# THE LOCATION OF RENIN IN THE KIDNEY

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Cook and Pickering (1959) have shown that glomeruli isolated from rabbit kidney contain much more renin than the remaining non-glomerular tissue of the cortex. They also found that glomeruli which retained a capsule and fragments of closely adjacent tissue, contained more renin than glomeruli without this additional material and concluded that the cells which form renin were in or near the glomeruli.

To locate renin more precisely, glomeruli were separated from the outer cortex of young rabbit's kidneys by the same magnetic method (Cook and Pickering, 1958). The kidneys were infused with a suspension of magnetic oxide of iron the particles of which lodged in the glomerular capillaries. The cortex was then fragmented, by pressing it through a fine sieve, suspended in physiological saline and allowed to flow past an electromagnet which picked out all the magnetic glomeruli. Those which retained a capsule and had the afferent arteriole filled with the magnetic oxide were then picked out with a micropipette and transferred to a miniature guillotine where they were cut into two parts, of about equal size, one of which always included the vascular pole region and the afferent arteriole.

*The Guillotine* (Fig. 1)

This consisted of a flat Perspex box with an inlet and an outlet for the circulation of ice-cold water. Over a large hole in the top was sealed a brass plate in the centre of which was a well 12 mm in diameter and 3 mm in depth. The well, which was of stainless steel and had a nylon bottom, was thus in intimate contact with the coolant and was kept at about 5°C during the cutting procedure. The knife, a sliver snapped from the edge of a stainless steel safety razor-blade, was mounted on a spring strip of phosphor-bronze which could be depressed by the expansion of the rubber bulb in the brass housing. Thus, when the large bulb was compressed underfoot, the knife was brought into contact with the nylon bottom of the well. The spring strip was constructed with a slight downward bend toward the tip and extra pressure on the bulb, above that required to lower the knife, tended to straighten it. This imparted a forward motion to the blade as well as downward pressure

against the nylon. On release of the pressure, the knife made a backward movement before lifting and in this way a sawing motion was produced which greatly improved the cutting action of the guillotine.

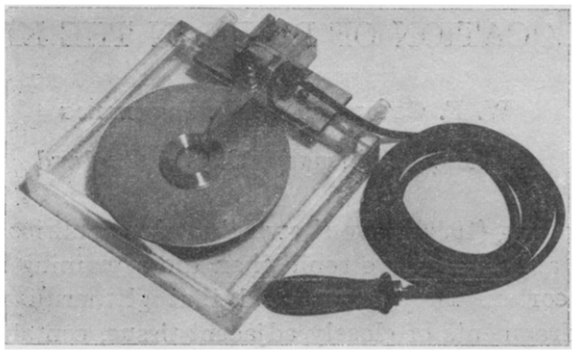

Fig. 1. The Guillotine – a general view. The cutting action was operated by compressing the large teat underfoot, whereupon the small teat in the brass housing (top centre) expanded and depressed the metal spring strip on which the knife blade was mounted.

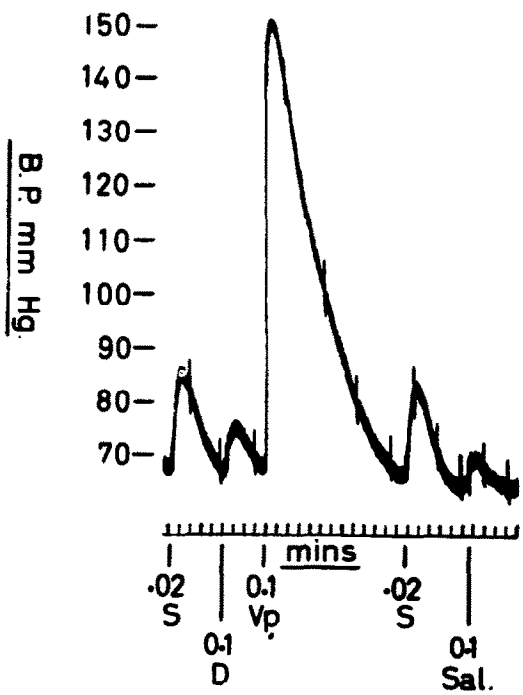


Fig. 2. Rat blood pressure record from the common carotid artery
S = 0·02 ml. of the standard renin
D = 0·1 ml. = the whole of the extract from 500 distal halves
Vp = 0·1 ml. = the whole of the extract from 500 vascular pole halves
Sal. = 0·1 ml. of approx. 0·5% NaCl

The glomeruli were cut in batches of 50, each in turn being manipulated with needles under the knife blade so that the afferent arteriole, easily identified by the filling of oxide, was on the right, and then bisected as accurately as could be judged by eye. The halves were collected in two piles; the vascular pole halves on the right of the knife and the distal halves on the left. When the batch of 50 had been cut, the collected halves were picked up with the micropipette and transferred to small tubes, one of which contained all the vascular pole halves and the other all the distal halves. The well of the guillotine was then washed out with fresh Krebs solution and another 50 glomeruli selected and cut. This procedure was continued for as long as possible, usually about 10 hr, the aim being to cut at least 500 glomeruli in each experiment. This number was not achieved in every case, however. The cut halves, in their respective tubes, were stored at $-20°C$ until required. They were then repeatedly frozen and thawed, suspended in about 0·1 ml of approximately 0·5% saline and centrifuged. The supernatant was taken off and injected, usually as a single dose, into a rat anaesthetized with sodium pentobarbital and treated with pentapyrrolidinium bitartrate. One such experiment is illustrated in Fig. 2 which shows the blood pressure record from the common carotid artery of the rat. In this experiment approximately 500 glomeruli were cut and the figure shows that almost all the pressor activity in the glomeruli was in the vascular pole halves.

In another experiment, approximately 450 glomeruli were cut and this time the vascular pole extract was divided into two equal parts. One part was injected into the rat directly and the other part was incubated with a preparation of angiotensinogen which consisted of a solution of the globulin fraction from the plasma of a rabbit totally nephrectomized 48 hr previously. The solution was completely freed from angiotensinase by adjusting to pH 3 for 30 min at room temperature.

Three tubes were set up, each containing 0·4 ml. of the substrate and 0·4 ml. of phosphate buffer at pH 7·4. To one tube was added half of the vascular pole extract; to the second tube was added the whole of the extract of the distal halves, and to the third tube was added the same volume of saline. The three tubes were left at room temperature for 60 min then placed in a boiling water bath for 5 min. They were then centrifuged for 30 min at 3000 rev/min. and 0·1 ml. of the supernatant from each tube was injected into the assay rat. Figure 3 shows the blood pressure record. Again a large pressor response was obtained from the vascular pole tissue which was also capable of producing angiotensin from an angiotensinogen preparation. Six experiments were done and in all of them renin was easily demonstrated in the vascular pole halves, but was barely detectable in the distal halves. Whether the distal halves actually contained a very small amount of renin or

whether this was introduced by faulty technique it is not possible to say, but there can be little doubt that the major portion of the renin in the glomeruli was contained in the vascular pole area. Precisely which cells are involved remains to be determined.

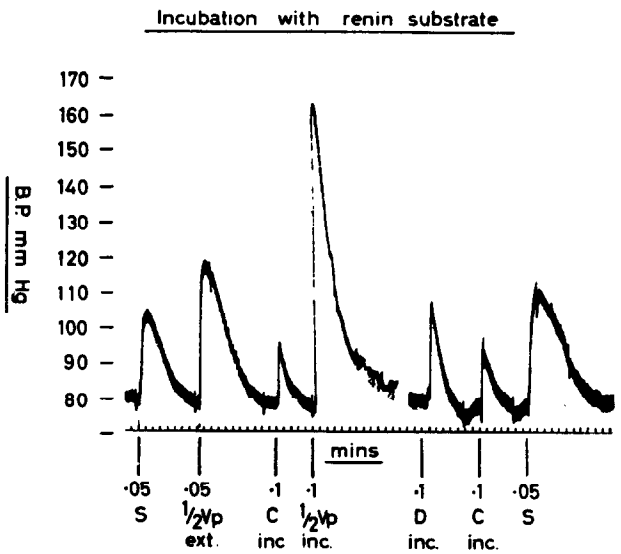


Fig. 3. Rat blood pressure record from the common carotid artery:
S = 0·05 ml. of the standard renin
$\frac{1}{2}$ Vp ext. = 0·05 ml. = half of the vascular pole extract
C = 0·1 ml. of the supernatant from the incubated control tube
D = 0·1 ml. of the supernatant from the tube in which the whole of the extract from the distal halves was incubated.
$\frac{1}{2}$ Vp inc. = 0·1 ml. of the supernatant from the tube in which half of the vascular pole extract was incubated.
The peculiarity in the record at the tail of the fourth response was due to the rider sticking in the manometer tube and the two minute break occurred when this was corrected.

In this region of the glomeruli are several groups of cells which are histologically distinct and which have no known function. They are the macula densa, the juxtaglomerular cells and a group of small flattened cells in the angle formed by the afferent and efferent arterioles. These latter cells have been the subject of some controversy and their nomenclature is confused. The macula densa has been implicated in the formation of renin by Bing and Kazimierczak (1960) and by Hess and Gross (1959).

The work of Pitcock *et al.*, (1959) and Tobian *et al.*, (1959) suggested a link between the granules of the juxtaglomerular cells and renin and we thought that if these granules were renin, or its storage form, then

it should be possible to separate, from the kidney cortex, particles containing a high concentration of renin by ultracentrifugation.

The outer cortex from the kidneys of young rabbits was homogenized in ice-cold 0·3 molar sucrose and fractionated by differential centrifugation according to the scheme outlined in Fig. 4. After removal of the sucrose by dialysis, the fractions were assayed for renin on the

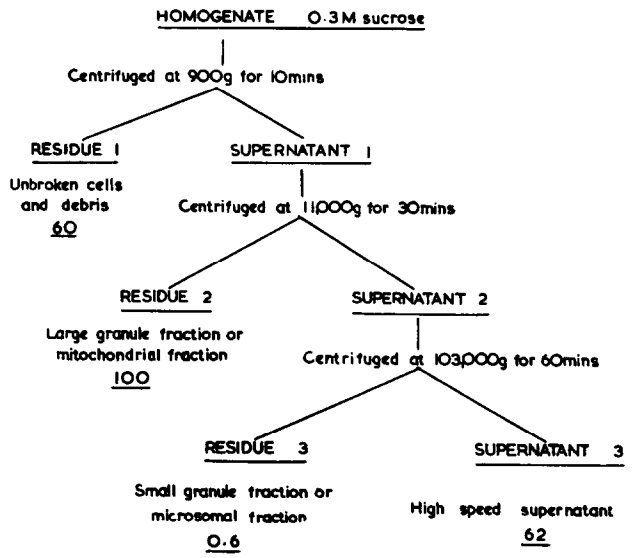


FIG. 4. Differential centrifugation of kidney cortex homogenate. The underlined figures represent units of renin obtained in one experiment.

rat's blood pressure as described previously (Cook and Pickering, 1959). The renin was divided between Residue (1), Residue (2) and the final high-speed supernatant in proportions which varied with the homogenization procedure. Vigorous treatment in the homogenizer resulted in most of the renin appearing in the final supernatant. Gentle treatment left most of the renin in the first residue of unbroken cells and debris. Optimum conditions resulted in about 50–60% of the renin appearing in the "large granule" or "mitochondrial" fraction. Only very small quantities of renin were recoverd from the "microsomal" or "small granule" fraction.

The large granule fraction obtained in this way was further fractionated by centrifugation on sucrose density gradients. This technique has been described by Anderson (1956). The gradients were prepared in 5 ml. plastic tubes by carefully pipetting 0·5 ml. portions of gradually decreasing concentrations of sucrose one over the other. The tubes were then left in the refrigerator at about 2°C for 18–20 hr before use. One millilitre of the large granule fraction was carefully layered over each of three gradients which were centrifuged at approximately

103,000 *g* for 60 min. The tubes were cut into six fractions which were analysed for renin, protein and succinic dehydrogenase. Renin was assayed as before on the rat blood pressure; protein was estimated colorimetrically by the method of Lowry *et al.*, (1951) and the succinic dehydrogenase was estimated by the rate of reduction of potassium ferricyanide in the presence of KCN as used by Kuff and Schneider (1954).

Particle Fractionation On Sucrose Density Gradient

| | gradient M sucrose | after cent. | fraction No. | volume ml | renin % | succinic d-ase % | protein mg |
|---|---|---|---|---|---|---|---|
| applied | | | | 15·0 | 100 | 100 | 5·9 |
| | 0·30 | | 1 | 2·9 | 4 | 0 | 0·1 |
| cut | LGF | | | | | | |
| | 1·20 | | 2 | 1·9 | 1 | 13 | 0·7 |
| cut | 1·30 | | | | | | |
| | | | 3 | 2·7 | 9 | 70 | 3·5 |
| cut | 1·40 | | | | | | |
| | 1·50 | | 4 | 2·7 | 58 | 8 | 1·1 |
| cut | 1·55 | | | | | | |
| | 1·60 | | 5 | 2·5 | 7 | 0 | 0·2 |
| cut | 1·65 | | | | | | |
| | 1·70 | | 6 | 1·8 | 1 | 0 | 0·2 |
| recovered | | | | 14·5 | 80 | 91 | 5·8 |

Fig. 5. Fractionation of the large granule fraction on a sucrose density gradient. The positions at which the gradients were cut were determined by measurement of the pieces of the tubes.

Fig. 5 shows one such fractionation. The enzyme activities of the fractions are expressed as a percentage of the activity in the large granule fraction applied to the gradients. The peak activity of each enzyme occurred in a different fraction and this probably indicates that the two enzymes occur in different subcellular particles.

It is concluded that renin in the rabbit kidney is normally associated with intracellular granules and that the cells concerned are located in the vascular pole region of the glomeruli. The juxtaglomerular cells would seem to be the most likely site of renin storage, but the other cell groups in this region cannot be excluded.

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