THE POSSIBLE SITES OF RENIN FORMATION IN THE KIDNEYS OF THE EXPERIMENTAL HYPERTENSIVE RABBITS

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SIX FIGURES

Two theories concerning the site of renin formation have been known. One is proposed by Goormaghtigh (1940, 47) who assumed juxtaglomerular cells to be responsible for the production of renin on the basis that the cells hypertrophy and the granulation in them augments in degree in the experimental hypertensive animals. The other is proximal convolution theory adovocated by Friedman and Kaplan (1942a, 42b, 42c, 43) from the experimental proof that renin disappears from the kidney of the subcutaneously administered rabbits with Na-tartrate, when proximal convolution epithelial cells undergo severe damage or necrosis. Supported by Selye (1952), this theory has been accepted by a number of investigators.

Recently, Yoshimura and Negishi (1954) substantiated that the vasopressor effect of renin extracted from the kidney of the rabbits injected with Na-tartrate would be neither enervated nor accelerated. Nevertheless, Yoshimura and Tsunoda (1955) confirmed the definite degenerative figures in proximal convolution epithelial cells of the same animals, casting a doubt to Friedman and Kaplans' view. Also they were successful in making the pronounced hyper granulation in juxtaglomerular cells by the chemical administration. This would enable the ground of the theory that juxtaglomerular cells produce renin to be anything but convincing.

Previously, Yoshimura and Nemoto (1953) found the particular dark-shaded cells charged with abundant mitochondria ("dark cell" of Yoshimura and Nemoto) within epithelium of the distal segments of the mammalian renal tubules. Especially in the amphibian kidney, according to Yoshimura and Sunaga (1952) the cells are characterized by the sign of endocrine activity which may be regulated under the anterior lobe of pituitary. In mammals, however, these cells lack in the endocrine figures. In present investigation the authors will examine the cytological alterations in three possible sites of renin formation in the experimental renal hypertensive rabbits with the moderate constriction of main renal artery.

Received for publication May 20, 1955.
MATERIAL AND METHODS

Operation Procedure Eighteen healthy adult male rabbits, weighing ca. 2 kg., were divided into three groups. The operations were performed without anesthesia as follows: In group 1 (12 animals) main artery of one kidney (left) was constricted moderately with silver wire. Among them only four were successfully provided with the marked elevation of blood pressure. In group 2 (3 animals) all were contralaterally nephrectomized (right) with the moderate constriction of main artery of one kidney by the same method, when secure hypertension was made in them without exception. Group 3 (3 animals) were for the control.

Measurement of Blood Pressure Systolic blood pressure of the rabbits was measured indirectly at auricular arteries; the location of measurement was constantly appointed at the fixed place of a small artery of 30-40 μ in diameter, which were distinguishable in structural characteristics of the wall, speed of blood stream and periodic pulsation from auricular vein. The practical way of measurement was utterly in accordance with Kawaguchi's method (1931). Ten times of measurements per one day were made at the interval of 90 minutes and each result recorded represents the mean value of ten times of measurements. Further in order to corroborate the data obtained by indirect method mentioned, blood pressure was measured directly, prior to sacrifice, at the carotid artery attached to a mercury manometer.

Histological Procedure Kidney tissues were removed immediately after decapitation and fixed with Levi's solution, Zenker-formol and 10% formol. They were embedded in paraffin cut at 3-4 μ thickness for serial sections, which were stained with Heidenhain's iron-hematoxylin, Kull's method, azan and hematoxylin (Hansen)-eosin, and for examination of glycogen they were stained with periodic acid-Schiff's reaction (PAS). Sections fixed with Zenker-formol were also stained with thionine for the purpose of detection of contained basophilic substances (ribonucleic acid, RNA) which was identified through hydrolysis test of Ely and Ross (1949).

OBSERVATION

Effect on Blood Pressure in the Experimental Rabbits
Systolic blood pressure of the control rabbits showed 60-30 mm. Hg. at auricular artery (indirect method) with animal differences and environmental affairs. In 4 rabbits among group 1, blood pressure elevated gradually within a period of 24-72 hours after the constriction of main artery of one kidney, reaching a maximum (90-120 mm. Hg., indirect method) in 7-10 days, then being inclined to drop (Fig. 1). The animals were sacrificed at the moment of a maximum of blood pressure showing 170-190 mm. Hg. at carotid artery (direct method).

In order to make the hypertension persist, it is necessary either moderately constrict main arteries of both kidneys or to do main artery of one kidney with contralateral nephrectomy. In all animals among group 2, blood pressure elevated slowly, usually within 24 hours, reaching a maximum (90-120 mm. Hg.) in 7-10 days, then hypertensive status was maintained or persistent without dropping (Fig. 1). The animals were sacrificed at the end of two weeks, blood pressure of carotid artery being calculated at 210-215 mm. Hg. (direct method) prior to sacrifice. These effects on blood pressure by the moderate constriction of main artery of one kidney, with or without contralateral nephrectomy, agree in general with the statements of Goldblatt (1948). Alterations in the kidneys of these actually hypertensive rabbits were histologically examined in succession.
Changes in Juxtaglomerular Cells

Smooth muscle layers of the media of glomerular afferent arterioles in normal rabbit consist of transparent spherical or ellipsoid epithelial cells losing myofibrils (Fig. 2, left). Between epitheloid and endothelial cells is seen the internal elastic membrane (elastic fiber type by Appelt, 1939). Also in the vicinity of vascular pole are present a mass of hypertrophied epithelial cells without the internal elastic membrane, which seemingly belong to Polkissen type of Zimmermann (1933). Both types of the cells have been named “juxtaglomerular cells” (cf. Goormaghtigh, 1940, 47). In normal condition they contain only a few number of delicate rod-shaped and minute granular mitochondria, but by no means large stainable granules (cf. Yoshimura and Tsunoda, 1955).

In a half number of hypertensive rabbits among group 1 and in all among group 2, juxtaglomerular cells hypertrophy and the hypergranulation occurs in their dark cytoplasm (Fig. 2, right). The rod-shaped or filamentous mitochondria in the cytoplasm become thicker, running principally parallel to the cell-axis, and the granular mitochondria also take place sparcely in the cell-bodies. The former
Fig. 2. Juxtaglomerular cells in normal (left) and hypertensive rabbits (right). G; glomerulus, L; lumen of afferent arteriole, M; macula densa. Juxtaglomerular cells in normal condition (arrow) show ellipsoid or spherical transparent epitheloid appearance, in contrast with the hypertensive condition under which these cells (arrow) become dark, hypertrophy and are packed with stainable granules. Fixed with Levi's solution, stained with iron-hematoxylin, 800 X.

Fig. 3. Cross sections of proximal convolution in normal (left) and hypertensive rabbits (right). Stainable granules or hyalin droplets appear in epithelial cells in hypertensive condition. Fixed with Levi's solution, stained with PAS-ironhematoxylin, 800 X.
has often small granular or dot-like swellings at their ends. The cell-bodies are, in this case, occupied by abundant stainable granules which fail, nevertheless, to actually occur in the other half of hypertensive animals among group I. The dimension of the stainable granules is variable; the largest ones are as large as coarse secretory granules and the minute as small as the size of swellings at the ends of mitochondria. This implies that the minute may be derived from the swellings which are in time detached from mitochondria. The minute granules increases gradually in size till they are transformed into coarse secretory granules. They are stained with iron-hematoxylin, Kull's method and azan. In some instances a few of them react on PAS and the reacting substances do not disappear by conventional saliva digestion, being identified with a certain substances but glycogen. In the Zenker-formol fixed and thionine stained preparations the smaller granules are colored in metachromatized tone, but the larger ones not stained. This may depend upon the possible cause of staining capacity of RNA-layer enclosing the smaller young granules in the cytoplasm. Goorgmaghtigh stated that in the initial phase of hypertension juxtaglomerular cells are diffusively stained, but acidophilic granules follow the development of hypertension, and finally basophilic ones arise in place of the former. Dunihue and Candon (1940), Dunihue (1941) and Kaufmann (1942) also mentioned that acidophilic, neutrophilic and basophilic granules arise, in turn, in them following the development of hypertension. In the authors' view, there might be no three kinds of granules in the same cells, but the differences in staining capacity may depend upon the unstable character of the same kind of granule according to cell function.

In the preceding report (1955), the authors demonstrated evident pictures of hypergranulation and internal secretion in juxtaglomerular cells of the rabbits by Na-tartrate administration. It is also the case with the present hypertensive animals that the pronounced hypergranulation under the same mechanism as exocrine glandular cells in general and the sign of endocrine phenomenon with little vacuolization were observed in these cells. The size and number of secretory granules in hypertensive animals are, however, much reduced, in comparison with those in the chemical administered rabbits.

Changes in Proximal Convolution Epithelial Cells

Epithelial cells are frequently more or less affected, despite some cells are not impaired in a certain nephrons. In the affected tubules the lumen are generally distended on account of flattened lining epithelial cells. In regard to the cytological changes in cells, it was learned in present investigation that basement striation is destroyed and its consistuent mitochondria are short, cut minutely or granulated (Fig. 3, right). In some cells all mitochondria show granular appearance with variable dimension. Their contours are obscure, but they are able to be preserved with Levi's solution and Zenker-formol and also stained faintly black and red with iron-hematoxylin and Kull's stain respectively. In the basal parts of some cells, however, relatively large granules are present, which are deeply colored with distinct contours. These are also sometimes seen at the supranuclear region, and when numerous, filling the whole cell-body. Also being strongly stained with PAS, they are considered identical with hyalin droplets.
Many faintly stained granules and hyalin droplets are often scattered from place to place in contrast with the normal picture that the cells include only a few number of stainable granules and vacuoles (Fig. 3, left). In some cases, faintly and firmly stained granules and vacuoles are found by mixture in a cell. Also large and small vacuoles with variable contour are frequently present at the every part of the cells. They are seemingly the degenerative vacuoles.

Furthermore, while the normal picture in some cells also often appear, of course, within the epithelium, exaggeration of apocrine secretion, discharge of nuclei and destruction or desquamation of the cell-bodies are sometimes visible in the another cells. The cytoplasm of some different cells become extremely cloudy and nuclei are either atrophied or discharged, presenting a necrotic pattern. Degeneration is advanced together with the degree of constriction of main renal arteries. The arterial occlusion or clamping in excess which failed to produce hypertension in group 1 caused sequentially the serious damage in many of epithelial cells. There occur no differences in degree of degeneration according to whether or not one kidney is removed; on the contrary, hyalin droplets and degenerative vacuoles were apparently observed in proximal convolution epithelial cells of the contralateral and unoperated kidney (Fig. 4, left).

Selye (1952) enumerated, as the morphological characteristics in so-called endocrine kidney, regeneration in proximal convolution epithelial cells, atrophies of renal tubules except proximal convolution, adhesion of the interstitial tissues and contralateral nephrosclerosis etc. The authors were able to corroborate Selyc's criteria in vain in present investigations, nor able to observe any vascular lesions.
like arterio- and arteriolosclerosis with thickening of the media and hyperplasia of elastic fiber and hyalinization of the intima, in the kidneys of hypertensive animals; in spite of this negative data, the animals were substantially in hypertensive status.

Changes in the Dark Cells in the Distal Segments of the Renal Tubules

The occurrence of characteristic dark-shaded cells has been primarily demonstrated by Yoshimura and Nemoto (1953) in the distal segments of renal tubules of various mammals (Fig. 5, right). In hypertensive rabbits they increase in number in junctional and collecting segments in medullary ray, hypertrophy and bulge in excess into the lumen (Fig. 5, left). The cytoplasm are full of delicate filamentous mitochondria with the dot-like swellings at their ends and fine granular ones. The smaller stainable granules with the same dimension as the granular mitochondria are scattered within the cell-body. No large stainable granules are, however, seen at any part of the cells. The small ones could augment in number, but in size. The dark cells often send the cytoplasmic eminences with various shapes into the tubular lumen. These eminences usually include a number of mitochondria and fine granules, but sometimes exclude mitochondria. The tips of them are deprived or desquamated as small fragments with embraced some inclusions. Thus, the dark cells conduct the pronounced apocrine secretion during the hypertensive status as well as in normal condition (cf. Yoshimura and Tsunoda, 1955).

Furthermore, several number of accumulated small vacuoles are seen at the basal parts of the cells, and since in the surrounding area of vacuoles there are faintly stained granules which are at the various stages of vacuolization, the
vacuoles may be resulted from liquefying stainable granules; the stainable granules are probably able to be transformed into the small vacuoles which increase gradually in size and aggregate in some measures, but are not converted into a huge vacuole. Often the vacuoles are distributed perpendicularly in a row to be identified with the intracellular canaliculi connected to the basement membrane, through which the content of vacuoles may be transported and flow out into the intertubular spaces, presenting a picture of exaggeration of the internal secretion (Fig. 6). Thus, it is worthy of notice that the constriction of main artery of one kidney endow surely an endocrine activity to the dark cell.

Epithelial cells of intercalated segment of renal tubules become higher and contain numerous filamentous mitochondria. They are also provided with the appearance of apocrine secretion conformed to the manner in the dark cells; intercalated epithelial cells are considered to be in intimate genetic interrelation to the dark cells in the distal parts of renal tubules.

In the unoperated and opposite side of kidney, epithelial cells of the distal segments of renal tubules were flattened and the dark cells occur infrequently (Fig. 4), where they are pressed by the adjacent cells to be atrophied, being sometimes spherical in shape, when mitochondria and granules are poor in them (Fig. 4), and the secretory function, in both internal and external, may be suspended or restrained.

Finally, in consideration as to the relationship between the dark cell and common pale cell in the distal segments of renal tubules (cf. Yoshimura and Nemoto, 1953), the authors have, in present investigation, much regard for the following observation that the pale cells with abundant mitochondria often occur within the epithelium of distal segments. This finding permits the authors to interpret
that the mitochondria-rich pale cells may assign their genetic origin to intercalated
cells and also may be probably transformed into the dark cells in case of hyper-
tensive condition, since the various kinds of direct transitional stages between the
dark cell and pale cell in the distal segments of renal tubules were observed in
present investigation.

DISCUSSION

As above alluded, Goormaghtigh elucidated that the experimental renal
hypertension induces hypertrophy and significant hypergranulation in juxtaglomerular
cells in dogs and rabbits, with consequent assumption that these cells are the site of
renin production. Juxtaglomerular apparatus theory has been approved afterwards
by Harrison et al. (1936), Pickering and Prinzmetal (1938). Notwithstanding that
Dunihue and Candon (1940), Dunihue (1941) and Kaufmann (1942) ascribe exclu-
sively the origin of experimental hypertension to these cells, Clara (1927),
Schumacher (1938) and Preg (1948) gave the contradictory view to Goormaghtigh;
these cells may secrete, according to them, acetylcholin- and histamin-like sub-
stances which only act as the regulating apparatus of the local circulation.
Yoshimura and Tsunoda (1955) were successful in making the pronounced hyper-
granulation by Na-tartrate administration, a though the chemical hardly influence
upon the renin content extracted from the administered kidney. In both hyper-
tensive and non-hypertensive animals in present experiments the granulation is
commonly made in juxtaglomerular cells, but all of the former were not always
provided with the hypergranulated cells. This fact supports a conception that
there is no particular correlation of granulation in juxtaglomerular cells with hyper-
tension. It was not susceptible of present experimental proof that the secretory
granules in juxtaglomerular cells may participate in the production of renin. The
authors could infer that the granulation is extremely labile and may be easily
subjected to various stimulative factors or agents.

Goldblatt (1948) emphasized, in his widespread works, the sign of nephro-
sclerosis in human essential hypertension. In the kidney of experimental renal
hypertension, however, he did not find any marked morphological alterations; in
the benign phase biopsies do not show special alterations in the kidney, and in
dogs, even after 6 years of persistent hypertension, no significant pathological
changes were observed in the aorta or in the large and small arteries in the other
organs. Goldblatt's experiments, therefore, afford no proof for the view that
hypertension by itself is a sufficient condition for the production of generalized,
true, simple arterial or arteriolar sclerosis. However, in the malignant phase,
even when it terminates fatally in as little as 48 to 72 hours, the most profound
changes have been observed in the wall of blood vessels. Microscopically, in the
aorta, there is only interstitial edema, mainly of the media; but the wall of
arteries and arterioles, in those organs in which the petchiae occur, is the seat
of necrosis and fibrinoid degeneration, with or without vascular and perivascular
inflammation characterized by the exudation of polymorphonuclear leucocytes
and some lymphoid cells. However, in the kidney vascular lesions do not occur
beyond the site of constriction of main renal artery. The morphological findings
in present experiments would presumably appertain to the benign phase, and vascular lesions seen in the malignant phase were not present in the arteries and arterioles in the other organs. Wilson and Byrom (1939) illustrated the modifications presenting the malignant phase in the contralateral kidney of hypertensive animals with the obliteration of main renal artery, but Goldblatt did not accept their results. Also above modifications were not obtained in the authors' observations.

Proximal convolution epithelial cells are easily affected in general by various unknown stimuli. Some degenerative signs were frequently recognized in the kidneys of hypertensive animals, in spite of a number of intact epithelial cells. Degeneration in renal tubules, especially in proximal convolution, may depend upon the degree of clamping of renal arteries, and the profound damage or necrosis occurs in epithelial cells by the higher obliteration with the renal excretory insufficiency. As concerns regeneration in proximal convolution epithelial cells making the ground of Selye's theory relating the site of renin formation, the authors could not approve of it, nor observe any mitotic figures in them. Goldblatt reported, in the kidney with the moderate constriction of main renal artery, that there is little or no interstitial fibrosis, and the reduced size of the kidney is due to disappearance of glomeruli and of cortical tubules as well as shrinkage of the tubules which have no distinct lumen and which are lined by greatly altered epithelium in which the sign of regeneration is commonly recognized. The authors could not, however, observe widespread necrosis or disappearance of parenchym cells, so that proximal convolution epithelial cells are no longer considered to be a source of renin production.

The dark cells in the distal segments of renal tubules increase exceedingly in number and hypertrophy in the initial phase of hypertension, and furthermore several number of vacuoles, sometimes in particular intracellular canaliculi, occur at the basal parts of the cells. An assumption that the dark cells may secrete renin would be naturally drawn up from the fact that the moderate constriction of renal arteries often produce the apparent sign of internal secretion in these cells. Standing on this hypothesis, the authors could conceive that the hypersecretion of renin may be of functional significance in the initial phase of hypertension. Also Taquini and Fasciolo (1946) noted, in biopsies, that amount of renin in the kidney increased rapidly after the clamping of renal arteries; this increase, according to them, usually appeared within the first 20 minutes, generally reached a peak within 60 minutes after the initiation of the ischemia. The authors hope to investigate the alterations in the dark cells, especially in the acute hypertension, shortly after the ligation of renal arteries. And that in the contralateral kidney the dark cells fall into atrophy, enables the authors to interpret it as the reflected or compensatory action upon the hypersecretion of renin in one kidney.

**SUMMARY**

Among 18 health male adult rabbits, 7 were made hypertensive by means of the moderate constriction of main renal artery with or without the removal of the contralateral kidney. Systolic blood pressure was measured indirectly and directly
June 1955  

SITES OF RENIN FORMATION  

at auricular and carotid arteries respectively. The possible sites of renin formation were histologically investigated in the kidneys of renal hypertensive rabbits.

In some of hypertensive animals the hypergranulation occurred in juxtaglomerular cells under the same mechanism as the exocrine glandular cells in general, while in the other hypertensive animals there is little or no alterations in juxtaglomerular cells. In the authors' view, the granulation is extremely labile and may be altered by manifold stimuli or agents; Yoshimura and Tsunoda (1955) were successful in making the hypergranulation in them by Na-tartrate administration, when renin content in the kidney was settled on the constant level. These preceding results and present experimental indices do not support a conception that the granules in juxtaglomerular cells may contain renin substance (Goormaghtigh's view).

Proximal convolution epithelial cells are easily affected in general by various stimuli; some degenerations are frequently recognized in epithelial cells of present hypertensive rabbits, in spite of a number of intact epithelial cells. The profound damage or necrosis in the epithelial cells responds to the higher obliteration with renal excretory insufficiency. Regeneration in proximal convolution, interstitial fibrosis and serious and widespread necrosis or disappearance of parenchym cells were not observed in the kidneys of present hypertensive rabbits. Also any kinds of vascular lesions, arterio- and arteriolasclerosis, take place in vain in the kidneys of the authors' experimental animals. These do not affirm that proximal convolution is a determined site in the formation of renin (Friedman and Kaplans' or Selye's theory).

The dark cells in the distal segments of renal tubules increase in number and hypertrophy in the initial phase of hypertension, and several number of vacuoles, sometimes in particular intracellular canaliculi, occur at the basal part of the cells. Thus renal experimental hypertension induced the sign of internal secretion in the dark cells. The authors would, therefore, conceive the hypothesis that the dark cell is a probable source of renin production and its hypersecretion may be of functional significance in the initial phase of hypertension.

REFERENCES

Dunihue, F. W.: Arch. Path. 30: 40, 1941.
Goormaghtigh, N.: Am. J. Path. 16: 409, 1940.