CHANGES IN NUCLEOLI OF TUBULAR EPITHELIAL CELLS IN EXPERIMENTAL NECROTIZING NEPHROSIS INDUCED BY MERCURIC CHLORIDE

V. P. Andreev

UDC 616.612-018.1:576.315.45]-02:[615.916. 546.49]-092.9-076.4

KEY WORDS: mercuric chloride-induced nephrosis, nucleolus, regeneration

An experimental model of toxic nephrosis induced by mercuric chloride is nowadays widely used in research in order to clarify the pathogenesis, method of treatment, and prevention of acute renal failure. Mercuric chloride (corrosive sublimate), if introduced into an animal, causes heterogeneous structural changes in the proximal part of the renal tubules, consisting of a whole spectrum of ultrastructural changes, which are difficult to detect, and which are manifested as different degrees of partial necrosis, up to and including death of the epithelial cells. During the morphological study of the damaged renal tissue, difficulties are constantly arising in connection with the establishment of viability and subsequent restoration of epithelial cells, which have undergone some degree of partial necrosis.

Since destructive and reparative changes depend on the intensity of protein synthesis, and since the nucleolus is a very important component of the protein-synthesizing system, we decided to undertake the investigation described below in order to establish correlation between the degree of partial necrosis of a cell, the state of its cytoplasmic structures, and ultrastructural changes in its nucleolus.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 180-220 g. The animals were divided into two groups: the rats of Group 1 (three animals) served as the control, those of Group 2 (six animals) were given a single subcutaneous injection of mercuric chloride in a dose of 0.4 mg/100 g body weight, dissolved in physiological saline. Pieces of kidney were removed from the animals, under ether anesthesia, 24, 48, and 72 h after injection of the mercuric chloride, fixed in 2.5% glutaraldehyde in phosphate buffer, and then postfixed with OsO_4 Individual tissue fragments were fixed in 1% OsO_4 solution only. The fixed tissue fragments were processed by the usual method for electron-microscopic study.

EXPERIMENTAL RESULTS

Interphase nuclei of the epithelial cells of the proximal part of the renal tubule in the control animals contain as a rule one nucleolus, arranged centrally or, much less frequently, near the nuclear membrane, The nucleolonemal structure of the nucleolus was ill defined, but fibrillar and granular components were clearly distinguishable. As a rule the nucleoli were surrounded to a large extent by perinucleolar heterochromatin, which sometimes was joined in some areas with the well defined peripheral heterochromatin. The number and size of the heterochromatin islets in the nuclei varied considerably (Fig. 1).

Department of Biology, Grodno Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 1, pp. 66-69, January, 1991. Original article submitted February 16, 1990.

0007-4888/91/0001-0090$12.50

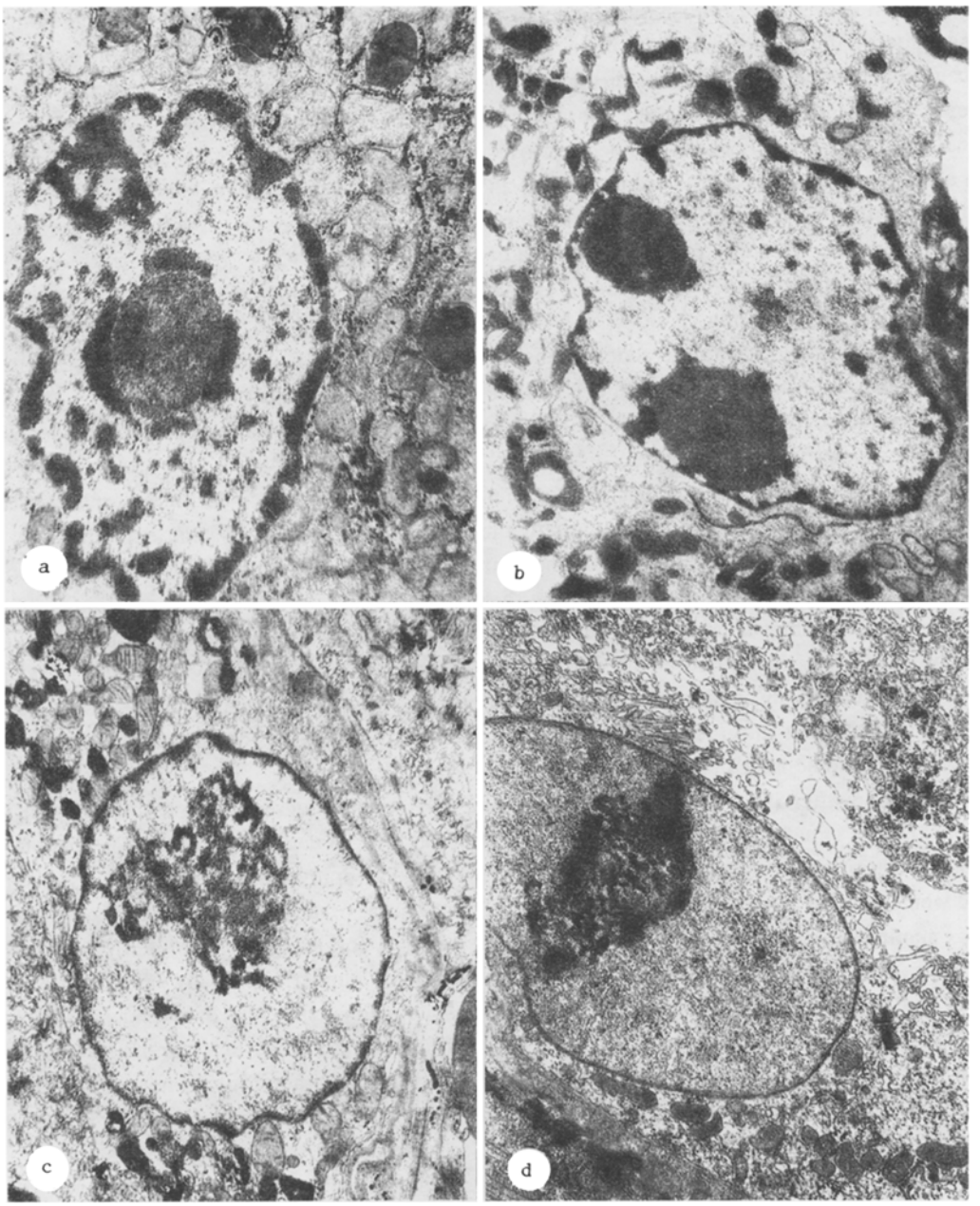

Fig. 1. Ultrastructure of interphase nucleus of epithelial cell in proximal part of urinary tubule in rat kidney: a) granular and fibrillar components present in central nucleolus; nucleolus largely surrounded by perinucleolar heterochromatin, which almost joins the well-marked peripheral heterochromatin. Control animal. 10,000×; b) degranulated, enlarged nucleoli, consisting of fibrillar material, located at periphery of nucleus. Perinucleolar and peripheral heterochromatin largely decondensed; proximal part of urinary tubule of rat kidney 24 h after poisoning with mercuric chloride. 9000×; c) a few elements of the newly formed, dense fibrillar component (arrows), located around periphery of enlarged nucleolus. 24 h after injection of mercuric chloride. 6000×; d) intensive accumulation of granular component in nucleolus with well developed nucleolonemal structure. A few mitochondria, free ribosomes, and elements of the rough endoplasmic reticulum can be seen in the cytoplasm. 72 h after injection of mercuric chloride. 5000×.

With this dose of the poison, the most marked destructive changes developed towards the end of the first day [5]. By that time the lumen of many renal tubules in the region of the thick segment of the descending part of the loop of Henle was dilated and filled with debris. As a rule among the debris there were clumps of cytoplasmic membranes, disintegrated mitochondria, and fragments of the endoplasmic reticulum. Free lysosomes were seen very rarely in the lumen of the renal tubules, evidently because of their rapid destruction. In some tubules, filled with debris, complete destruction of the epithelial cells was observed over a considerable length of their course. In segments in which the epithelium was preserved, the ultrastructural changes indicated that the epithelial cells had been exposed to the action of two injurious factors. Some changes were due to the direct action of mercuric chloride on the epithelial cells and were characterized by condensation of the matrix of the mitochondria in connection with their calcification. The other changes were evidently associated with the response of the epithelial cells to the developing hypoxia, resulting from a disturbance of the blood supply to the renal cortex through spasm of the afferent arterioles. The hypoxic character of damage to the epithelial cells was demonstrated by the presence of myelin figures in the cytoplasm and vacuolation of the mitochondria.

In epithelial cells subjected to the action of these factors to a varied degree, four principal [2] ultrastructural modifications of the nucleolus were found: segregation, degranulation, fragmentation, and hypertrophy.

Incidentally, the degree of degranulation and segregation of the nucleoli varied considerably depending on the intensity of the destructive processes in the cytoplasm.

For example, in epithelial cells which had completely or partially lost their brush border and the apical parts of their cytoplasm, but still contained some mitochondria with the normal ultrastructure, partial degranulation and condensation of components of the nucleolus was observed, sometimes with preservation of fragments of the perinucleolar heterochromatin. Meanwhile considerable degenerative changes affecting the nucleus and nucleolus, which are incompatible with life of the cell, develop in cells not undergoing partial necrosis, but having an almost totally damaged mitochondrial apparatus. In such cells the whole of the chromatin is concentrated at the periphery of the nucleus and is highly condensed, marked translucency of the nucleoplasm is observed, with dispersion of components of the nucleolus and, ultimately, rupture of the cytoplasmic membrane and release of the cell contents into the lumen of the tubule. Destructive processes of this kind are most marked toward the end of the first day, but they arise later in individual epithelial cells.

On the first day after injection of mercuric chloride, enlarged, condensed nucleoli consisting of fibrillar material and having a regular oval or circular shape are frequently found in partially damaged cells (Fig. 1b). Vacuolated mitochondria are often found in the cytoplasm of these cells. During this period, in some epithelial cells which have lost the apical part of their cytoplasm and which contain mitochondria with a normal ultrastructural organization, active rRNA synthesis is observed in the nucleoli, and is manifested morphologically as the appearance of newly formed elements of dense fibrillar material (Fig. 1c).

Later (the 2nd-3rd day after injection of mercuric chloride) the number of partially damaged cells with nucleoli containing elements of the dense fibrillar component increases, the nucleolonemal structure of the nucleoli becomes more distinct in many cells, and the quantity of the granular component rises considerably, resulting in a marked increase in size of the nucleoli. Frequently nucleoli whose granular component borders on the nuclear membranes on two opposite surfaces, can be seen in the preparations (Fig. 1d). The number of mitochondria is reduced in the cytoplasm of these cells, the digital folds of the plasmalemma in the basal parts of the cell disappear, and the intercellular spaces are widened.

Another characteristic feature of the partially damaged cells is fragmentation of their nucleoli. In some epithelial cells up to five small nucleoli, sometimes with signs of active transcription of ribosomal genes, is found 72 h after damage to the kidneys.

The appearance of a granular component, consisting of RNP-particles (proribosomes), 10-25 nm in diameter, at different stages of maturation, is usually considered to be a morphological sign of reactivation of the nucleolus after exposure to some factor leading to inhibition of rRNA transcription. The granular component, surrounding the dense fibrillar material (newly synthesized 45S pre-rRNA) in narrow zones, molds the nucleolonemal structure of the nucleolus.

The presence of large nucleoli, consisting of dense fibrillar material, in partially damaged epithelial cells is evidence that the process of formation of proribosomes is mainly suppressed in such nucleoli, while ability to synthesize rRNA is preserved to some degree.

Our previous electron-microscopic and autoradiographic investigation [2] showed that RNA synthesis is not completely suppressed in nucleoli consisting of fibrillar material with decondensed heterochromatin.

The present investigation thus shows that nonspecific (i.e., developing also in other pathological processes and in other cells) changes in the nucleolus take place in damaged epithelial cells, in the form of segregation, degranulation, fragmentation, and hypertrophy. None of these changes are fatal, and after cessation of the action of the damaging agent, they can undergo regression with restoration of the normal structure of the nucleolus [3, 4].

It must be particularly emphasized that comparison of the data described above with our previous observations [1] leads to the conclusion that the destructive changes described in the nucleolus, in the same way as partial necrosis while some normal mitochondria and part of the protein-synthesizing apparatus in the cytoplasm remains intact, do not prevent the cells with partial necrosis from embarking upon a phase of DNA synthesis. New DNA formation is the initial phase of development of intracellular regeneration, namely hyperplasia of the genome. The latter, in turn, brings about hyperplasia of intracellular structures at the early stage, and an increase in the number of cells at a later stage. In other words, our results demonstrate that injury, if not too severe, acts as a stimulus activating a regenerative response which, in the epithelial cells of the tubules, is exhibited in both intracellular and cellular form.

LITERATURE CITED

1. V. P. Andreev and A. A. Pal'tsyn, Byull. Éksp. Biol. Med., No. 8, 241 (1987).
2. V. P. Andreev and A. A. Pal'tsyn, Byull. Éksp. Biol. Med., No. 3, 357 (1989).
3. A. G. Lomagin, Usp. Sov. Biol., **103**, No. 1, 81 (1987).
4. M. I. Moskvin-Tarkhanov, N. E. Cherni, and N. N. Belyaeva, Tsitologiya, No. 5, 529 (1989).
5. T. Z. Gritzka and B. F. Trump, Am. J. Path., **52**, 1225 (1968).

SEASONAL DIFFERENCES IN EFFECT OF THYROID HORMONE DEFICIENCY ON INDOLEAMINE METABOLISM IN THE RAT PINEAL GLAND

E. S. Rom-Bugoslavskaya and L. A. Bondarenko

UDC 612.826.33.015.3:547.751].06:612.444.06:613.13

KEY WORDS: pineal gland, thyroid gland, serotonin, melatonin, thyroid hormones

An excess and deficiency of thyroid hormones in the body are known to cause diametrically opposite effects on indoleamine metabolism in the rat pineal gland: saturation of the body with thyroxine activates melatonin formation in the pinealocytes, whereas thyroidectomy inhibits this process [1]. It has been shown that in the presence of an excess of thyroid hormones, serotonin utilization in the pineal is disturbed predominantly toward N-acetylation and subsequent O-methylation, with the formation of N-acetylserotonin (N-aS) and melatonin, whereas in thyroid deficiency, it is shifted toward relative predominance of oxidative deamination, against the background of marked inhibition of methoxyindole biosynthesis. In other words, thyroid hormones affect mainly processes of N-acetylation and O-methylation of pineal indoles. Subsequently, in a study of seasonal differences in the effect of thyroxine on the metabolic pathways of serotonin in the rat pineal gland [3] it was shown that excessive saturation of the body with thyroid hormone causes biochemical disturbances aimed at stimulating the production of pineal melatonin independently of the season, although under conditions of short daylight (winter) these changes were much more marked [3].

Laboratory of Biochemistry of Endocrine Diseases, Khar'kov Research Institute of Endocrinology and Hormone Chemistry.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Korzh'.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 1, pp. 69-70, January, 1991. Original article submitted December 29, 1989.

0007-4888/91/0001-0093S12.50