Ultrastructural Reorganization of Rat Adrenal Cortex after Whole Body Hyperthermia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 5, pp. 591-596, May, 2008
Original article submitted March 11, 2008

Intracellular reorganization of mouse adrenocorticocytes after single whole body hyperthermia is determined by the two main events: development of the general adaptation syndrome and inhibition of the regeneratory plastic reactions in cells resultant from endotoxication and redistribution of plastic resources between organs and tissues. Stress-induced changes manifest in significant exhaustion of lipid droplets in the adrenocorticocytes of all adrenocortical zones, particularly in the zona fasciculata. Intracellular changes in adrenocorticocytes reflecting the development of regeneratory plastic insufficiency manifest in annular transformation of the nucleoli, segregation of the nucleolonema into granular and fibrillar components, intensification of autophagocytosis, and destructive changes in mitochondria and Golgi complex elements. The most pronounced changes were observed in adrenocorticocytes of the zona reticularis.

Key Words: *hyperthermia; post-thermal restitution; adrenocorticocytes; ultrastructure*

Whole body hyperthermia, similarly as whole body hypothermia, can cause significant structural and functional reorganization of organs and tissues. It is assumed that these changes are caused by the development of nonspecific stress reaction (common adaptation syndrome) and tissue hypoxia and endotoxication, processes specific for whole body hyperthermia [4,6,8]. The development of general adaptation syndrome is realized through the pituitary—adrenal interactions and is paralleled by elevation of blood glucocorticoids and catecholamines.

Intensification of the adrenal function during the development of stress reaction or simulation of this condition by injection of ACTH is associated with structural changes in the cortical matter and medulla, sometimes used as criteria of the intensity of stress exposure without consideration for the nature of the stress factor [1,7]. These criteria were also used for evaluating the stress effects of hyperthermia [4,5,9]. Evaluation of the compensatory adaptive reserve of the adrenals, specifically, of the type of intracellular reorganization in adrenocorticocytes (ACC), is an interesting approach to evaluation of the adaptive potential of the organism. Evaluation of the type of ultrastructural changes in ACC over the course of post-thermal restitution is particularly interesting from this viewpoint, because publications on the problem are scanty.

We studied the type of intracellular reorganization of mouse ACC in the course of restitution after single whole body hyperthermia.

MATERIALS AND METHODS

Experiment was carried out on 36 male CBA mice exposed to single whole body hyperthermia at 43°C for 35 min. This duration of exposure corresponded to the threshold period, after which mass mortality was observed. The material for morphological study was collected 30 min and on days 3, 7, and 14 after total hyperthermia. Control group consisted of 8 animals. All animals were kept under standard

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0007-4888/08/1455-0650

vivarium conditions with free access to water and food.

Adrenal specimens for electron microscopy were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, treated routinely, and embedded in epon and araldite mixture. Ultrathin sections were prepared on an LKB-III ultratome, contrasted with uranyl acetate and lead citrate, and examined under a JEM-1010 electron microscope at accelerating voltage of 40 kV. Morphometric study was carried out on semithin sections stained with Azur II and prepared from the same blocks as the ultrathin sections.

RESULTS

Single whole body hyperthermia caused no appreciable shifts in body weight and adrenal weight of mice over the entire period of post-thermal restitution [4]. According to morphometry data, the width of the adrenal cortex increased significantly (by 17.4% *vs.* control: from 0.46±0.03 to 0.54±0.01 mm) by day 3, but on day 7 of post-thermal restitution it decreased (by 15.2%, to 0.39±0.01 mm); on day 14 this parameter virtually did not differ from the control. Transitory changes in the width of the adrenal cortex during the early period after hyperthermia were mainly determined by the increase of the blood content of the organ [5]; then destructive processes predominated in the adrenal cortex (especially in the zona reticularis), while by the end of the experiment, reparative processes progressed.

The ultrastructure of ACC in intact CBA mice corresponded to that of other mammals, but mitochondrial crystae in ACC in the zona fasciculata were shaped as vesicles and tubules (Fig. 1, *a*). Lipid inclusions were seen in ACC of all adrenocortical zones, but were most abundant in zona fasciculata cells. Lipid inclusions had irregular shape, heterogeneous structure, and were surrounded by myelin-like membrane structures.

Thirty minutes after single whole body hyperthermia, structural and functional heterogeneity of ACC in the zona fasciculata and zona reticularis appeared, which was seen from the presence of cells with light and electron-dense cytoplasm. The number of lipid droplets decreased significantly in ACC of both types; myelin-like transformation of these cells was often observed, with the formation of myelin-like residual bodies, released into extracellular space. These changes in the ACC ultrastructure were attributed to intensification of corticosteroid synthesis [1,7]. Intracellular reorganization of ACC in the zona reticularis and zona fasciculata during this period was characterized by a significant dilatation of vesicles of the agranular cytoplasmic reticulum, as a result of which the cell cytoplasm looked “foamy” (Fig. 1, *b*). The most pronounced ultrastructural changes were observed in mitochondria (MC), particularly in the zona reticularis ACC: concentrical transformation of the crystae was seen in some MC (Fig. 1, *c*), the crystae were unevenly dilated, in some cases myelin-like degradation of organelles with the formation of residual bodies was seen.

The most pronounced changes in the ACC ultrastructure were recorded on day 3 of post-thermal restitution. High heterogeneity of ACC in the zona fasciculata and zona reticularis persisted (“clear” and “dark” cells; Fig. 1, *d*). The dark cells contained numerous MC, electron-dense cytoplasm contained numerous glycogen granules, vesicles, and tubules of the agranular and granular cytoplasmic reticulum. Greater phenotypical heterogeneity of ACC can reflect different cell sensitivity to destructive factors and an increase in the subpopulation of newly formed ACC, in other words, activation of the regeneratory processes [2,3].

The number of lipid inclusions decreased significantly in all ACC during this period; they were highly polymorphic, and numerous residual bodies with membrane formations appeared (Fig. 2, *a*). In many ACC in zona reticularis lipid inclusions were absent. Mitochondria with partially lyzed matrix and significant vacuolation of the agranular cytoplasmic reticulum were often seen in “clear” cells (Fig. 2, *b*). Autophagocytosis processes progressed, small zones of the cytoplasm sequestration appeared, which resulted in the appearance of an appreciable number of myelin-like structures in cell cytoplasm, extracellular space, and sinusoidal lumens (Fig. 2, *c*). Sites containing no ACC were seen in the zona reticularis.

Ultrastructural changes in the main cytoplasmic compartments were paralleled by changes in the nuclear system of ACC, particularly in the nucleoli. Pronounced polymorphism of ACC nuclei was noted: in some cells, marginally located heterochromatin predominated, while in others only euchromatin was present. Very often annular nucleoli were seen in these nuclei (Fig. 2, *d*), which can be regarded as an ultrastructural equivalent of reduced rRNA synthesis and inhibition of biosynthetic processes in the cells. The number of monocytes and lymphocytes in the sinusoidal lumens increased, fibroblasts, lymphocytes, and macrophages were seen perivascularly.

On day 7 after hyperthermia, ACC cytoplasm in the zona fasciculata and zona reticularis looked mainly condensed, electron dense, and contained

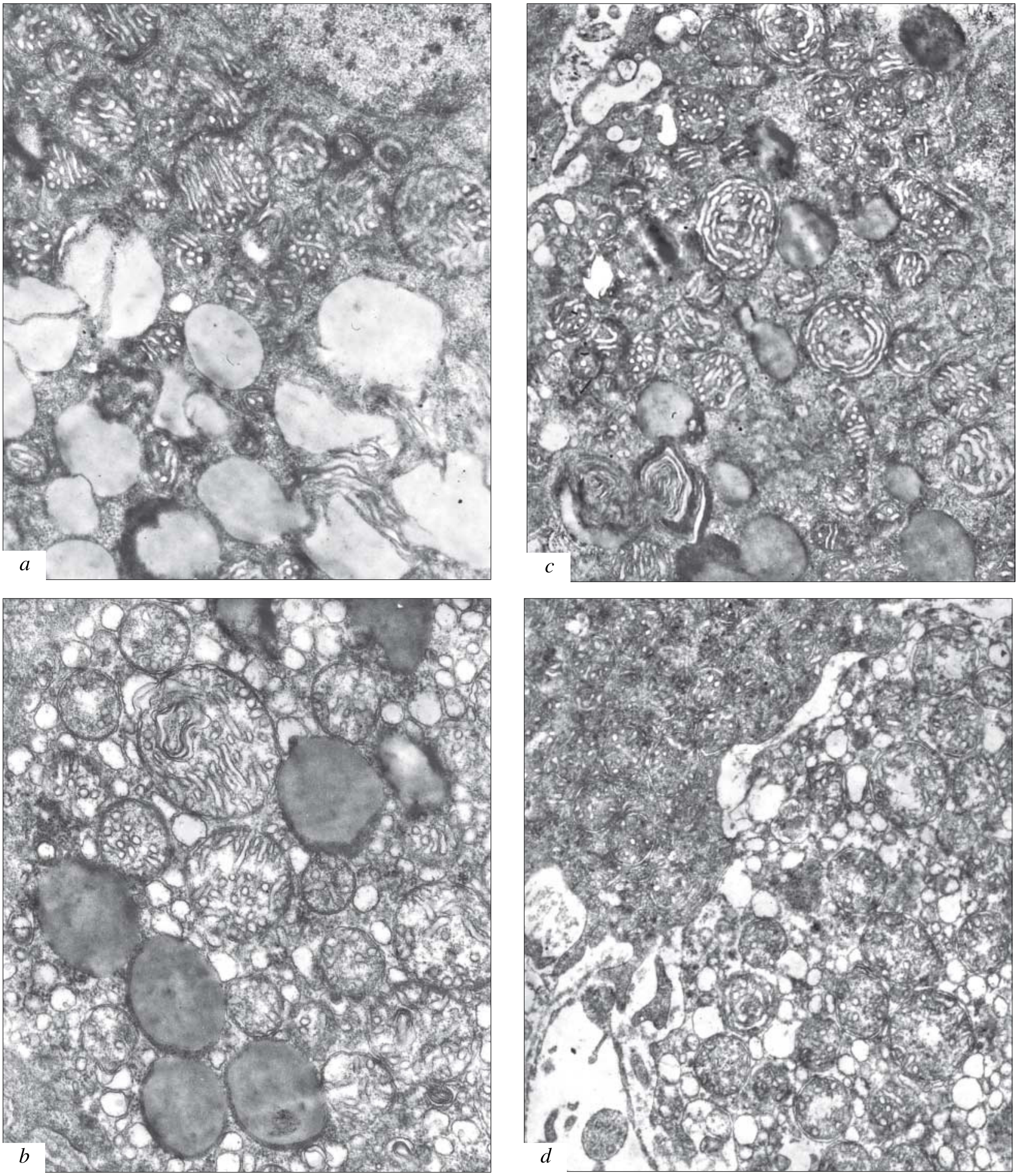

Fig. 1. Ultrastructure of mouse ACC early after whole body hyperthermia. *a*) ACC in zona fasciculata of an intact animal, ×8000; *b*) dilatation of agranular cytoplasmic reticulum vesicles 30 min after hyperthermia, ×10,000; *c*) concentrical transformation of MC crystae, formation of myelin-like structures 30 min after whole body hyperthermia, ×8000; *d*) "clear" and "dark" ACC in the zona reticularis 3 days after whole body hyperthermia, ×10,000.

densely packed MC, cytoplasmic reticulum vesicles, and glycogen granules. ACC in the zona fasciculata were markedly polymorphic by the content of lipid inclusions and their ultrastructural characteristics (appearance of "exhausting" lipid droplets, myelin-like and membranous forms). Destructive changes in MC progressed; focal lysis of their matrix and partial reduction of crystae were obser-

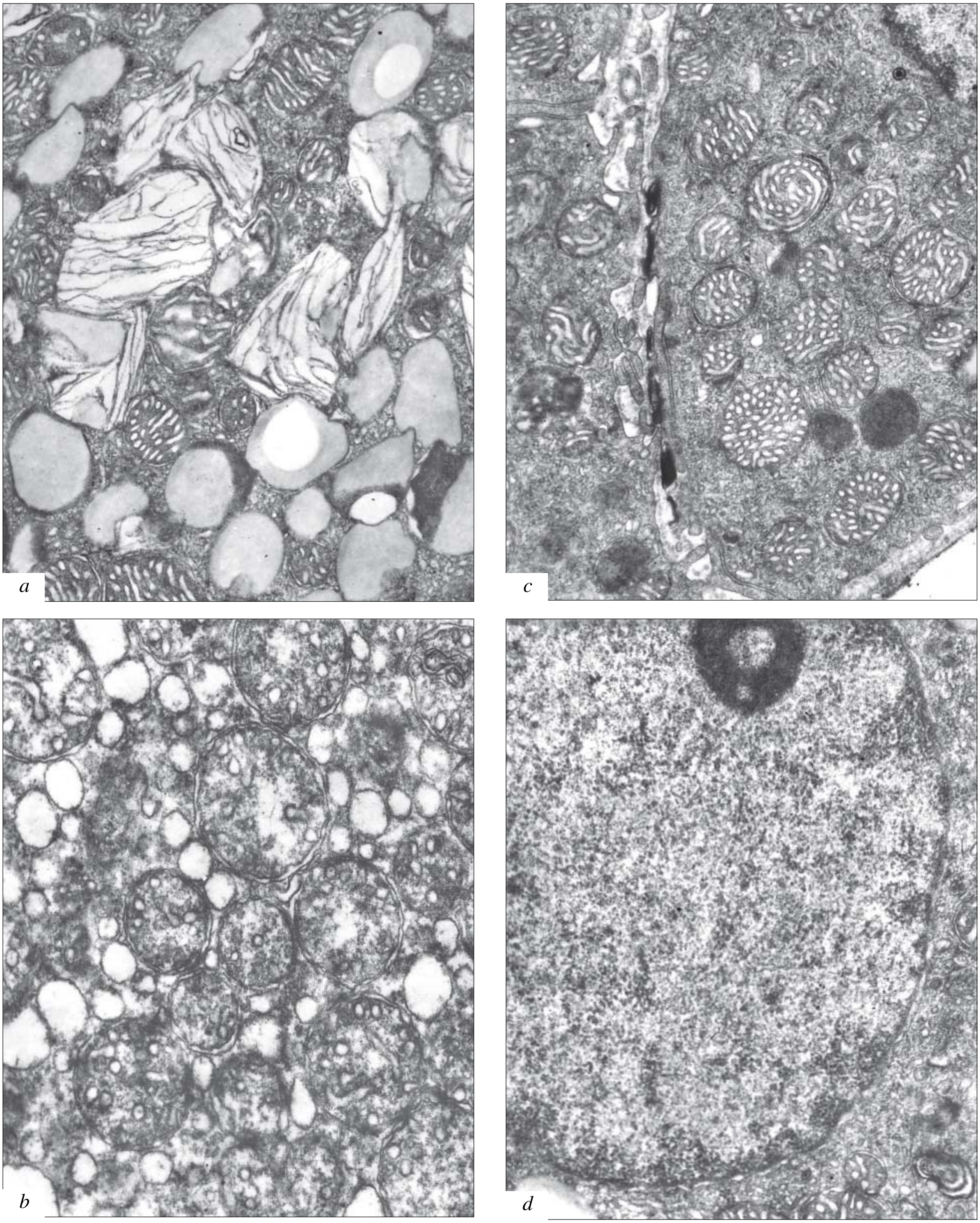

Fig. 2. Ultrastructural manifestations of compensatory adaptive reactions and regeneratory plastic deficiency in ACC 3 days after whole body hyperthermia. *a*) heterogeneity and exhaustion of lipid inclusions, ×10,000; *b*) destructive changes in MC and dilatations of agranular cytoplasmic reticulum, ×12,000; *c*) complete exhaustion of lipid inclusions and appearance of myelin-like structures in extracellular space, ×10,000; *d*) annular nucleolus in ACC of the zona reticularis, ×10,000.

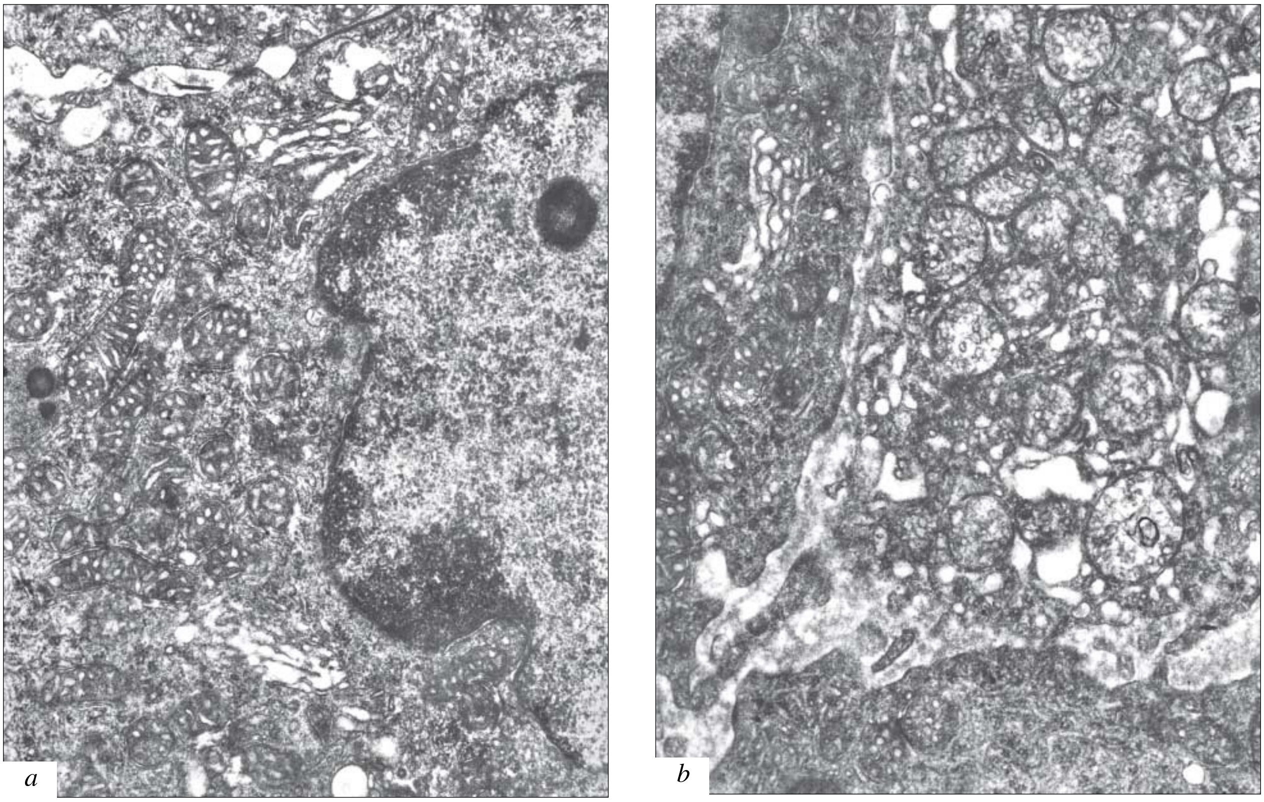

Fig. 3. Ultrastructural characteristics of mouse ACC 14 days after whole body hyperthermia. *a*) annular nucleolus and uneven dilatations of the Golgi complex dictiosomes in ACC of the zona reticularis, ×10,000; *b*) persisting destructive changes in MC and dilatation of agranular cytoplasmic reticulum in ACC of the zona fasciculata, ×10,000.

ved; concentrical transformation of the crystae and their pronounced dilatation were often noted. It is noteworthy that many ACC contained well-developed Golgi complex.

By day 14, the most pronounced changes were observed in ACC of the zona fasciculata and zona reticularis. The cells in both zones often had electron-dense cytoplasm virtually without lipid inclusions, but with numerous well-discernible small MC, Golgi complex, and sometimes secondary lysosomes. Golgi complex was often modified, dictiosomes were significantly dilated and their membranes were partially destructed (Fig. 3, *a*). Vacuolation of the agranular cytoplasmic reticulum was still observed in many ACC (mainly in the zona fasciculata), destruction of MC was more pronounced (Fig. 3, *b*). Autophagocytosis processes were less pronounced than during previous periods, but small osmiophilic myelin-like structures (residual bodies) were constantly present in the ACC, extracellular spaces, and sinusoidal lumens. In the zona reticularis, ACC were virtually absent or presented by necrobiotic forms, particularly in areas adjacent to the medulla; at the same time, numerous small ACC appeared in the zona reticularis. The nuclei in some ACC had annular nucleoli (Fig. 3, *a*) with segregation of the granular and fibrillar components of the nucleolonema.

Hence, ultrastructural reorganization of ACC after single whole body hyperthermal exposure is determined by such basic events as the development of the general adaptation syndrome and inhibition of the regeneratory plastic reactions in cells induced by endotoxication and possible redistribution of plastic resources between organs and tissues. Intracellular changes in ACC caused by the development of the general adaptation syndrome manifest in significant exhaustion of the lipid droplets in ACC in all adrenocortical zones, especially in the zona fasciculata. It should be emphasized that these changes persisted for 14 days after single whole body hyperthermia. Intracellular changes in ACC reflecting the development of regeneratory plastic insufficiency manifest in annular transformation of the nucleoli, segregation of the nucleolonema into the granular and fibrillar components, intensification of autophagocytosis processes, and destructive changes in MC and Golgi complex elements. These changes (cell shrinkage, more pronounced destructive changes, cell elimination) are more pronounced in ACC of the zona reticularis. The changes detected in ACC indicate lasting consequences of stress reaction and appearance of ultrastructural signs of exhaustion of the adaptation and compensatory reactions.

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