Ultrastructural Peculiarities of Intranuclear Inclusions in Some Target Cells during Endotoxin Shock

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 5, pp. 589-594, May, 2002
Original article submitted January 31, 2002

Intranuclear inclusions detected in hepatic, renal, and cerebral cells during endotoxemia can be subdivided into true and pseudo inclusions. True inclusions have filamentous and crystalline structure and are rarely found in control animals. However, the number of true inclusions increases during pathology and can serve as a marker of molecular pathology in the cells provoked by endotoxin.

Key Words: *intranuclear inclusions; ultrastructure; endotoxic shock*

The mechanism of formation and the role of intranuclear inclusions (INI) of nonviral etiology remain poorly understood. It is hypothesized that the formation of these inclusions depends on endo- and exogenous factors such as gene mutations, age-related changes, and various diseases [2,6,11]. Moreover, excessive accumulation of intranuclear complexes is considered as a surrogate marker of molecular pathology [10].

Our aim was to study INI in cells of the main target organs during endotoxic shock (ES).

MATERIALS AND METHODS

The study was carried out on 39 mongrel dogs (body weight up to 10 kg), 26 male Chinchilla rabbits weighing 3 kg, and 39 male rats weighing 200-250 g. The animals were maintained under vivarium conditions on standard ration and water *ad libitum*. The dogs and rabbits were intravenously injected with *E. coli* endotoxin (5 mg/kg). In rats, the endotoxin was injected into the caudal vein (20 mg/kg). The animals were sacrificed by Nembutal overdose 30 min (initial period of ES), 5 hours (intermediate period of ES), and 3 days postinjection (late endotoxemia stage). The control animals (6 rabbits, 9 dogs, and 9 rats) received an equal volume of sterile NaCl (0.9%). Fragments of the anterotemporal cortex were isolated. Examination of V-VI layers under an electron microscope revealed the presence of large neurons functionally equivalent to neurons of the sensorimotor area [1].

The specimens of the sensorimotor cortex, liver, and kidneys were fixed in 2.5% glutaraldehyde on phosphate buffer, then in 1% OsO_4 on the same buffer, and after dehydration embedded in Epon. Morphological alterations were initially examined on semithin (1-2 μ) sections stained with Toluidine Blue. Ultrathin sections (LKB-8800 ultramicrotome) were contrasted with uranyl acetate and lead citrate and examined under a JEM-100S electron microscope.

RESULTS

In control and test animals, INI looking like vacuoles and cavities of various size and shape were detected in neurons of the sensorimotor cortex, podocytes, and proximal epithelial cells of the nephron. Electron microscopy showed that these structures represent invaginations of the karyolemma of irregular shape, which looked like INI under the light microscope. Under electron microscopy these structures looked like ""transparent'' cytoplasmic fragment with standard organelles and inclusions (Fig. 1, *a*, *b*). These pseudo inclusions in cells of the cerebral cortex, podocytes, and renal epithelium were seen at all sages of ES, but most often they were found in late endotoxemia.

True INI in cells of the examined organs were presented by two basic types. Type 1 INI consisted of

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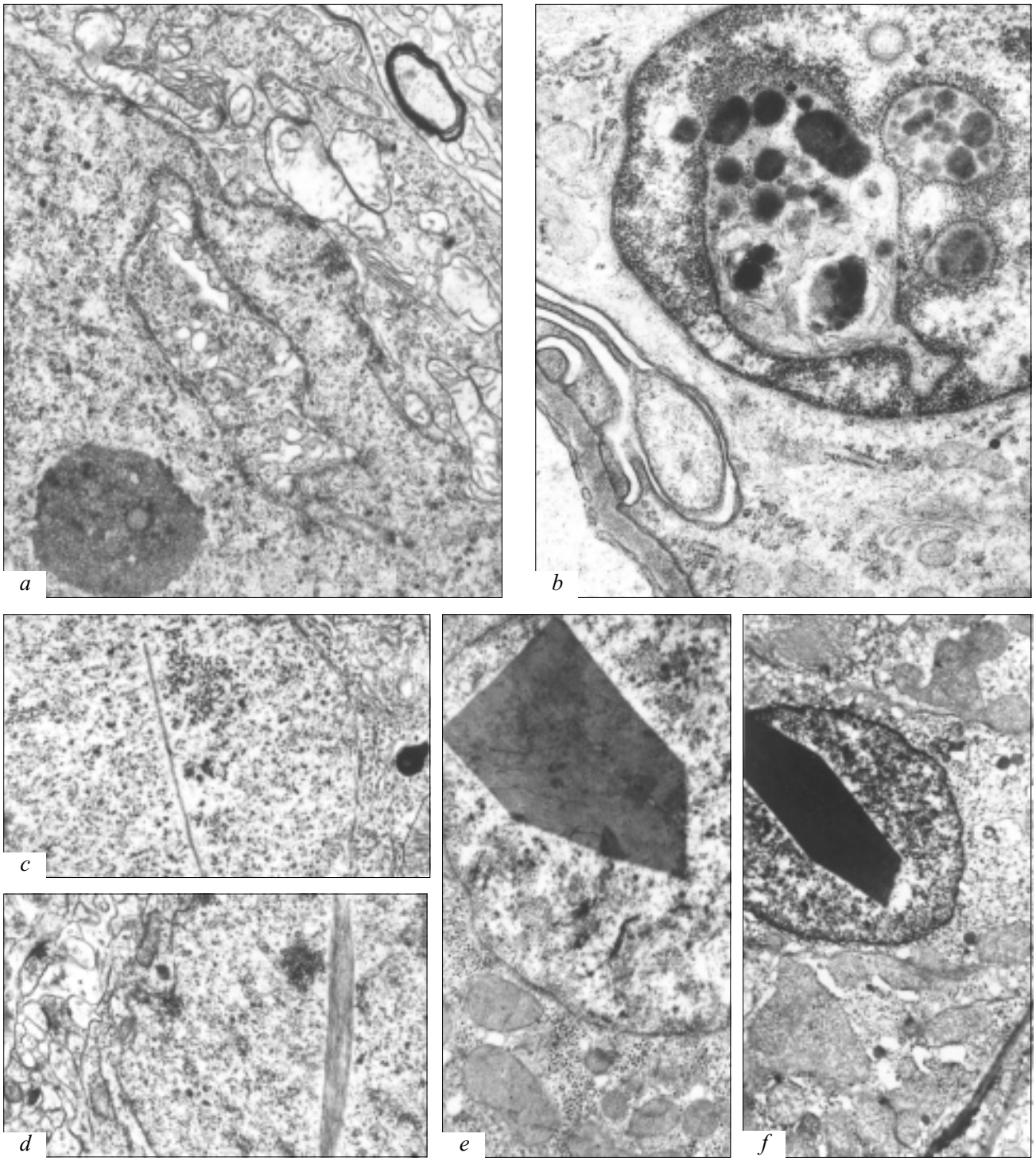

Fig. 1. Ultrastructure of intranuclear inclusions in control rats (*a*, *b*, *d*), rabbits (*c*), and dogs (*e*, *f*): ×7000 (*a*-*c*), ×10,000 (*d*), ×5600 (*e*, *f*). *a*) pseudo inclusions in a neuron; *b*) pseudo inclusion in a podocyte; *c*) true filamentous INI in a neuron; *d*) INI in the form of filamentous fascicle in a neuron; *e*) INI in a hepatocyte; *f*) INI in epithelial cell of proximal renal tubule.

filamentous matter and were found in neuronal nuclei (Fig. 1, *c*, *d*). Type 2 INI looked like osmiophilic conglomerates in hepatocyte and proximal nephrocyte nuclei (Fig. 1, *e*, *f*).

In control rats, nuclei of sensorimotor neurons had filamentous INI 0.54-350 nm thick (Fig. 1, *c, d*) consisting of filament bundles 14 nm in diameter. The karyoplasm around INI was clarified. In dogs INI of peculiar hexagonal or elongated rectangular shape were found in hepatocyte nuclei (Fig. 1, *e*), in epithelial cells of proximal renal tubules of control dogs they looked like homogenous osmiophilic conglo-

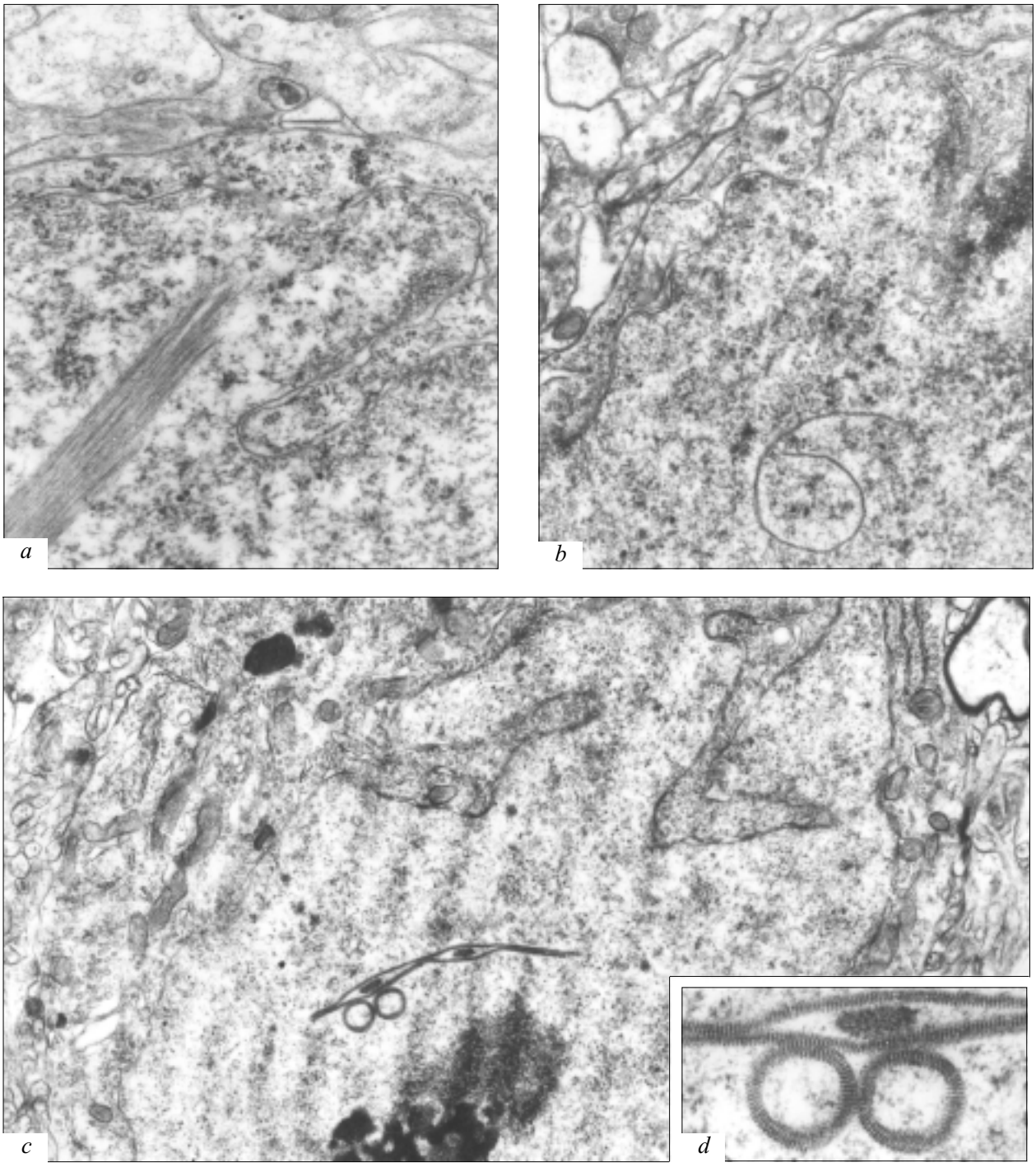

Fig. 2. Ultrastructure of intranuclear inclusions in neurons of sensorimotor cortex in rats (*a*, *b*) and dogs (*c*, *d*) during endotoxemia. *a*) initial period of ES, ×10,000; *b*) intermediate period of ES, ×14,000; *c*) late endotoxemia, ×5600; *d*) a part of the panel *b* at ×50,000.

merates occupying a great part of the karyoplasm (Fig. 1, *f*).

In initial period of ES, INI presented by thick (470 nm) bundles of finest fibrils were seen in the karyoplasm of sensorimotor neurons (Fig. 2, *a*). These INI never contacted with nucleoli or karyolemma.

In intermediate period of ES, filamentous INI were observed against the background reactive alterations in nervous cells, but the thickness of bindles decreased to 60 nm. Sometimes, helical structures with a small period and thickness of about 57 nm were found (Fig. 2, *b*). Reactive changes in neurons are usually reversible. However, the formation of these INI in neurons with destructive changes was irreversible and was accompanied by disintegration of organelles, deformation and osmiophilia of the nucleus, homogenization

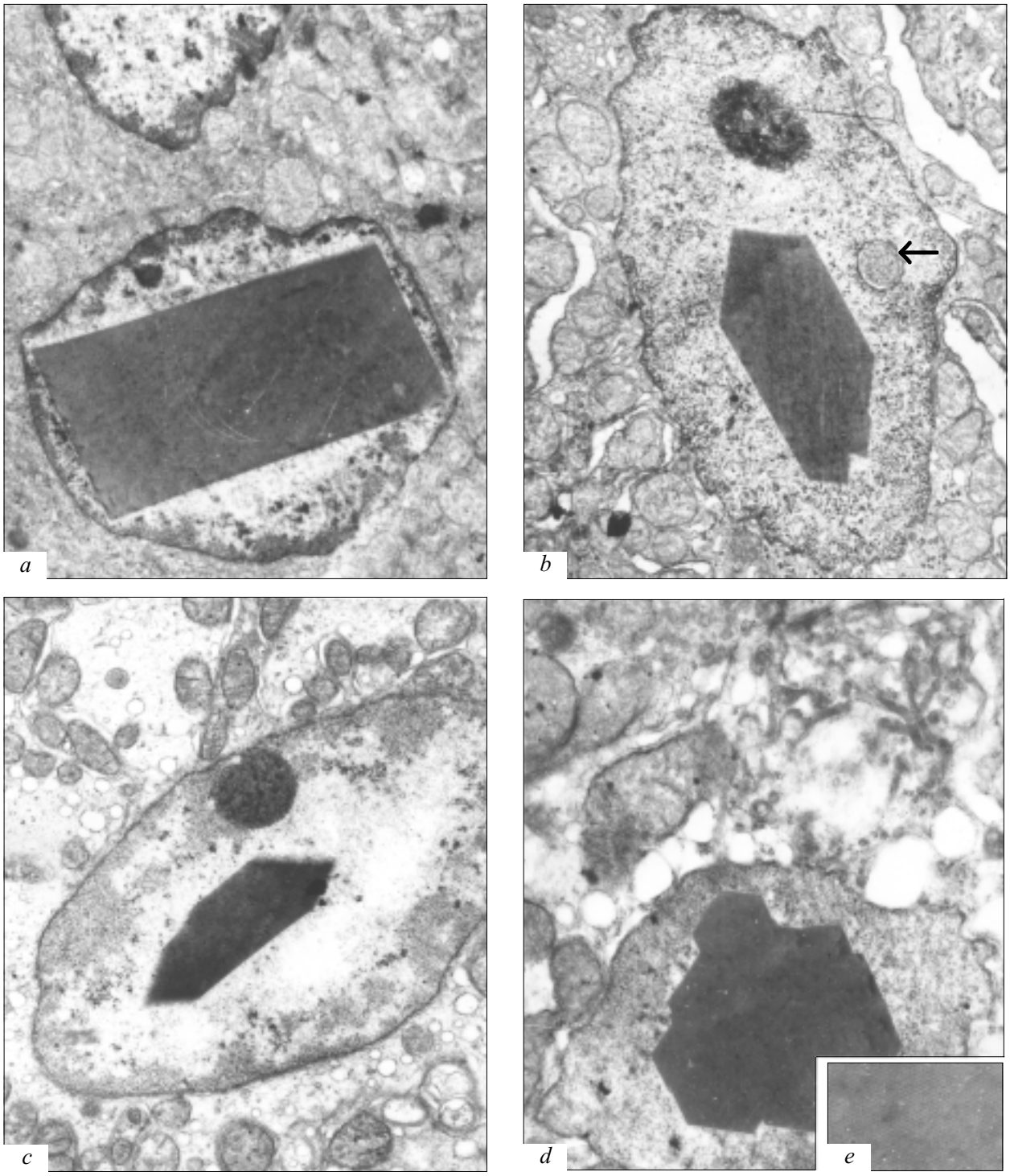

Fig. 3. Ultrastructure of intranuclear inclusions in canine liver (*a*) and kidney (*b*-*e*) during endotoxemia. *a*) INI in a hepatocyte during intermediate period of ES, ×4200; *b*) pseudo (arrow) and true INI in epithelial cell of proximal renal tubule during the initial period of ES, ×5600; *c*, *d*) INI in a nephrocyte during intermediate period of ES, ×5600; *e*) fragment of INI shown in panel *d*, ×60,000.

of chromatin, alteration of nuclear and plasma membranes, destruction of mitochondria, fragmentation of cisterns of the granular and agranular reticulum, formation of large cavities in the cytoplasm, *etc*.

During late endotoxemia, an unusual INI presented by single helical filament was observed apart from filamentous inclusions described above (Fig. 2, *c*, *d*). Electron micrograph most likely shows a cross-section of this helix.

In hepatocytes INI was detected only at the intermediate stage of ES. It looked as regular rectangle with ideally straight sides (Fig. 3, *a*).

In proximal epithelial cell of convoluted renal tubules, INI were seen at all stages of ES. During the initial period, they occupied a large part of the karyoplasm (Fig. 3, *b*), while in the intermediate period of ES they looked like 1.25×3.60 μ irregular hexagons (Fig. 3, *c*). A larger INI with similar density was found in dogs with late endotoxemia (Fig. 3, *d*). Interestingly, rosette-like molecular globules arranged as a crystal lattice were sometimes seen in these INI despite their high osmiophilia (Fig. 3, *e*). The mean size of these globules was 7.0 nm, the mean distance between their centers along the lattice axes was 14.8 nm, and lattice axes formed an angle of 65° (Fig. 3, *e*).

Immunohistochemical methods showed that during some neurodegenerative disorders, INI of cortical neurons located predominantly in layers II, V, and VI, and in ependymocytes consist of β-tubulin, the main class III cytoskeleton protein [11]. During various asphyxia-induced states, intranuclear clusters containing ubiquitin, a heat shock protein can be detected in midbrain neurons [8]. Ubiquitin and ataxin-3 are synthesized and expressed in INI of substantia nigra neurons during portal hepatic encephalopathy [5]. Of particular importance is the fact that the presence of ataxin-3 in intranuclear complexes initiates apoptosis [10]. In addition, immunohistochemical methods showed that during myosites, the intranuclear aggregates of the myocytes contain apoptosis control proteins (Bc1-2, Bc1-x, and Bax), which corroborates the possibility of participation of the mechanisms of programmed cell death in neurotoxicity and muscle degeneration [7].

Experiments on animals showed that appearance of neural INI is preceded by metabolic dysfunctions in cells provoked by oxidative stress and exotoxins [4]. In this period, the regions of brain degeneration demonstrate enhanced levels of malonic dialdehyde, 8-hydroxydeoxyguanosine, 3-nitrotyrosine, and heme oxygenase, and also an increase of free radical production.

Interesting data were obtained in the study of ultrastructure of hepatocytes of African gray parrots after sudden death. Hepatocyte nuclei of these birds contained nonviral inclusions consisting of loop-like filamentous complexes occupying a great part of the karyoplasm [9]. During various intoxications, numerous electron-dense intranuclear bodies were also revealed in the epithelium of proximal convoluted renal tubules, hepatocytes, and pneumocytes [3].

Thus, cell nuclei contain true and pseudo inclusions. True inclusions are filamentous or homogenous structures with occasional striation looking like crystal lattice, while pseudo inclusions are a result of peculiar course of section plane via complex invaginations of the karyolemma; they often contain usual cell organelles. INI can be found in control animals, but their accumulation during pathologic states, in particular ES is a marker of cell damage.

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