SECRETION OF THE EPITHELIUM OF RENAL COLLECTING TUBULES UNDER THE EFFECT OF THE ANTIDIURETIC HORMONE

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The mechanism of action of the antidiuretic hormone, according to present-day concepts, is based on the fact that under the effect of this hormone the epithelium of collecting tubules begins to secrete according to the apocrine type. Apparently, the enzyme hyaluronidase enters the composition of this secretion. This enzyme depolymerizes the hyaluronic structures of the interstitium and the intercellular cementing tissue, with the result that the wall of collecting tubules becomes permeable to water [3, 4, 6, 7].

The phenomenon of apocrine renal secretion has been described a number of times in various morphological and electron-microscope works [15, 16], but only A. G. Ginetsinskiy and his co-workers [3, 4] established the connection of this phenomenon with the action of the antidiuretic hormone.

We thought it interesting to investigate the effect of the antidiuretic hormone on the content and distribution of the nucleic acids (RNA and DNA) in the epithelium of the collecting tubules, since it is known that nucleic acids participate in protein synthesis; including the synthesis connected with the process of secretion [1, 2, 5, 8, 9, 10, 12, 13, 14].

METHOD OF EXPERIMENTS

The study was carried out on 16 adult white rats, weighing from 200 to 250 gm, and on 32 ratlings, one to 33 days old which were in a state of hydration and antidiuresis. The kidneys were obtained for examination within an hour after the introduction of water into the animals' stomach in a ratio of 5 ml per 100 gm body weight, or within 30 minutes after a subcutaneous injection of 15-30 m. u. of pituitrin. The material was fixated in Carnoi fluid. The processing for RNA determination was carried out by means of methylgreen-pyronine according to Brachet, or with hallocyanine as per Einarson [11] with an obligatory control by means of ribonuclease. DNA was determined by the Felgen method [11].

In a number of cases, for the histochemical characterization of the secretion, we carried out the reaction on protein as per Daniel-Pierce-Barston [11] which enabled us to elicit simultaneously certain amino acids (tryptophan, tyrosine, and histidine) which enter into the molecular composition of all proteins investigated up to the present time.

RESULTS OF EXPERIMENTS

In hydrated adult animals the collecting tubules are lined with cells of cuboidal epithelium with a centrally located nucleus.

The height of cells is almost uniform, with the result that the lumen of the tubule possesses clearly defined and even outlines. RNA in the cells is found in very small quantities and is evenly distributed over the entire cytoplasm (Fig. 1, a). Thanks to the fine dispersion characteristics of RNA particles an impression is created of a diffuse coloration of the cells' cytoplasm. The RNA content of the epithelium of the collecting tubules is uniform over the entire length of the tubules. Results obtained in eliciting RNA particles as per Brachet and Einarson are completely identical. Upon treatment of the sections with ribonuclease, it is possible to completely split the RNA in all segments of the collecting tubules, excepting the top of the papilla where a very slight coloration of cells' cytoplasm is partially preserved, due to the apparently non-specific character of the cells.

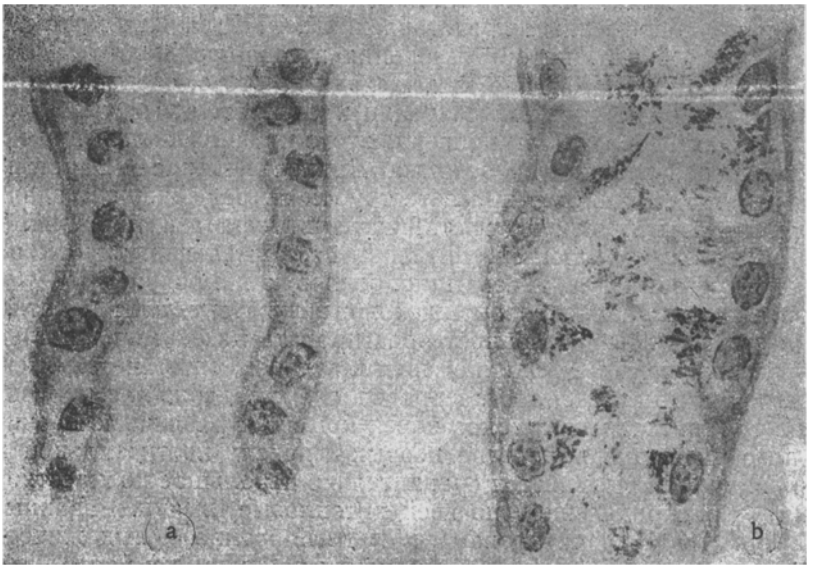

Fig. 1. RNA distribution in the epithelium of collecting tubules of adult rats. a) Under conditions of aqueous diuresis; b) after pituitrin injection. RNA elicited by the Brachet method. Magnification: oc. 10×, obj. 60×, immersion. Drawing made by means of a drawing apparatus.

Under the effect of pituitrin, in the majority of epithelial cells of the initial segments of the collecting tubules situated in the medullar rays, a definite apocrine secretion takes place. The apical cellular ends have indistinct, somewhat loose contours. In some areas a separation of the entire apical pole of the cell into the tubular lumen can be seen. In a number of secretory cells, in their apical part, one can observe formation of fairly large, compactly situated RNA granules. It can also be seen that, in apocrinia, together with the separation of the apical part of cells, there is also a secretion of colored granules in the lumen of the tubule, later elicited in the composition of the secretion which fills the collecting tubules (Fig. 1, b).

Within 30 minutes after the injection of pituitrin, no secreting cells can be found in the epithelium of the tubules at the base and medium third of the papilla, although their lumen is filled with secretion. The epithelial cells in this section are flattened, their nuclei oriented along the tubule and are separated from the lumen by a narrow, thin plasmatic rim. The RNA content in the cytoplasm of these cells is considerably higher than in the corresponding segments of the tubules of hydrated animals. At the apex of the papilla the character of epithelial lining and the RNA quantity in the cellular cytoplasm is not altered.

In contrast to what can be observed in the RNA study, the change in the content and distribution of DNA, determined according to Felgen in the membrane and chromatin of the epithelial nuclei of the collecting tubules following injection of pituitrin, could not be ascertained.

According to modern concepts, RNA serves as a matrix for the synthetized protein, and its formation is always preceded by an increased RNA concentration in the cells. On this basis, we would expect the formation of a protein secretion also under apocrine secretion. Upon verification of this assumption via histochemical

reaction on protein as per Daniel–Pierce–Barston, it was found that the secretion obtained in the tubular lumen had a large quantity of amino acids of histidine, tryptophan, and tyrosine. Thus, the secretion of the epithelium of collecting tubules, under antidiuretic conditions, is undoubtedly of protein nature.

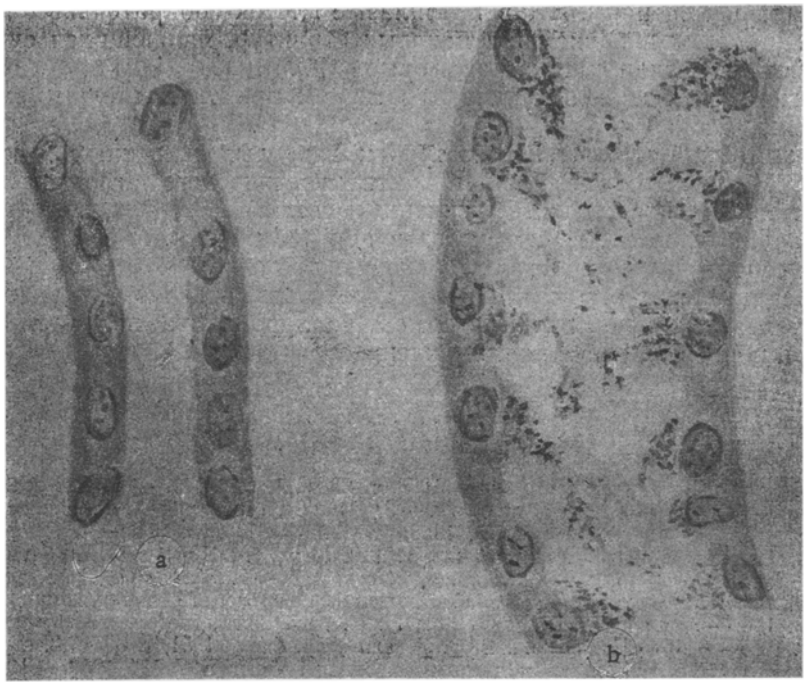

Fig. 2. Development of renal reaction to the antidiuretic hormone during ontogenesis. a) Absence of a reaction to the injection of pituitrin in the collecting renal tubules of a 13-day-old ratling; b) apocrine secretion of the epithelium of collecting renal tubules of a 31-day-old ratling after the hormone injection. RNA elicited by Brachet method. Magnif.: oc. 10×, obj. 60×, immersion. Drawing made by means of drawing apparatus.

It is known that kidneys of newborn are insensitive to the antidiuretic hormone, and that the property of reacting to the hormone by means of a canalicular antidiuresis originates only during the postnatal ontogenesis. In this connection, we studied the distribution and content of nucleic acids in the renal collecting tubules at various periods of postnatal development. During the first three weeks of postnatal life a small quantity of RNA, evenly distributed over the cellular cytoplasm, is elicited in the epithelium of the tubules. No difference is yet observable during this period in the RNA content in hydrated and dehydrated animals. Neither are there any changes noted after the injection of pituitrin (Fig. 2, a). Only starting on the 22nd day, the hormone injection induces an enhanced RNA synthesis in the epithelium of the collecting tubules.

During the same period of time characteristic pictures of apocrine secretion make their appearance.

Toward the 31st-33rd day these changes are as clearly expressed as in adult animals (Fig. 2, b). The coincidence should be noted of the periods of appearance of RNA changes in the cellular cytoplasm of the collecting tubules and the formation of renal hyaluronic structures which represent the action substrate of the antidiuretic hormone [6].

In summarizing all above-stated, we can arrive at the following conclusion.

In the epithelium of the collecting tubules of adult animals and ratlings over 20 days old, under the effect of the antidiuretic hormone a redistribution and enhanced synthesis of RNA takes place; this process precedes the formation of the secretion discharged into the lumen of the tubule. Histochemical analysis of the secretion confirmed its protein nature.

Thus, the idea that the secretion, discharged by the epithelium of the collecting tubules, contains a hyaluronidase enzyme finds its new, indirect corroboration.

LITERATURE CITED

1. J. Brachet, Uspekhi Sovrem. Biol., 29, 1 (1950) p. 140.
2. J. Brachet, in the book: Nucleic Acids [in Russian], (1957) p. 320.
3. A. G. Ginetsinskiy, M. G. Zaks, and L. K. Titova, Doklady AN SSSR, 120, No. 1, (1958) p. 216.
4. A. G. Ginetsinskiy and L. N. Ivanova, Doklady Akad. Nauk SSSR, 119, 5 (1958) p. 1043.
5. S. S. Debov, Uspekhi Biol. Khimii, 2, (Moscow, 1954) p. 115.
6. M. G. Zaks, T. V. Krestinskaya, and L. K. Titova, in the book: Evolution of Physiological Functions [in Russian] (Moscow-Leningrad, 1960) p. 166.
7. M. G. Zaks and L. K. Titova, Arkh. Anat., Gistol. i Émbriol., No. 7, (1959) p. 19.
8. B. V. Kedrovskii, Uspekhi Sovrem. Biol., 32, 3, (1951) p. 38.
9. B. V. Kedrovskii, Cytology of Protein Synthesis in the Animal Cell [in Russian] (Moscow, 1959).
10. I. Lesli, in the book: Nucleic Acids [in Russian] (Moscow, 1957) p. 7.
11. É. Pirs, Theoretical and Applied Histochemistry [in Russian] (Moscow, 1956).
12. V. A. Askonas, J. L. Simkin, and T. S. Work, in the book: Biochemical Society Symposia, (Cambridge, 1957), No. 14, p. 32.
13. J. Brachet, Biochemical Cytology (New York, 1957).
14. T. Casperson, Symposia Society exp. Biol., No. 1, (1950) p. 127.
15. W. Möllendorff, Handbuch der mikroskopischen Anatomie des Menschen, 7, 1, 1 (Berlin, 1930).
16. I. Rhodin, Intern. Rev. of Cytol., 7, (1958) p. 485.

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.
