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THE ASSAY OF RENIN IN SINGLE GLOMERULI IN THE NORMAL RABBIT AND THE APPEARANCE OF THE JUXTAGLOMERULAR APPARATUS

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Though there has been considerable controversy concerning the location of renin in the kidney, there is now general agreement that it is situated in some part of the juxtaglomerular apparatus (see review by Bing, 1963). Cook, Gordon & Peart (1957), Bing & Wiberg (1958) and Cook & Pickering (1959) all found, in the rabbit, using microdissection techniques combined with biological assay, that renin is in, or close to, the glomeruli, and that little is present elsewhere in the kidney. The work was extended (Bing & Wiberg, 1958; Cook, 1960) to show that the assayable renin is located in the juxtaglomerular apparatus. The terminology of the structures (including the granular cells of the afferent glomerular arteriole, lacis cells and macula densa) which comprise the juxtaglomerular apparatus is discussed by Bing (1963) and Cook (1963). These workers were agreed that more renin is found associated with the glomeruli lying superficially in the cortex than with those from deeper zones.

In the biological assay systems cited, pooling of considerable numbers of glomeruli was necessary for the renin assay. The development of a sensitive enzyme-kinetic method (Lever, Robertson & Tree, 1963, 1964) has enabled the renin associated with single rabbit glomeruli to be measured. The present study was therefore undertaken to obtain more detailed information concerning the distribution of renin within the kidney and its correlation with histological features. Preliminary reports of this work have previously appeared (Brown, Davies, Lever, Parker & Robertson, 1963*a*, *b*).

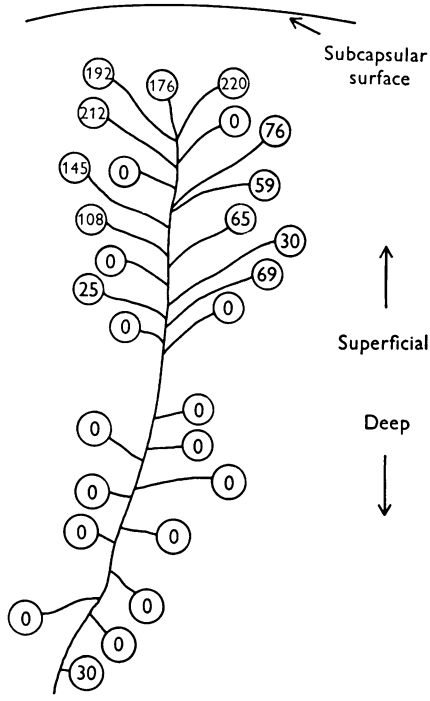
In this paper the term 'renal cortex' indicates that part of the kidney extending from the subcapsular surface to the deepest glomerular zone. The term 'superficial' refers to the outer half, and 'deep' to the inner half of the cortex.

METHODS

Normal male rabbits weighing 1·5–3 kg were killed with intravenous pentobarbitone and the kidneys removed immediately.

Dissection of single glomeruli

Transverse slices of renal cortex (1–2 mm thick), extending from the capsule to the cortico-medullary junction, were cut freehand from the zone opposite the hilum of the kidney. These were kept moist with 0·15 M phosphate/saline buffer pH 5·7 (Lever *et al.* 1964). Under the dissecting microscope (× 25) the glomeruli were readily identified as red



Text-fig. 1. Diagrammatic representation of renin content of twenty-eight glomeruli along the course of a single interlobular artery in a normal rabbit. Renin in units × 10,000.

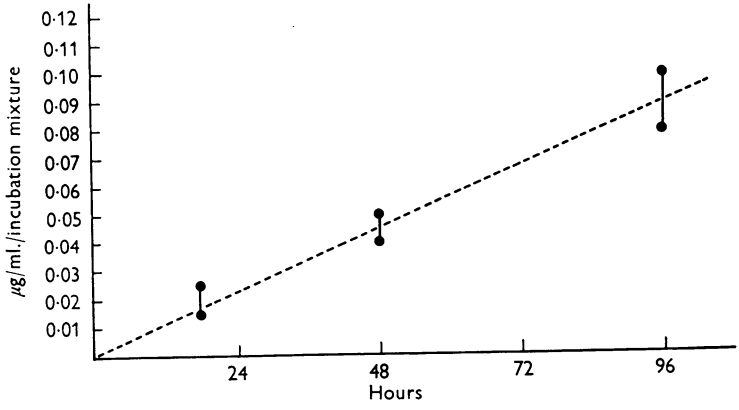
dots due to the presence of numerous erythrocytes in the capillary loops. Using fine mounted needles, single glomeruli were teased out and examined to confirm that the macula densa and the terminal part of the afferent arteriole remained attached (Pl. 1, fig. 1). Each glomerulus was then picked up on a small piece of paper and transferred to a polyethylene tube containing 1 ml. of the 0·15 M buffer pH 5·7 with 0·2 % neomycin sulphate.

In some cortical slices, 20–40 glomeruli were dissected out individually along the course of an interlobular artery (Text-fig. 1). In other cases, a more limited examination was made by taking six glomeruli from the most superficial and six from the deepest glomerular layer of each cortical slice. Whichever procedure was used, the order of dissection of the

glomeruli was varied, sometimes the most superficial and sometimes the most deep being dissected first, so that if any loss of renin occurred during the dissection a systematic error would be avoided.

Renin assay

The single glomeruli in buffer were frozen at − 20° C, thawed, and 4 ml. of prepared ox serum substrate (Lever *et al.* 1964) added. These mixtures were then incubated at 37° C for periods varying up to 96 hr. Samples were removed at intervals from each incubation mixture, and the angiotensin concentration assayed biologically against synthetic asp[1]-β-amide val[5] octapeptide angiotensin (Hypertensin CIBA) on the rat blood pressure preparation. The initial velocity of angiotensin formation in each incubation mixture (Text-fig. 2)



Text-fig. 2. Incubation progress curve for a single glomerular extract incubated with ox serum substrate. Assay bracket limits are shown.

was proportional to the renin concentration, which could then be determined and expressed in arbitrary units. One unit of renin is that amount which when incubated at 37° C with 4 ml. of prepared ox substrate in a total volume of 5 ml. at pH 5·7 forms angiotensin at a rate of 0·1 μg/ml./hr. This renin assay technique has been described in detail elsewhere (Lever *et al.* 1963, 1964; Brown, Davies, Lever, Robertson & Tree, 1964). The accuracy of the method requires the elimination of endogenous substrate and angiotensinase from the incubation mixtures. Absence of endogenous substrate from the dissected glomeruli and their attached fragments was checked by taking groups of 6–8 glomeruli from a zone containing renin and incubating them alone in buffer, without added substrate. Angiotensin was not formed in these circumstances, showing that endogenous substrate was not included. Absence of angiotensinase was shown in a similar manner by adding synthetic angiotensin (0·5 μg/ml.) to groups of six or more glomeruli in buffer and incubating for 96 hr. In all cases angiotensin survival was greater than 80 %. The absence of angiotensinase was further shown in individual incubation mixtures by the observation that angiotensin concentration increased progressively until substrate was exhausted, and then remained constant. The velocity of angiotensin formation did not deviate measurably from rectilinear whilst the substrate concentration remained above 20 units/ml. (Text-fig. 2) (Lever *et al.* 1964; Brown, Davies, Lever, Robertson & Tree, 1964).

Histology

Thin slices of kidney were fixed in formal-saline solution and in Helly's fluid. The part of the cortex opposite the hilum of the kidney was used throughout this investigation, to avoid possible variation around the circumference of the kidney. Sections were stained with

haematoxylin and eosin. Juxtaglomerular granules were demonstrated with Bowie's stain (Pitcock & Hartroft, 1958) using the Helly-fixed material. Diphosphopyridine nucleotide (DPN) diaphorase and triphosphopyridine nucleotide (TPN) diaphorase were demonstrated by the method of Wachstein & Meisel (1959) using formal calcium as fixative at 4° C for 24 hr. The following quantities were used in the incubation solution: DPNH or TPNH (5 mg/ml.), 2 ml.; Nitro BT (1 mg/ml.), 7 ml.; 0·2 M phosphate buffer (pH 7·4), 8 ml.

Juxtaglomerular granulation was estimated by the method of Hartroft & Hartroft (1953), grade 4 indicating the most dense granulation and grade 1 the least. The juxtaglomerular cell granulation index (JGI) was calculated by the method of Hartroft & Hartroft (1955). Two hundred glomeruli were counted in each rabbit in the present investigation.

RESULTS

Renin distribution

The renin distribution had a consistent pattern in all rabbits. The glomeruli lying deepest in the cortex usually contained little or no extractable renin, this being in the majority below the sensitivity limit for the method. Glomerular renin, increased progressively along the course of the interlobular artery, though the renin content often differed considerably in adjacent glomeruli. The most superficial glomeruli were usually richest in extractable renin. The results of glomerular renin assays in twenty-eight glomeruli from one interlobular artery are shown in Text-fig. 1. Essentially similar results were obtained in six other rabbits. The contrast between the renin content of the superficial and deep glomeruli is shown in Text-fig. 3, which gives the results of renin assays on 254 glomeruli from fifteen normal rabbits.

The quantities of renin found in comparable cortical levels varied very considerably from one rabbit to another, confirming the work of Pickering, Prinzmetal & Kelsall (1942) and Cook & Pickering (1959), who found that the assayable renin might vary 25- to 100-fold between the kidneys of different rabbits.

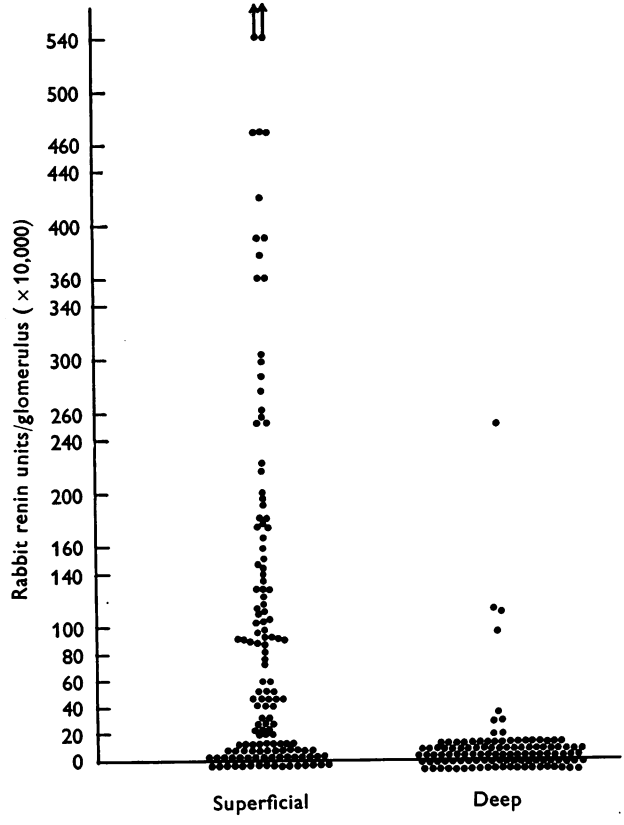
Anatomical and histological differentiation within the renal cortex

Approximately 50 % of the glomeruli in the deep half of the renal cortex (comprising about 15 % of the total glomeruli) are larger (Pl. 1, fig. 2) than other glomeruli. These large glomeruli predominate in the zone nearest the medulla, and presumably correspond to the 'juxtamedullary' glomeruli which Trueta, Barclay, Daniel, Franklin & Prichard (1947) define as possessing especially wide efferent arterioles which continue as the vasa recta circulation of the renal medulla. In the rabbit the afferent arterioles of these glomeruli are wide and frequently short; these arise either from the arcuate or interlobular artery (Trueta *et al.* 1947; Fourman & Moffat, 1964). The smaller superficial glomeruli (Pl. 1, fig. 3) are supplied by narrower and often longer afferent arterioles. Seventy per cent of the

glomeruli are found in the superficial half of the cortex, where they are consequently closer together than in the deep half.

Juxtaglomerular granulation

Goormaghtigh (1945) and Vickhert & Serebrovskaya (1964) state that juxtaglomerular granules are more plentiful in the superficial cortex of the rabbit than in the deep part. Since a glomerulus is much larger than its

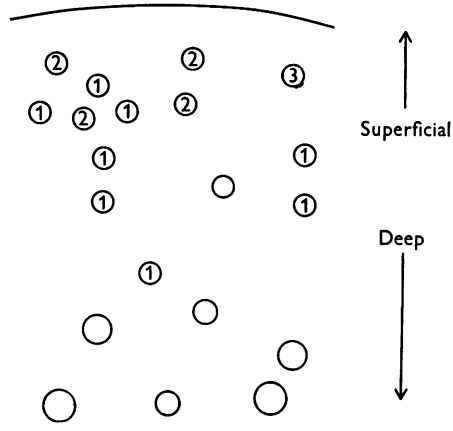


Text-fig. 3. Renin extracted from 254 superficial and deep glomeruli of 15 normal rabbits. These values are obtained from glomeruli dissected by the two techniques described in methods section.

juxtaglomerular cells, and since many of the deep glomeruli are larger than the superficial ones, absence of juxtaglomerular granules adjacent to a glomerulus in a single section does not necessarily mean that that glomerulus is without associated granules. A study of serial sections of several hundred Bowie stained glomeruli from two rabbits confirmed that

juxtaglomerular granular cells occurred more often in relation to glomeruli in the superficial half of the cortex than in the deep part.

Text-figure 4 shows the amount of granulation associated with twenty glomeruli, thirteen being in the superficial half of the cortex and seven in the deep. Twelve of the thirteen superficial glomeruli, but only one of the seven deep glomeruli, possessed juxtaglomerular cells with granules. Four of the deep non-granulated glomeruli were larger than the rest. Only very rarely was a large deep glomerulus found with associated granules. The greatest amount of granulation was always seen in the most superficial glomeruli.



Text-fig. 4. Diagrammatic representation of juxtaglomerular granulation in twenty glomeruli followed in serial sections. Grade 3 indicates most dense granulation.

For the reasons given, calculation of the juxtaglomerular index on single microscopic sections is inherently a less accurate method of determining differential granulation at various cortical levels than is a study of serial sections. Nevertheless, results obtained by both techniques agreed closely. The juxtaglomerular index for the superficial half of the cortex in twenty-two rabbits was 21 per 100 glomeruli (range 14–38), and for the deep half four (range 0–8). Thus, assessed by both methods, the decrease in granulation from superficial to deep cortical layers closely followed the decrease in glomerular renin.

Macula densa

In fixed tissue the macula densa appears to protrude into the tubular lumen (Pl. 1, fig. 4) and its nuclei are closer together than those of the adjacent tubular cells. Although readily stained by haematoxylin and eosin, the macula densa is more selectively stained by the DPN and

TPN diaphorase methods (Pl. 1, fig. 5). Using these techniques the macula densa cells of the superficial half of the cortex showed a cytoplasmic band of staining of equal intensity and of about the same width on each side of the row of nuclei (Pl. 1, fig. 5). Both the size and staining characteristics of some maculae from the deeper half of the cortex were similar to those of the superficial cortex. In others there were fewer cells, with unaltered staining characteristics. In some instances (Pl. 1, fig. 6) the intensity of staining was less marked on the luminal side of the deep macular cells. Over-all, however, there was less variation in the appearances of the macula densa at different cortical levels than in the other components of the juxtaglomerular apparatus, or in the quantity of extractable renin.

DISCUSSION

Considerable evidence in recent years has suggested the existence of a humoral regulator of aldosterone secretion, and has indicated that the renin-angiotensin system is probably responsible (Gross, 1958; Denton, Goding & Wright, 1959; Laragh, Angers, Kelly & Lieberman, 1960; Genest, Biron, Koiw, Nowaczynski, Boucher & Chretien, 1961; Ganong & Mulrow, 1961; Davis, 1962; Brown, Davies, Lever & Robinson, 1963, 1964*a*; Scornik & Paladini, 1964). Whether the renin-angiotensin system also serves an intrarenal regulatory function is uncertain, although this idea also has received considerable attention (Schmid, 1962; Lever & Peart, 1962; Bing, 1963; Leyssac, 1963; Tobian, 1964; Brown, Matthew & Robertson, 1964).

The possible function of the juxtaglomerular apparatus, and especially the relation of its various components to renin formation and secretion, has consequently excited much interest. Bing (1963) has recently reviewed this field, and has developed the theory of the macula densa as the site of renin formation. According to this view, renin is then transferred across the lacis to the granular cells in the wall of the afferent arteriole. Bing's work is based largely on direct renin assays of dissected fragments of the juxtaglomerular apparatus, and is supported circumstantially by both histochemical studies (Fisher, 1961; Hess, 1963) and by electron microscopy (Oberling & Hatt, 1960; Latta & Maunsbach, 1962). The opinions of Hartroft, Sutherland & Hartroft (1964) conflict somewhat with those of Bing, since these workers, using a fluorescent antibody technique, suggest that renin is located in the granules of the afferent arteriolar epithelioid cells, rather than in the macula densa. The use of a fluorescent antibody technique in the localization of renin has been criticized on the grounds that the antigen used is impure. The criticism of these and other studies of the localization of renin (Nairn, Fraser & Chadwick, 1959) is discussed by Hartroft *et al.* (1964).

Any theory of the function of the juxtaglomerular apparatus and of the factors controlling renin secretion should account for both the gradation of juxtaglomerular renin seen in these and earlier experiments, and the close relation between the three major components of the juxtaglomerular apparatus (macula densa, lacis cells and granular cells of afferent glomerular arteriole).

Tobian (1960, 1964) has suggested that the afferent arteriole acts as a stretch receptor, releasing renin from the granular cells in response to changes in stretch. This theory could account for the gradation in juxtaglomerular renin from superficial cortex to corticomedullary junction, in that the pressure drop in the wide and often shorter afferent arteries of deep glomeruli could well be less than that in the narrow and often long afferent arteries of superficial glomeruli. It could also account for the change in juxtaglomerular renin which follows experimental constriction of a renal artery (Brown *et al.* 1963*b*; Brown, Davies, Lever & Robertson, 1964*b*; Parker, 1964). The way in which a stretch receptor might respond to those changes in sodium metabolism known to affect plasma-renin concentration is less clear. The stretch theory is also unable to account for the close contact between granular cells in the afferent artery and the macula densa. A possible explanation of this relation, which was considered by Latta & Maunsbach (1962), is that the macula densa acts as a sensory receptor which modifies renin secretion in response to a change in the osmolality or sodium content of tubular fluid. We have recently suggested (Brown, Davies, Lever & Robertson, 1964*c*) that if the macula densa is sensitive to osmolality in this way, a mechanism might exist in which a salt load reduced renin secretion by reducing the molality of fluid in the early distal tubule. Guyton (1963) and Thurau (1964) have also recently proposed that the macula densa might act as a sensory receptor, and that subsequent changes in renin secretion could be responsible for autoregulation of the glomerular filtration rate.

Lastly, there exists the possibility that the macula densa is secretory, whilst the granular cells are responsible for the disposal of renin arriving via the afferent arteriole. The kidney could well both remove and secrete renin; the recent demonstration of a high concentration of a renin-like enzyme in normal urine (Brown, Davies, Lever, Lloyd, Robertson & Tree, 1964) might be relevant to this suggestion. It is possible that the close anatomical relations described serve to regulate differential renin secretion into renal lymph (Lever & Peart, 1962), renal venous plasma or urine in various circumstances.

SUMMARY

1. Renin distribution in the normal rabbit kidney was studied by assaying renin extracted from single glomeruli with their attached juxtaglomerular fragments.

2. Renin was absent or low in glomeruli nearest the medulla, and tended to rise progressively in glomeruli along the course of the interlobular artery.

3. This gradation in renin content was parallelled by histological and anatomical differences in various parts of the juxtaglomerular apparatus. The most marked association was between the granularity of the afferent glomerular arteriole and the juxtaglomerular renin content. A less marked association existed between renin content and the staining characteristics of the macula densa.

4. The possible significance of these relations is considered.

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EXPLANATION OF PLATE

Fig. 1. Teased out single glomerulus with attached juxtaglomerular apparatus (unstained × 300).

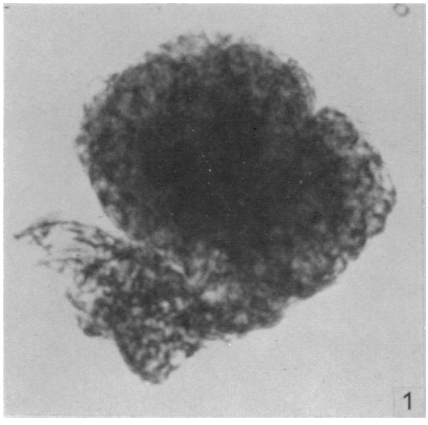
Fig. 2. Large glomerulus from deep half of cortex (H. & E. × 250).

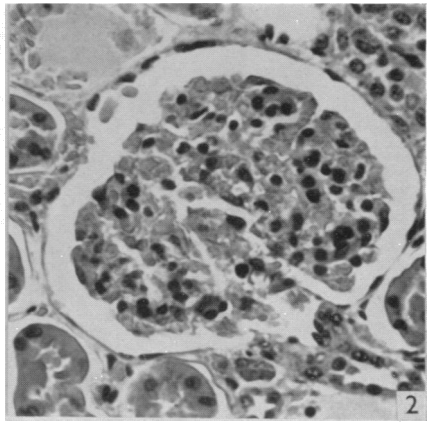
Fig. 3. Small glomerulus from superficial half of cortex (H. & E. × 250).

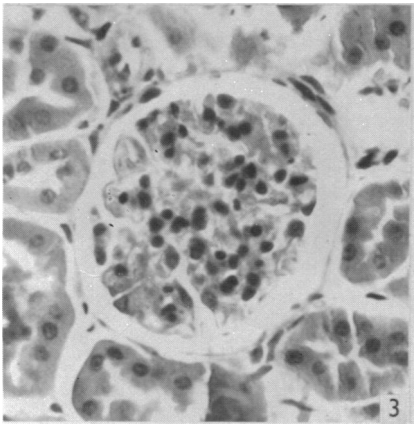
Fig. 4. Macula densa projecting into lumen of tubule (H. & E. × 540).

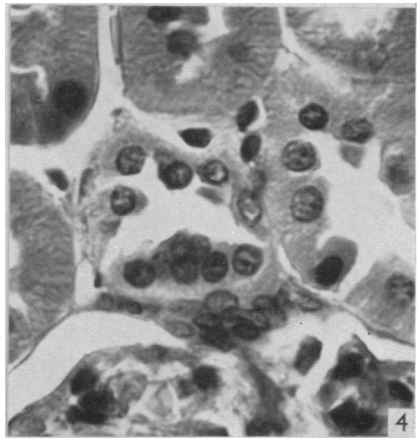
Fig. 5. Macula densa from superficial half of cortex with band of staining of about equal width on each side of its row of nuclei (TPN diaphorase × 700).

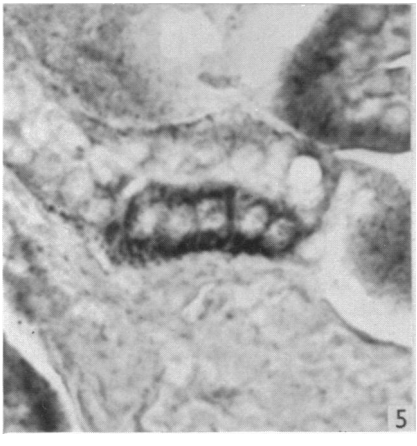
Fig. 6. Macula densa from deep half of cortex with luminal band of staining less marked (TPN diaphorase × 700).

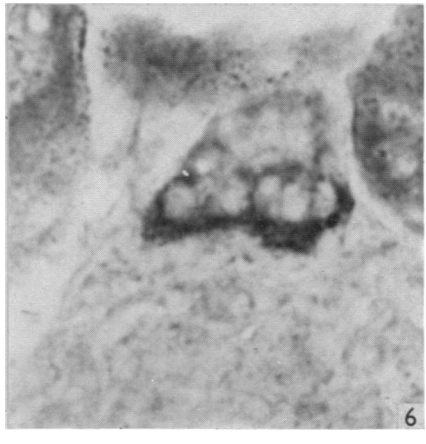












(*Facing p.* 428)