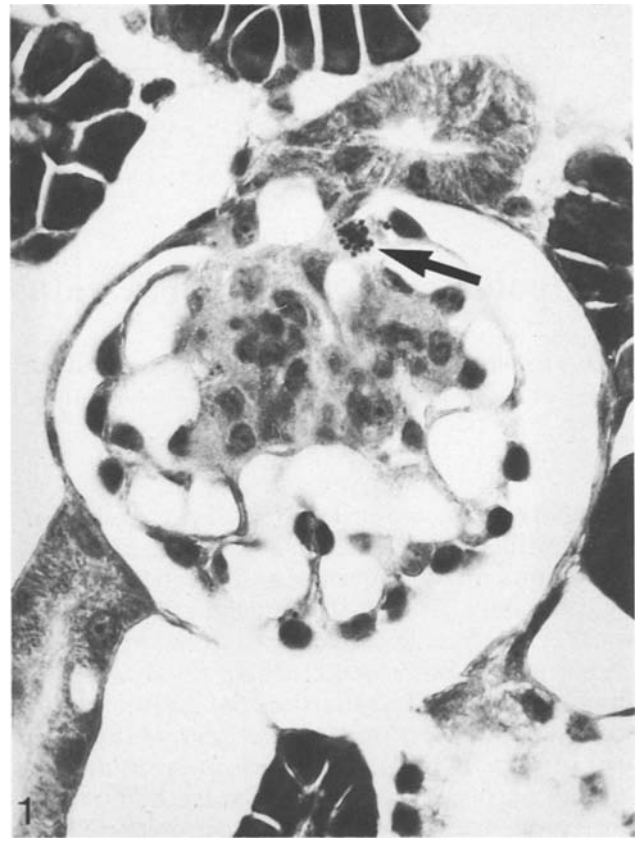

Fig. 1. Reptilian type glomerulus from a normal chicken. One heavily granulated peripolar cell is seen in typical position at the glomerular hilus (*arrow*). MSB trichrome. × 1000

Electronmicroscopic studies were made on material fixed in 2% glutaraldehyde, block contrasted with uranyl acetate and embedded in araldite. The ultrathin sections were cut on a Reichert Ultratome OM 2, stained with lead and uranyl acetate, mounted on slot grids and examined on a Zeiss Elmi 109.

The results of the trials were analyzed with two-sample and Student's *t*-tests (STATISTIX, Version 1.0, IBM, NH Analytical Software, St. Paul, MN, USA). A P value less than 0.05 was accepted as significant.

Results

In the normal birds granulated cells were found in hilar position in nearly every glomerulus examined (Fig. 1; Table 1). These cells fit the description of peripolar cells in other species.

The four chickens in the furosemide treated group showed a rapid fall in their blood pressure after the first injection. The blood pressures after 36 h are given in Table 2. The chickens in the control group did not show any variation in their blood pressures in this short period. The weight which is usually stable in chickens of this age

Table 1. Number of peripolar cells in normal and under experimental conditions

	Normal state	After furosemide	After DOCA
Reptilian type glomeruli	1.883±1.62	3.033±1.56 $p=0.0050$	1.200±1.13 $p=0.0841$
Mammalian type glomeruli	1.233±1.19	5.967±2.55 $p<0.0001$	1.033±0.96 $p=0.4783$

Podocytes with intracytoplasmatic granules are not considered in the table. The p-values represent comparison between normal controls and birds treated with furosemide and between normal controls and birds treated with DOCA (two-sample t-test). The cell numbers are expressed as mean±SD. $N=30$

showed a concomitant fall (Table 2). There was no fall in the plasma total protein or in the albumin concentrations. In the same short period of examination the EVF of the furosemide treated chickens rose from 33.5±1.7 to 44±3.5 (Table 2).

The total number of peripolar cells in furosemide treated, hypovolemic chickens are listed in Table 1. Increase in the number of peripolar cells was found both in the mammalian and reptilian type glomeruli. The peripolar cells were found on the epithelial side of the glomerular tuft, at the border between the visceral and the parietal sheet of the Bowman's capsule. In a few glomeruli in the control group and in several glomeruli of the furosemide treated chickens, rather heavily granulated epithelial cells of the glomerular capillaries (podocytes) were found. These cells had the same light microscopic appearance and were stained in a fashion similar to peripolar cells. They were located both peripherally and near the glomerular hilus. All granulated cells were located on the epithelial side of the capillary loops and were never found in the mesangium. The granulated content of the peripolar and the epithelial glomerular cell was different from the granules seen in granulated epithelioid cells of the arterioles. Renin granules of the epithelioid cells in the chickens vary very little in size. The granules of the peripolar and the podocytic cells however, varied considerably in shape and size.

In the DOCA group the birds started to lose weight a few days after the first injection and the weight loss continued throughout the experiment. The blood pressure and the EVF were elevated (Table 2). The rise in the EVF was however, not as high as in the furosemide group. Considerable changes occurred in the glomeruli of the chickens treated with DOCA. There were crescent formations in the glomeruli with fibrosis and hyalinization. Proliferation of the mesangial cells and increase of the mesangium matrix were seen together with mitotic figures in the mesangial cells and in the podocytes. There also was an increase in the number of visible arterioles within the mesangium, especially in the mammalian type. However, the area of the capillary loops was not measured.

The number of peripolar cells in the DOCA group was not increased (Table 1). Again the peripolar cell was defined as a cell located in the hilar region within two cell diameters of the border between the visceral and the parietal sheet of Bowman's capsule. There were numerous other granulated cells in the glomeruli of the chickens treated with DOCA. In many glomeruli, granulated cells in the parietal sheet of Bowman's capsule were found. These cells were slender and long, had the morphology of capsular cells and were mostly located near the glomerular hilus. In both the mammalian and reptilian type glomeruli the podocytes were heavily granulated. The granules were stained by toluidine blue, but were most distinct with the MSB trichrome (Lendrum et al. 1962) which gave the granules a brilliant red colour. With the light microscope the granules seemed to be of the same type as seen in the peripolar cells.

The number of granulated podocytes were so numerous that they could not be counted. Nearly

Table 2. Blood pressure, weight and EVF before and at the end of the experiment

	Before furosemide	After furosemide	Before DOCA	After DOCA
Blood pressure	122 ± 12.6	85± 27.4 $p=0.0435$	137 ± 8.3	174 ± 21.6 $p=0.0085$
EVF	33.5± 1.7	44± 3.5 $p=0.0066$	29.2± 1.9	35.0 ± 2.6 $p=0.0001$
Weight	1654 ±274.6	1490±215.8 $p=0.0156$	1594 ±135.2	1254 ±143.9 $p=0.0069$

The p-values are based on comparison of measurements before and after furosemide and of measurements before and after DOCA (paired Student's t-test). Number of birds in the furosemide group $N=4$, in the DOCA group $N=5$. The results are given as mean ±SD

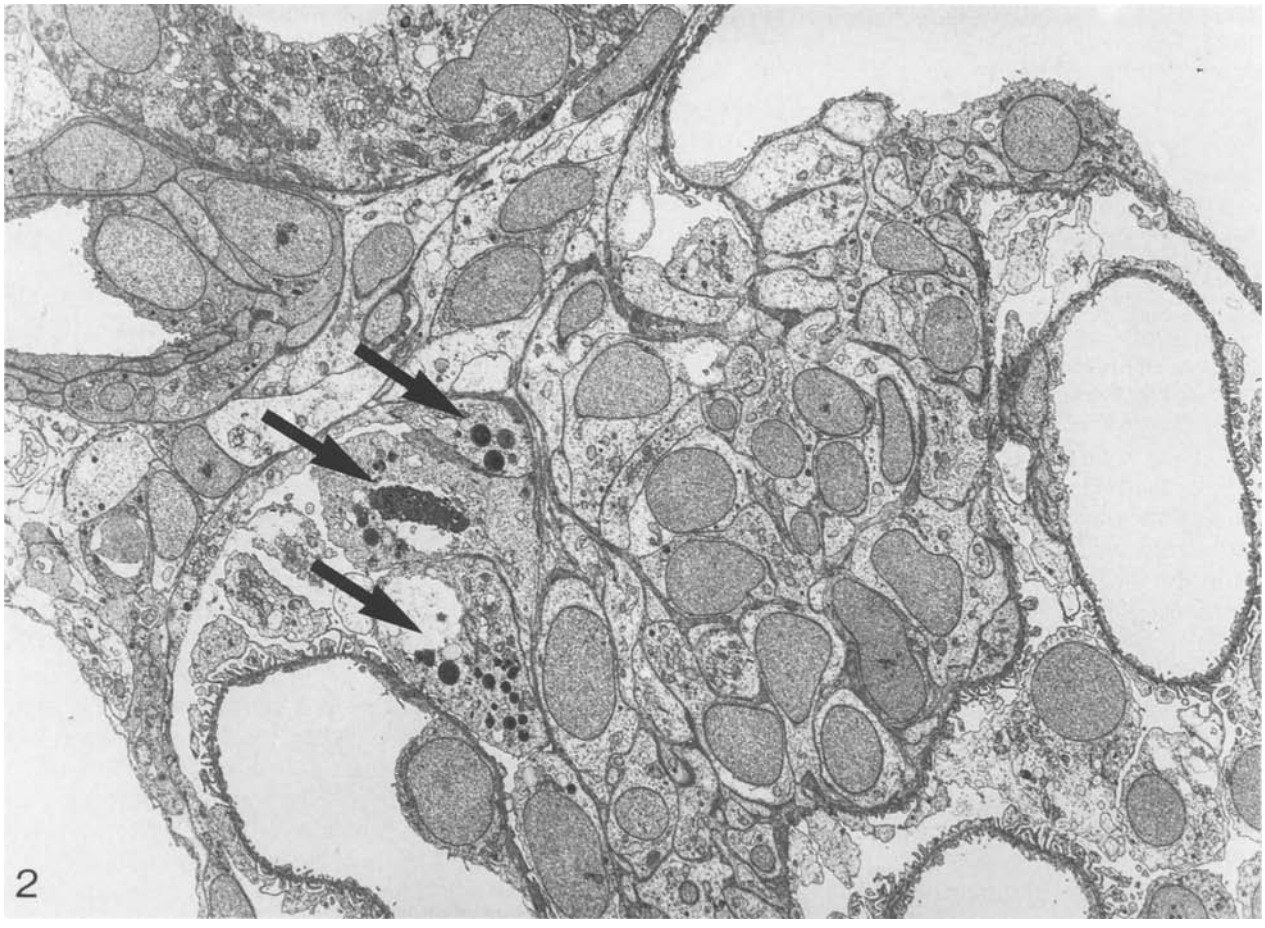

Fig. 2. Reptilian type glomerulus from a hypovolemic chicken. Three granulated peripolar cells are seen (*arrows*). Note the large variation in size of granules. Electron micrograph × 2600

all podocytes showed granules of different size in their cytoplasm. No granulated cells were found in the mesangium. There were continuity between granulated podocytes, peripolar cells and the granulated cells in the parietal sheet of Bowman's capsule. Often it was difficult to differentiate between the different cell types.

Ultrastructurally there was no difference in the peripolar cells encountered in normal birds when compared with the two experimental groups. Most granules were membrane bound and varied considerably in size and shape. However, granules without a definite membrane were also found. The granules contained material with variable electron density, often with more dense areas in the center of the granules. In some of the granules small vacuoles could be seen, mostly close to the membrane of the granule. No crystalline substructure could be detected in the granules. The granules in the podocytes did not differ from the granules in the peripolar cells or from the granules in the cells of Bowman's capsule.

In the chickens treated with DOCA the glomerular basement membrane and the foot processes of the podocytes were normal in all glomeruli examined (Fig. 3).

The plasma electrolyte concentrations in the DOCA group showed elevated sodium and chloride whereas plasma potassium concentration was reduced. The plasma albumin concentration fell from 19.10 ± 1.60 g/l to 15.20 ± 2.15 g/l ($P = 0.0009$) in the DOCA group. In the controls and in the furosemide group the albumin concentration was unchanged.

Discussion

The recognition of the peripolar cell in the kidney is relatively new and few relevant reports have been published. Several mammalian species have been investigated (Gall et al. 1986) and peripolar cells have also been found in non-mammalian species (Hanner and Ryan 1980). To our knowledge peripolar cells have never been reported in birds.

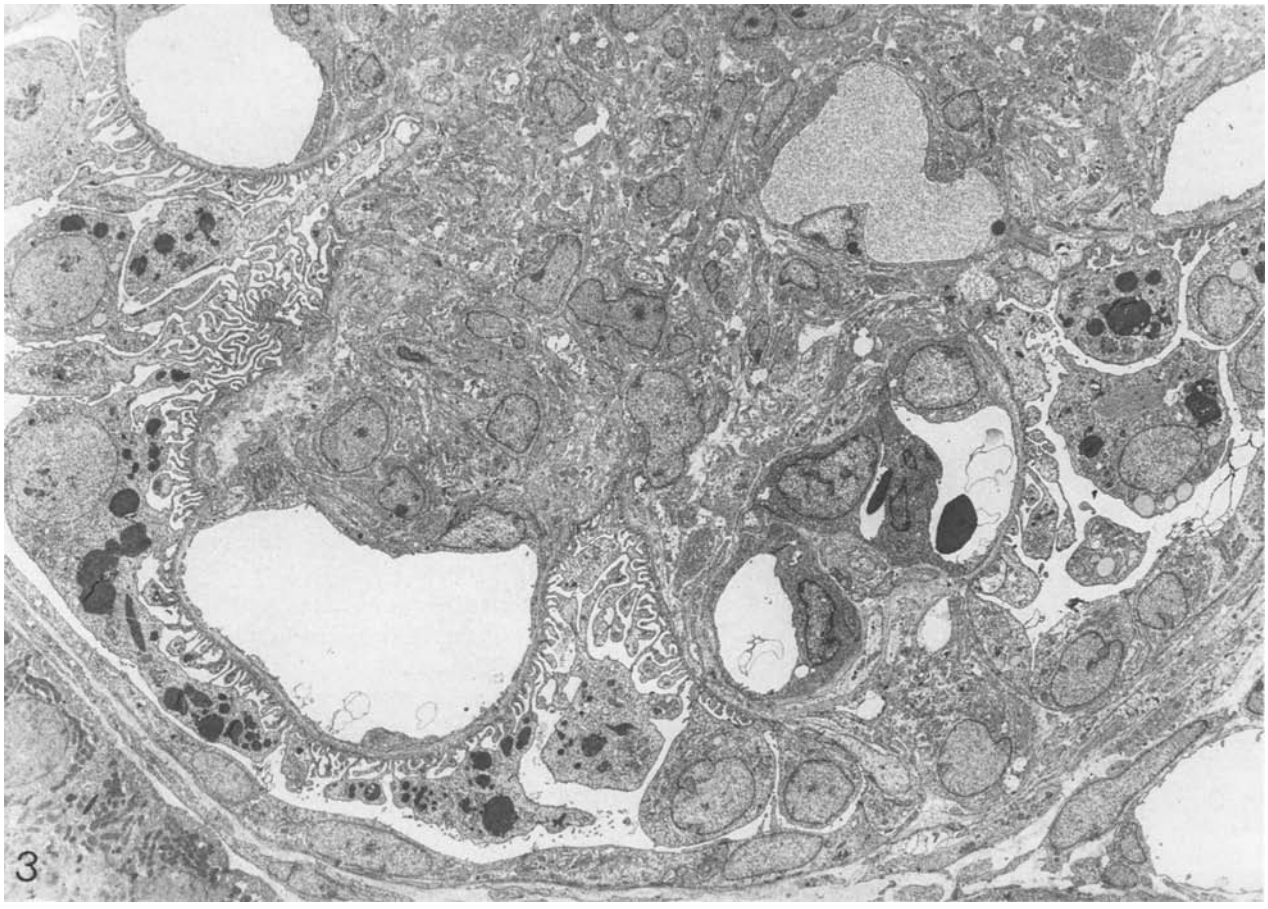

Fig. 3. Mammalian type glomerulus from a chicken treated with DOCA. Numerous granules are seen in the podocytes. The podocytic foot processes and the basement membrane of the capillary walls are optically intact. Electron micrograph × 2600

The studies of Hill et al. (1983) gave valuable information regarding the possible function of the peripolar cells as these authors observed exocytosis in peripolar cells after sodium depletion. The significant increase of peripolar granulated cells in furosemide treated sodium depleted and hypovolaemic chickens in our study may be consistent with this. This may indicate production and possibly secretion of a substance into the Bowman's space. Sodium depletion in chickens leads to an increase in granulated epithelioid cells in the afferent arterioles (Morild et al. 1987) which was interpreted as evidence of renin production in order to maintain normal blood pressure. The increased number of granulated peripolar cells may indicate another regulatory mechanism in the hypovolaemic state. Increased synthetic activity and marked heterogeneity of granule density was shown in sheep peripolar cells after sodium depletion while sodium loaded sheep showed no consistent peripolar cell changes (Hill et al. 1983). A factor added to the glomerular ultrafiltrate may modulate the reabsorptive and secretory function of the tubular cells. Häberle and Shigai (1978) found that proximal tubular fluid in the rat contained a factor which promoted volume reabsorption in the proximal tubule in response to increased glomerular filtration rate. It was proposed that an increased volume reabsorption resulted from increased proximal tubular sodium reabsorption. Artificial ultrafiltrate of plasma did not contain this factor indicating that it was added to the tubular fluid after plasma filtration across the glomerular capillary wall. Release phenomenas have been observed in peripolar cells discharging their granulated contents into the urinary space in the axolotl following immersion in distilled water (Hanner and Ryan 1980). Exocytotic release of peripolar cell granule material into the urinary space demonstrated by Hill et al. (1983) was also taken as support for the concept that peripolar cells play a role in sodium homeostasis. Similar observations have been made in newborn lambs subjected to acute volume expansion and diuresis following intrave-

nous infusion of dextrose solution (Alcorn et al. 1981). Observations of Gall et al. (1984) have indicated that peripolar cells of sheep may contain a kallikrein-like polypeptide. However this observation has only been presented as an abstract. It has also been suggested that the renal kallikrein-kinin system may participate in the control of renin release (Carretero and Beierwaltes 1984).

In an effort to characterize the granules in the peripolar and the other granulated cells within the glomerulus, we made immunocytochemical investigations in a preliminary study. Rat antibodies were applied against the cathepsins B, D, L and H on paraffin and electronmicroscopic sections. None of these reactions were positive in peripolar cells. In the granulated epithelioid cells of the juxtaglomerular apparatus, positive reaction was seen after using rat antibodies against cathepsin B. This points to the lysosomal nature of the renin granules as found in the rat (Taugner et al. 1985a, b). Incubations with human, rat and mouse antirenin were all negative. Antikallikrein was not tested because this antibody was not available.

In the present study we recorded an increased number of peripolar cells in sodium depleted chickens with low blood pressure. In the sodium loaded chickens with high blood pressure a large number of podocytes showed intracytoplasmic granules or droplets. These changes were also found in some cells in the parietal sheet of Bowman's capsule. Similar changes have been described earlier in humans suffering from high blood pressure (Fahr 1925). Ryan and Karnovsky (1975) found a similar type of intracytoplasmic droplet in the podocytic epithelium after aminonucleoside induced nephrosis. Caulfield et al. (1976) attributed these changes in the podocytic epithelium to lysosomal action. Discharge of lysosomal contents into the urinary space by a mechanism identical to the exocytosis found in peripolar cells was also shown in this study (Caulfield et al. 1976). Activation and exhaustion of the podocytic lysosomal system have also been shown in the early phase of experimental hypertension (Szokol et al. 1979). The great number of lysosomal bodies in the podocytes under such conditions can be attributed to the increased amount of proteins penetrating through the glomerular barrier. The exhaustion of the lysosomal system is also probably a consequence of the increased protein load (Caulfield et al. 1976). In our study with DOCA the plasma albumin concentration fell significantly indicating protein loss. We believe the changes in the podocytes are due to protein absorption and concentration related to lysosomal action. Microscopically we were not able to find any structural differences between the granules in the podocytes and the granules in peripolar cells. The microscopical appearance of the granules in the peripolar cells are consistent with lysosomes. Taugner et al. (1985a, b) demonstrated lysosome-like properties of secretory granules in the epithelioid cells in rats and mice. Their results further emphasize the similarity between lysosomes and secretory granules.

Gall et al. (1986) used the "peripolar cell index" to characterize different mammalian species with respect to their content of peripolar cells in the kidneys. In our experience it is extremely difficult to classify a cell as being peripolar when the glomerulus is not sectioned through the hilus. The occurrence of other granulated cells than the peripolar cells in the glomeruli of the chickens proves that is necessary to study the glomeruli on serial sections.

The nature and function of the peripolar cell has not been fully identified. The results of the present study raise the question if the peripolar cell is a specific cell type. In chickens, cells similar to the peripolar cells are found as podocytes on the epithelial side of the glomerular capillaries. Even if they were found in a much larger number in the birds treated with DOCA, they were also found in the control birds. To solve this problem, immunocytochemical studies with specific avian antibodies are required.

Acknowledgements. The authors are grateful to Norske Hoechst A/S, Oslo, Norway and Ciba-Geigy Limited, Basle, Switzerland for their generous supply of furosemide and desoxycortone trimethylacetate respectively. The immunocytochemical investigations in the preliminary study was carried out by Prof. Dr. R. Taugner, I. Institute of Physiology, University of Heidelberg, Federal Republic of Germany. We also thank Prof. M. Farstad at the Department of Clinical Biochemistry, University of Bergen, Norway for the technical help with autoanalyses of the blood samples. Carol Church corrected the English language. Inger Søborg possessed the microscopical specimens with great skill. Tore Jacob Raa and Asbjørn Nilsen carefully handled the birds.

References

Alcorn D, Chesire GR, Coghlan JP, Ryan GB (1984) Peripolar cell hypertrophy in the renal juxtaglomerular region of newborn sheep. Cell Tissue Res 236:197–202

Carretero OA, Beierwaltes WH (1984) Effect of Glandular Kallikrein, Kinins and Aprotinin (a Serine Protease Inhibitor) on Renin Release. J Hypertension 2: (suppl 1): 125–130

Caulfield JP, Reid JJ, Farquhar MG (1976) Alterations of the glomerular epithelium in acute aminonucleoside nephrosis. Evidence for formation of occluding junctions and epithelial cell detachment. Lab Invest 34:43–59

Christensen JA, Fosse RT, Halvorsen OJ, Morild I (1987) Com-

parison of various anesthetic regimens in the domestic fowl. Am J Vet Res 48:1649–1657

Fahr T (1925) Die kompensierte benigne Nierensklerose (Reine arteriosklerotische Nierenveränderung im Stadium der Dekompensation) In: Henke F, Lubarsch O (eds) Handbuch der speziellen pathologischen Anatomie und Histologie. vol VI/1, Springer, Berlin Heidelberg New York, pp 400–405

Gall JAM, Alcorn D, Coghlan JP, Johnston CI, Ryan GB (1984) Immunohistochemical detection of urinary kallikrein-like material in juxtaglomerular peripolar cells. Proc. IXth Int Cong Nephrol, p 451 A (Abstract)

Gall JAM, Alcorn D, Butkus A, Coghlan JP, Ryan GB (1986) Distribution of glomerular peripolar cells in different mammalian species. Cell Tissue Res 244:203–208

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

Hanner RH, Ryan GB (1980) Ultrastructure of the renal juxtaglomerular complex and peripolar cells in the axolotl (Ambystoma mexicanum) and toad (Bufo marinus). J Anat 130:445–455

Hill PA, Coghlan JP, Scoggins BA, Ryan GB (1983) Ultrastructural changes in the sheep renal juxtaglomerular apparatus in response to sodium depletion or loading. Pathology 15:463–473

Häberle DA, Shiigai T (1978) Flow-dependent volume reabsorption in the proximal convolution of the rat kidney: The role of glomerular-born tubular fluid for the maintenance of glomerulotubular balance, In: Vogel HG, Ullrich JK (eds) New Aspects of renal function, Excerpta Medica, Amsterdam, pp. 198–207

Kjærheim A (1969) Studies of adrenocortical ultrastructure. 1. Aldehyde perfusion fixation of the domestic fowl. Acta Anat 74:424–453

Lendrum AC, Fraser DS, Slidders W, Henderson R (1962) Studies on the character and staining of fibrin. J Clin Pathol 15:401–413

Mitchell GM, Stratford BF, Ryan GB (1982) Morphogenesis of the renal juxtaglomerular apparatus and peripolar cells in the sheep. Cell Tissue Res 222:101–111

Morild I, Christensen JA, Halvorsen OJ, Farstad M (1987) Effect of volume depletion of the afferent arterioles in the avian kidney. Virchows Arch [Pathol Anat] 411:149–155

Ryan GB, Karnovsky MJ (1975) An ultrastructural study of the mechanism of proteinuria in aminonucleoside nephrosis. Kidney Int 8:219–232

Ryan GB, Coghlan JP, Scoggins BA (1979) The granulated peripolar epithelial cell: a potential secretory component of the renal juxtaglomerular complex. Nature 277:655–656

Ryan GB, Alcorn D, Coghlan JP, Hill P, Jacobs R (1982) Ultrastructural morphology of granule release from juxtaglomerular myoepithelioid and peripolar cells. Kidney Int 22:(suppl 12):S3–S8

Szokol M, Soltèsz M, Varga S (1979) Ultrastructural changes of the rat renal glomeruli in the early phase of renovascular hypertension. Acta Morphol Acad Sci Hung 27:117–132

Taugner R, Whalley A, Angermüller S, Bührle CP, Hackenthal E (1985a) Are the renin-containing granules of juxtaglomerular epithelioid cells modified lyosomes? Cell Tissue Res 239:575–587

Taugner R, Bührle CP, Nobiling R, Hirschke H (1985b) Coexistence of renin and cathepsin B in epithelioid cell secretory granules. Histochemistry 83:103–108

Accepted November 16, 1987