HYPERGRANULATION IN GLOMERULAR SURFACING CELLS OF THE RAT KIDNEYS IN RESPONSE TO DCA ADMINISTRATION

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FOUR FIGURES

A special attention has been given upon the secretory activity of glomerular surfacing cells (visceral glomerular epithelial cells, Deckzellen) by Yoshimura and Sunaga (1952) on the cytological basis that some of these cells in the amphibian glomeruli participate in secretory activity attributed to cell function by itself but to physical filtration mechanism. Their observations support the contradictory view of Tamura (1936) to physical filtration theory dealing with the glomerular function. The secretory phenomenon in surfacing cells has been thereafter observed not only in mammalian kidneys, but also in human ones (Yoshimura and Sunaga, 1953; Sunaga, 1955 a). However, in normal condition accumulated secretory granules have been infrequently observed in mammalian glomeruli. Present authors will communicate that DCA administration often induces pronounced hypergranulation in surfacing cells of the rat kidneys and discuss its functional significance.

MATERIAL AND METHOD

For the present work, eleven male adult albino rats in Wistar strain weighing 130-160 g. at autopsy were used. Animals in litter mates constantly maintained at 28°C room-temperature were enteratined with a commercial laboratory chow (Lot # R 41, Oriental & Co.) plus some vegetables, and given freely with drinking water. Three rats in the first lot were subcutaneously administered daily 2.5 mg. of desoxycorticosterone acetate (Schering's Cortate) per one rat for seven days, four rats in the second lot, a half of which were unilaterally nephrectomized at the third administration day, were injected daily with the same dosage of the drug per one rat for thirty days, and the remainders were for the control with or without unilateral nephrectomy. Animals were sacrificed by decapitation at the end of six hours after the final injection. Kidney tissues were fixed, immediately after the sacrifice, with Levi's solution, Zenker-formol and 10% formol, and embedded in paraffin to make the serial sections at 3-4μ thickness. Sections were stained with Heidenhain's iron-hematoxylin, Kull's and azan stains.

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OBSERVATIONS

**Glomerular Surfacing Cells in Control Rats**

Surfacing cells are usually based on the ground membrane of glomerular capillary loop and face the subcapsular space of Bowman (Fig. 1.) Since boundaries between cells are obscure, their shapes are not recognizable in biopsy except when the staining capacity differs respectively between neighboring cells, where they show irregular and complicated appearances. The poor cytoplasm surrounding the large nuclei which are scanty in chromatin are pale in general and include a few number of delicate filamentous or rod-shaped mitochondria, distributed approximately parallel to the cell-axis, and of fine granular ones whose occurrence is not easily demonstrated in common preparations insufficiently fixed. The former has frequently the spherical swellings at their ends. Ranking with mitochondria there are also rarely found the stainable granules coloured red and black with Kull's stain and iron-hematoxylin respectively; the minute one is as small as the spherical swellings at the ends of mitochondria. This infers the derivation of the minute granules: they may be followed by the removal of the spherical swellings from the mitochondria. The minute granules possibly formed after this manner pressumably remain in indifferentiated stage with little increasing size, since large stainable granules and vacuoles hardly appear in the cell-body.

However, it has been learned in present observation that surfacing cells accumulating lots of stainable granules with various dimensions are exceptionally

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Fig. 1. Normal picture of renal-corpuscle in control rat. **CS**; common surfacing cells containing no granules, **D**; dark surfacing cells packed with secretory granules, **B**; basement membrane of capillary loop, **CB**; epithelium of capsule of Bowman, **S**; subcapsular space of Bowman. Fixed with Levi's solution, stained with iron-hematoxylin. 1200×.
present even in normal condition (Fig. 1). This kind of the cell is usually dark, hypertrophies and is so packed with stainable granules (named “the dark surfacing cell” by Yoshimura and Sunaga, 1953). These granules differ in both size and staining capacity; the largest are identical with coarse droplets either deeply or faintly stained, being out of lipid or lipofuchsin nature, because they are also well preserved by Zenker-formol and having not native yellow tone in the preparations fixed with 10% formol. The minute granules which may be derived from the spherical swellings of the mitochondria augment in both size and number with consequent result of occupying the whole cell-body. Although it would not be perceptible of the picture of direct depletion into subcapsular space, these granules may be, in the authors’ view, practically released into space with little vacuolization. The granules in the dark surfacing cells are therefore nothing but secretory granules whose formation is in agreement with that in exocrine glandular cells in general (cf. Yoshimura and Sunaga, 1953). Obtained findings would appreciably show that, even in normal condition, dark surfacing cells are, to some degree, able to be provided with secretory activity probably conditional on the promotion of cell function.

**Epithelial Cell of Capsule of Bowman in Control Rats**

The epithelium of capsule of Bowman consists, in general, of flattened squamous epithelial cells. Their cytoplasm are sometimes so flattened that they appear as a line of capule of Bowman but in the vicinity of nuclei. And the cells contain a

![Fig. 2. High columnar and dark epithelial cells of capsule of Bowman in renal-corpuscle of control rat. P; proximal convolution at the urinary pole, B; brush border, D; dark surfacing cell including several number of secretory granules. The cells are characterized by the occurrences of brush border, basal striation and granule formation. Fixed with Levi’s solution, stained with iron-hematoxylin. 1200×.](image)
few number of mitochondria but no stainable granules. However, there often appear among them, singly or in group, large dark epithelial cells which resemble, in most, proximal convolution epithelial cells with brush border, granule formation and basal striaation. These cells arise more frequently near the urinary pole. The occurrence of them has been ascertained in human renal-corporuscle by Sunaga (1955 a), who has not, however, enumerated the equipment of the brush border in them as one of the similar characteristics. In rats, dark epithelial cells of Bowman are often provided with the brush border even in normal condition (Fig. 2). Since these cells and proximal convolution epithelial cells are considered by a number of investigators to have the same developmental origin (cf. Zimmermann, 1915, 29; Mc Gregor, 1929), it is naturally accepted that both cells show the identical appearances. Also the authors could confirm the figures of apocrine secretion in the epithelial cells of capsule of Bowman, supporting Sunaga's statement in men (cf. Sunaga, 1955 a).

Changes in Surfacing Cells in DCA Administered Rats

DCA administration for as long as seven or thirty days hardly influenced upon the degree of granulation in surfacing cells, so far as the alternative kidney was not removed. These cells in rats unilaterally nephrectomized were fairly affected by the persistent DCA administration, in contrast with non alterations in control rats with bilateral kidneys. As the results of administration plus operation, surfacing cells rich in secretory granules appear more frequently in glomerulus. These granule-rich cells are usually observed in most of glomeruli; when the granules are abundant, the cells look to be masked with them (Fig. 3); inversely

![Fig. 3. Hypergranulation in dark surfacing cells in rats with persistent DCA administration. D; dark surfacing cells masked with secretory granules, V; vacuoles in dark surfacing cells, B; basement membrane of capillary loop, E; erythrocyte. Fixed with Levi's solution, stained iron-hematoxylin. 1200X.](image-url)
when they are reduced in number, only a few of them take place in parts of the
cells. That the granule-rich cells contain no mitochondria would tell the exhaustion
of them following the elevation of secretory action. The cells really hypertrophy
to some degree, but are not inclined to be proliferous. The extraordinary pale
cytoplasmic eminence sometimes bulging into the subcapsule space also include
several number of stainable granules. The tips of them were occasionally thrown
off or desquamated as small fragments containing granules which are either de-
creased in stainability or liquifying themselves. The authors would interprete this
to be a mere discharge of cytoplasm rather than the real apocrine secretion.
Present experimental proofs do not afford us to understand that the direct release
of secretory granules with or without vacuolization is common with most of sur-
facing cells, but to consider that it is limited in dark surfacing cells charged with
abundant granules.

Direct Cytoplasmic Combination Between the Epithelium of Capsule of Bowman and the
Glomerular Surfacing Cell Layer in DCA Administered Rats

DCA administrations for seven days produce no conspicuous alterations in
many of common epithelial cells of capsule of Bowman in rats with or without
unilateral nephrectomy. It is also the case with the dark high columnar epithelial
cells similar to proximal convolution epithelial cells in its internal structure.

Fig. 4. Direct cytoplasmic combination between the surfacing cell layer and the epithelium
of capsule of Bowman and its diagram (right). DC; direct cytoplasmic combination
full of secretory granules, CB; epithelium of capsule of Bowman, S; sub-
capsular space of Bowman. Fixed with Levi's solution, stained with iron-hematoxylin.
1200 X.
However, in rats administered with the overdosage of the drug, it is rarely found that the epithelial cells of capsule of Bowman send the prolonged and slender cytoplasmic projection into the subcapsular space (Fig. 4). Furthermore they extend themselves deeply between the glomerular lobuli along the surface of them, being replaced with the surfacing cell layer. At the basal part of the projection somewhat thick filamentous mitochondria arranged irregularly are associated with lots of stainable granules. The projection is full of stainable granules and thick filamentous mitochondria resembling those in dark surfacing cells. In fact, all granule-rich cells take place, as a rule, toward the extension of the projection as illustrated in figure 4, which may be susceptible of indication that the prolonged and slender projection is a sort of cytoplasmic direct combination between the epithelium of capsule of Bowman and the surfacing cell layer. The view that the former reflects on the latter at the vascular pole is believed in general mainly on the basis of embryology, and further it was known that the direct combination is made somewhere between two cell films excepting at the vascular pole; Bargmann (1936) demonstrated the occurrence of it in reptilian glomeruli. A possibility is also naturally arising that the combinations may be present in rat glomeruli even in normal condition. But they neither hypertrophy nor contain stainable granules, so that it is controversial that they are able to be sufficiently distinguished from the artificial productions shed in the subcapsular space under mounting the optical preparations. Anyhow, the present investigation elucidated the fact that the direct cytoplasmic combination is brought to in the sufficiently demonstrable condition by DCA administration.

Three kinds of cells, that is, surfacing cells, epithelial cells of capsule of Bowman and proximal convolution epithelial cells, are considered to belong to a continuous cell layer with the same developmental origin and preceding two of them may participate together in excitative secretory activity in response to DCA administration.

DISCUSSION

Upon the demonstrations of hypertension, polyuria, nephrosclerosis, cardiac nodule and periarteritis nodosa in the unilaterally nephrectomized rats administered with DCA and fed on high sodium diet, Selye et al. (1943) established the hypothesis that mineralo-corticoid is a sort of origins of hypertension. It has been also supported by recent investigators (Friedman and Friedman, 1949; Prado, 1950; Creen et al., 1952; Salgado, 1953). As the changes in the kidneys by means of DCA administration, Selye et al. adduced hyalin degeneration of glomerulus and hypertrophy of epithelium of capsule of Bowman. In the present experimental condition that animals are kept on out of high sodium diet, we could obtain no sign of hyalin degeneration in them.

DCA appears in general to be concerned primarily with regulation of electrolyte balance, a function which is mediated entirely by promoting the reabsorption of sodium in the renal tubules; when injected into normal animals, the drug also induced abnormal retention of sodium and a loss of potassium. Thus, DCA possesses such sodium retaining power and increases the intracellular sodium concentration, while it has no effect on potassium excretion (Thatscher and Hart-
Otherwise, in regard to glomerular filtration rate, it has long been recognized that the administration of isotonic saline increases the glomerular filtration rate in dogs. Schmitz (1932) observed increases of 27 to 89 per cent in dogs after the intravenous administration of quantities no larger than 100 to 150 cc. This observation has been supported by many investigators (Shannon, 1942; Balwin, Kahana and Clarke, 1949). In fact, massive doses of DCA in normal rats lead to the development, in a few weeks, of polydipsia and polyuria, which are exaggerated by the dietary administration of excess salt (cited from Smith, 1951). The increased urine flow induced by large dose of DCA is frequently likened to diabetes insipidus, but the parallel is unsafe. Nevertheless, various factors are presumably involved in this diuresis which is too complex to make simple interpretation. The question of electrolyte distribution between tissues and extracellular fluid, the concentration of sodium in the plasma, changes in the filtration rate and sodium load delivered to the distal tubules, and a possible toxic effect of large doses of DCA on the tubules themselves, all require further investigation before a physiologically specific "diuretic" activity can be attributed to DCA or any of the natural hormones. Consequently it is yet unapparent whether or not the glomerular filtration rate by itself augment in degree by the administration of large doses of DCA, in spite of apparent conspicuous sodium retention.

That, also morphologically, pronounced hypergranulation were certainly observed in surfacing cells is probably considered to be due to condition of more or less diuretic action because in the light of present investigation we understood morphologically that they may participate in exaggeration of glomerular secretory function. It is also much interesting that analogous hypergranulation is known to occur in the amphibian glomerulus especially during hibernation (Yoshimura and Sunaga, 1952).

Apart from this phenomenon in rats with alternative kidney, the hypergranulations have not been observed in rats with the present DCA administration so far as the bilateral kidneys remain. Little was known about the reason of this opposite result, but it is also commonly considered that DCA is much effective on the kidney in compensatory hyperfunction on account for the contralateral nephrectomy.

SUMMARY

The morphological influences of DCA administration upon glomeruli of rats with or without unilateral nephrectomy were cytologically investigated. Some of glomerular surfacing cells in rats have more or less the secretory activity even in normal condition. Administration of 2.5 mg./day for seven or thirty days did not affect in any significant measures surfacing cells in rats with bilateral kidneys, while thirty days administration produced, in so far as animals were unilaterally nephrectomized, pronounced hypergranulation in dark surfacing cells, when the direct cytoplasmic combinations between surfacing cell layer and the epithelium of capsule of Bowman were rarely recognized. They extend themselves deeply between the glomerular lobuli upon the glomerular surface, being replaced by
the surfacing cell layer packed with the secretory granules. Thus DCA administration upon the unilaterally nephrectomized rats made hypertrophy and hypergranulation in dark surfacing cells, showing exaggeration of secretory activity.

REFERENCES

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