CYTOCHEMICAL CHANGES IN THE URINIFEROUS TUBULES OF THE KIDNEY UNDER THE ACTION OF MERKUSAL

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Changes in the structure of the kidney and in its enzyme content under the influence of mercury and its derivatives, including mercurial diuretics, have been repeatedly investigated by us [4, 6, 9, 10, 12]. However, we have not given sufficient attention to a more detailed study of the cytochemical shifts observed under these conditions in the cells of all parts of the nephron.

The aim of our work was an investigation of the structural and cytochemical changes in the kidney under the action of merkusal.

Merkusal was selected in connection with the fact that its administration made it possible to produce the picture of chronic and acute mercury poisoning and, further, we hoped to obtain some additional data on the mechanism of action of this diuretic.

EXPERIMENTAL METHOD

We carried out our experiments on white mice which weighed 18-20 g. Merkusal was administed intravenously in therapeutic doses (3 mg. per 1 kg. of weight) daily for 30 days, and, also, in single doses of twice to ten times the therapeutic dose.

Materials were fixed in a mixture of Carnoy's, Zenker's, Champy's, and Regaud's fluids and alcohol with formalin. Microscopic sections were stained with Boehmer's hematoxylin and eosin, iron hematoxylin, acid fuchsin of Bensley and Kull, and also methyl green-pyronine by Unna's method, and with Sudan IV. We carried out Feulgen's reaction; part of the preparations were developed in 5% solution of trichloroacetic acid (at 90°C for a period of 15 minutes to remove ribonucleic acid (RNA) and desoxyribonucleic acid (DNA)). We stained the total proteins with fast green at pH 2.2, the basic proteins with fast green at pH 8.0. Polysaccharides were determined by the Shabadash-Hotchkiss method.

EXPERIMENTAL RESULTS

The morphological structure and physiological importance of the various sectors of the nephron have been described in special handbooks and monographs [1, 2, 3], hence only a short description of the cytochemical characteristics of the normal kidney will be presented below.

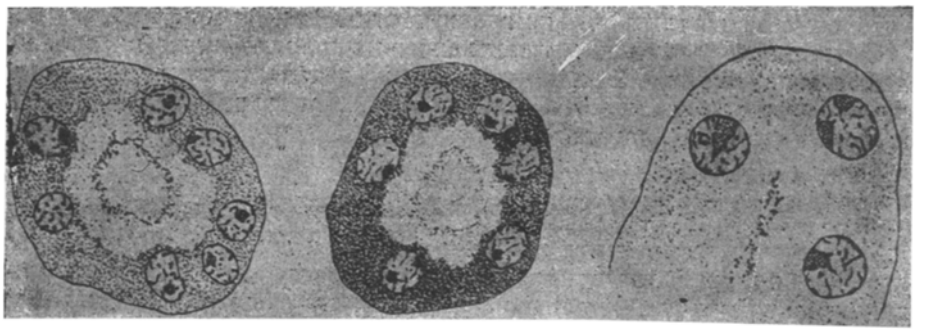
In normal kidneys of the white mouse, the nuclei of the cells of the uriniferous tubules give an intense reaction for DNA, their content of basic and acid proteins is low and approximately the same in all sectors. The nucleoli are rich in RNA and contain a small amount of acid proteins; basic proteins are not found in them.

Particularly large quantities of RNA and acid proteins are present in the cytoplasm of the main portions of the tubules; their content gradually decreases in the distal portions of the nephron.

As a rule, the basic mass of RNA and of the acid proteins is concentrated at the point of disposition of the chondrioma.

Polysaccharides were found in a significant amount in the "brush border" of the main portions, in the basal membrane and in the interstitial tissue, and we observed pale staining in the basal parts of the cells of the convoluted tubules of the main portions.

After a single administration of a therapeutic dose of merkusal, we observed only an insignificant increase in the content of RNA in the cytoplasm of the cells of the convoluted part of the main portion of the tubules. Within three days after daily injections of the diuretic, the cytoplasm of the cells of the main portions, especially their convoluted part, became intensively pyroninophyllic (see Sketch, a, b). Then the intensity of the pyroninophilia gradually decreased and on the 7th-8th day reached the level characteristic of the intact kidney, or even somewhat lower. Under these conditions, we observed the formation of minute foci of undifferentiated connective tissue developing between the tubules of the cortical and medullary layers. Large protein vacuoli appeared in the cells of individual straight tubules of the main portions and the mitochondria were swollen. We observed dilation of the vessels.



a b c

Structure of the kidney of the white mouse: a) the normal animal; the cytoplasm of the epithelium of the convoluted part of the main portion of the uriniferous tubules shows a significant pyroninophilia; b) 3 days after daily administration of merkusal. The pyroninophilia of the main portion is clearly increased; c) the kidney, a month after daily administration of merkusal; the pyroninophilia of the cytoplasm of the hypertrophied cells has been diminished. Fixation with Carnoy's mixture, staining with methyl green and pyronine.

After administration of merkusal for a period of 30 days, rather thick mitotic figures appeared in the cortical substance of the kidney. We observed extensive growth of connective tissue in all layers of the kidney up to the papilla. The majority of the tubules, surrounded by connective tissue, became atrophied, the "brush border" in the main portions was destroyed. The intensity of the pyroninophilia of the cytoplasm of the cells of these tubules was not diminished; however their content of acid proteins was notably decreased, the mitochondria were disintergrated into tiny fragments or disappeared.

In the remaining portion of the parenchyma of the kidney, we observed a swelling in the mitochondria of the cells of the main portions; the nuclei of individual cells of the convoluted tubules became pycnotic, the cells disintegrated. We observed a significant increase in the size of the nuclei (on the average, three times, in comparison to the controls) and an elevation of the cells of the main portions of a number of nephrons. The nucleoli were also enlarged and fused, forming 1-2 large, not infrequently odd forms of a nucleolus, generally abutting upon the membrane of the nucleus. The pyroninophilia of the hypertrophied cells decreased, but this was, evidently, associated not with a decrease in the amount of RNA, but with its distribution in the large volume of the cytoplasm (see Sketch a, c); the content of acid proteins also did not decrease. In the cytoplasm, not infrequently, we observed large protein vacuoles and fat droplets. An accumulation of DNA and basic proteins took place initially in the enlarged nuclei; afterward, some of these lost color and were dissolved; the RNA-rich nucleoli which were lying free in the cytoplasm disintegrated and the cells died.

The reaction of the epithelium of the nephrons to the administration of one and the same dose of merkusal was not always the same. Evidently, this is explained by different individual sensitivities of the animals.

Usually after the administration of a dose which was twice as strong as the therapeutic dose, we observed some increase in the content of the RNA in the cytoplasm of the main portion, rare cariokinetic figures appeared; the nuclei of individual cells clearly enlarged in volume, while in other cells they became pycnotic. On administration of six times the therapeutic dose, but more often on injection of eight times the dose of merkusal, the cells of a large part of the convoluted tubules of the main portions became necrotic. In the apical parts of the cells of

the main portions usually in the straight tubules, fine vacuoles appeared (hyaline drops) containing acid proteins and polysaccharides; the mitochondria in this case did not change in form. Gradually the number of hyaline drops was increased; they became large and filled all the cell, and the mitochondria were surrounded and swollen. The height of these cells was raised, their RNA contents was significantly lessened and we found it only in the fine, pale layers between the hyaline droplets. The amount of acid proteins in the cytoplasm surrounding the hyaline droplets was also lowered, the nuclei became pycnotic, the cells died and desquamated into the lumens of the tubules.

In the cytoplasm of the cells of the little-changed main portions, the content of RNA was a bit higher, the amount of acid proteins was not changed. In the remaining sections of the nephron we did not observe evident changes. It is difficult to pass judgment on the dynamics of the change in the content of DNA and basic proteins in the nuclei since the degree of these changes varied strongly: the nuclei of some cells became pycnotic, the nuclei of others became larger and were dissolved.

We observed hyaline cylinders in the lumens of the tubules.

The above-mentioned observations force us to join the opinion of the research workers who regard as incorrect the conclusion[8] that the cells of the ascending limb of the loop of Henle are the most sensitive to the action of mercurial diuretics. The cells of the main portions of the nephron appeared to be the most sensitive to the administration of merkusal.

The distinct increase in the intensity of the pyroninophilia of the cytoplasm of the main portions after 3-day administration of therapeutic doses of merkusal is possibly related to the changes in the functional condition of the cells; the majorority of research workers assume that merkusal realizes its effect in the main portions. The subsequent lowering of the RNA-content, evidently, is related to the toxic action of the accumulating mercury.

The chronic poisoning by mercury leads to atrophy of the epithelial lining of many of the tubules of the cortical and medullary layers and a growth of connective tissue. Hypertrophy of the cells of the main portions, evidently, represents a vicarious hypertrophy, thanks to which the function of the atrophied part of the kidney is compensated. A regeneration takes place in the epithelium of the convoluted tubules by way of a mitotic multiplication of intact cells.

Acute poisoning by mercury produces necrosis of a number of the proximal convoluted tubules and development of pictures of hyaline-drop distrophy in the epithelium of the main portions. The ascending limb of the loop of Henle is damaged slightly. The rapid action of mercury compounds has been described by authors [10] who have observed the manifestation of degenerative changes in the epithelial cells of the kidney even within 60 minutes after the administration of large doses of mercuhydrin.

In our work we were not able to connect the formation of hyaline droplets with the transformation in the mitochondria through swelling and fusion, as a number of research workers have done [5, 7, 11]. We were able to observe an apocrine secretion by the hyaline droplets [3]. This is possibly connected with the sudden and intense damage to the cells by mercury, which does not ignore the possibility of their adaptation under new conditions. The first hyaline droplets are observed in the apical part of the main portions, at the same time that the mitochondria in the cells of the convoluted tubules are being arranged basally. Furthermore, the appearance of these formations would always have to be accompanied by swelling in the mitochondria; however, the form of the latter is not changed if the number of the hyaline droplets is small.

SUMMARY

A study was made of the structural and cytochemical changes occurring in the uriniferous tubules of albino mice under the action of various doses of merkusal. The cells of the main nephron portions proved to be most sensitive to merkusal action; under observation were both the structural changes and various cytochemical shifts.

LITERATURE CITED

1. A. V. Akulov, and M. V. Kazakova, Transactions of the Moscow Veterinary Academy [in Russian] 9 (1955) p. 120.
2. P. A. Weiner, Russk. Arkh. Anat. Gistol. i Embriol., 5, No. 1 (1926) p. 11.
3. P. Weiner, Z. Mikr.-Anat. Forsch., 13 (1928), p. 197.
4. I. V. Davydovskii, Pathological Anatomy and Pathogenesis of Human Disease, [in Russian] 1 (Moscow, 1956) p. 471.

5. A. A. Zavarzin, and S. I. Shchelkunov, Manual for Histology [in Russian] (Leningrad, 1954).
6. B. D. Kravchinskii, Modern Principles of Kidney Physiology [in Russian] (Leningrad, 1958).
7. A. N. Trifonova, Dokl. AN, SSSR, 61, No. 5 (1948), p. 917.
8. A. Ecker, and R. Wiedersheim, Anatomie des Frosches, Braunschweig, No. 3 (1904), p. 238.
9. A. Farah, E. J. Cafruny, and H. S. Stefano, J. Histochem. Cytochem., 3 (1955), p. 271.
10. F. Gagne, Laval. Méd., 20 (1955), p. 330.
11. K. V. Lodge, and A. S. Wodcock, Am. J. Path., 30 (1954), p. 361.
12. K. K. Mustakallio, and A. Telkkä, Science, 118 (1953), p. 320.
13. E. G. Rennels, and A. Ruskin, Proc. Soc. Exp. Biol., 85 (New York, 1954), p. 309.
14. E. G. Starling, Fundamentals of Human Physiology [in Russian] 2 (Moscow, 1933).
15. M. Wachstein, and B. Meisel, Experientia, 10 (1954), p. 495.
16. H. U. Zollinger, Schweiz Z. Allg. Path., No. 18 (1955), p. 155.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
