EXPERIMENTAL BIOLOGY

Regeneration of Adrenal Zone Reticularis during Hyperthermia

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The appearance of cells with initial corticocyte differentiation signs beneath the internal capsule of the adrenal gland after exposure to exogenous hyperthermia is demonstrated. Ultrastructure of these cells and transformation of their organelles during further differentiation to typical zona reticularis corticocytes are described. The labeling index (administration of ^{3}H-thymidine) in these cells is considerably higher that in typical zona reticularis corticocytes.

Key Words: *adrenal glands; regeneration; hyperthermia; ^{3}H-thymidine*

Exogenous hyperthermia in a widespread adverse environmental factor, which influences inhabitants of arid regions, miners, hot plant metallurgy workers, military operators, and cosmonauts [1,2]. In the present study we investigate the effect of exogenous hyperthermia on corticocyte (CC) regeneration in the zona reticularis.

MATERIALS AND METHODS

Experiments were carried out on 52 adult CBA mice. The animals were daily exposed to 4-h exogenous hyperthermia (38.5-39.3°C) in a thermocontrolled chamber for 7, 14, 21, 30, and 60 days (25 mice served as the control), after which ^{3}H-thymidine was injected to control and experimental animals in a dose of 10 μCi/g body weight. The mice were decapitated under ether anesthesia 1 h after label injection and the adrenal glands were embedded in Epon-Araldite. Autoradiographs of semithin and ultrathin (for electron microscopy) sections were prepared as described elsewhere [5,6]. Ultrathin sections were examined under a CM 10 electron microscope (Philips). In median serial semithin sections 2000 cells for each animal and all cells with initial signs of CC differentiation were counted. The total labeling index (LI) for the zona reticularis cells and LI for light and dark cells. Morphometric study was carried out on an Axiofot microscope coupled with an IBAS-2000 image analysis system (Opton) using KONTRON bildanalyse. The cross-sectional area and perimeter of blood capillaries and the parameters (cross-sectional area, maximum and minimum diameters, and shape factor) of 30 CC for each animal were measured. The data were processed statistically using Student's *t* test.

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RESULTS

Seven-day hyperthermia induced total dystrophy of the zona reticularis manifested as hydropic degeneration. In some cells ballooning degeneration culminating in necrosis of some CC was seen. A tendency toward an increase in LI of typical CC was noted, which was primarily due to an increase in LI of light cells (Table 1). Degenerative changes occurred in the microcirculatory bed: endotheliocyte desquamation, stasis, sludge, and considerable capillary dilation (the cross-sectional capillary area increased by 65.42%).

Under these conditions, small dark cells with signs of initial CC differentiation appeared around blood capillaries beneath the adrenal capsule. These cells did not form dense layer and were diffusely scattered.

On day 14, the number of cells with signs of initial CC differentiation and untypical ultrastructure increased in the inner layers of the zona reticularis (Fig. 1, *a*). They had numerous mitochondria of irregular

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TABLE 1. Dynamics of LI of Dark and Light Cells and Total Number of CC and Cells with Signs of Initial CC Differentiation in the Zona Reticularis after Injection of ^{3}H-Thymidine (‰, *M*±*m*)

Duration of hyperthermia, days	Total number of CC in zona reticularis	LI of dark cells	LI of light cells	Number of cells with signs of initial CC differentiation
Intact animals	1.98±0.12	2.06±0.15	1.92±0.13	—
7	2.05±0.14	1.13±0.11*	3.25±0.26*	28.44±1.23
14	1.11±0.08*+	0.81±0.07*+	1.14±0.09*+	21.87±1.17+
21	2.39±0.13*+	2.61±0.19*+	2.04±0.17+	24.13±1.20
30	1.36±0.13*+	0.75±0.06*	2.09±0.23+	15.63±1.11+
60	0.78±0.08*+	0.62±0.07*	0.83±0.09*+	10.91±0.94+

Note. Data are significant: *in comparison with intact animals; +in comparison with previous term.

shape (cord-, dumbbell-, and ring-shaped) with dense matrix and solitary small vesicular cristae, developed rough endoplasmic reticulum with dilated cisterns, and very small lysosomes compactly arranged on one side of the nucleus.

On day 21, in some regions of the zona reticularis we observed cells with various degree of subcellular differentiation arranged in layers. Single small dense cells with signs of initial CC differentiation are seen beneath the internal capsule (Fig. 1, *b*). Cells with intermediate subcellular structure are arranged on the outside of these cell. The outermost layers consist of large light cells, typical CC with moderate degenerative changes.

Single cells with intermediate subcellular structure first appeared on day 14, their number peaked on

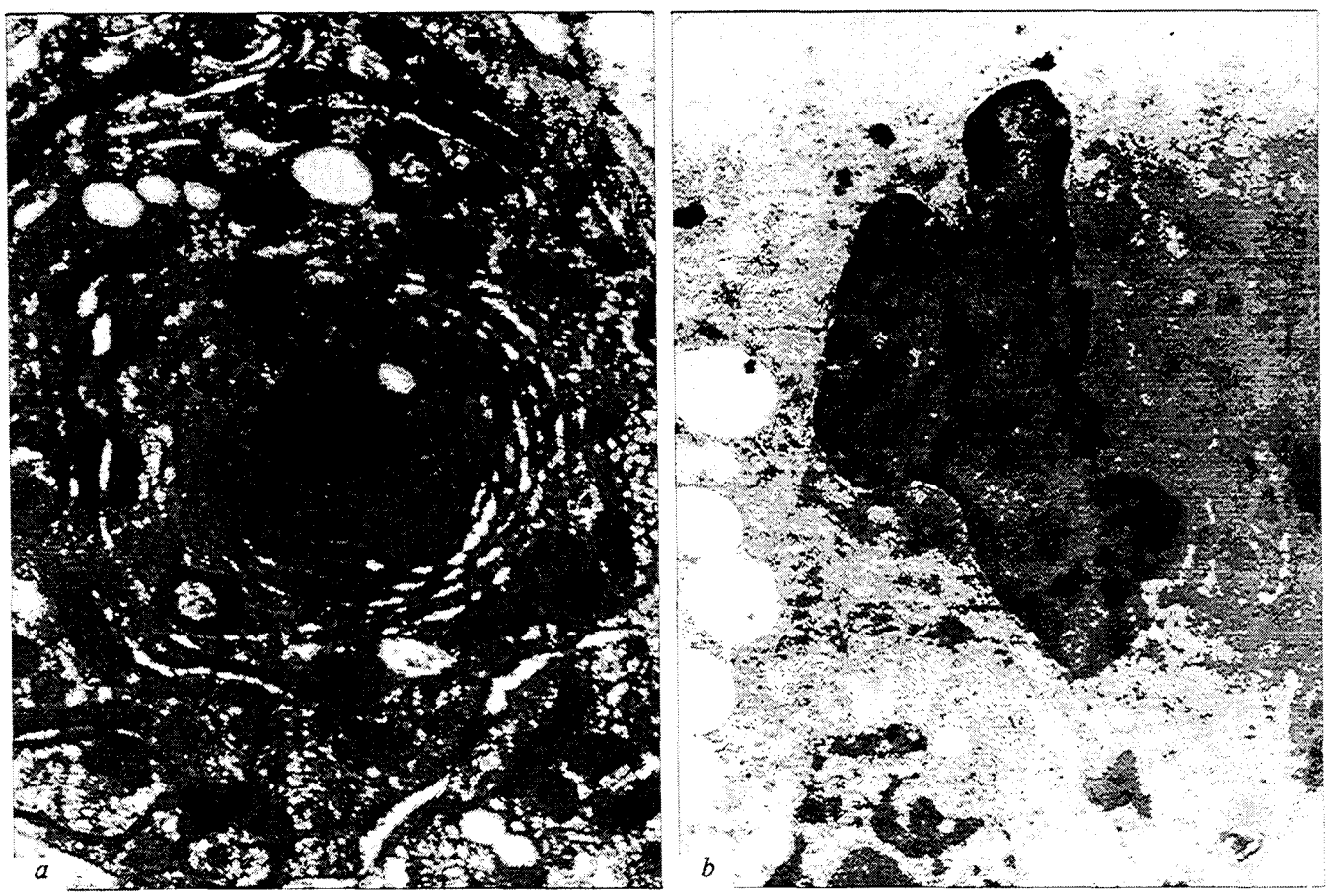

Fig. 1. Adrenal zona reticularis on day 14 of exogenous hyperthermia. *a*) ultrastructure of cell with corticocyte differentiation signs and uncommon organelles, ×25,000; *b*) electron microscopic autoradiograph of DNA synthesis in the nucleus of cell with initial corticocyte differentiation signs, ×30,000.

Fig. 2. Adrenal zona reticularis on day 21 of exogenous hyperthermia. Ultrastructure of cell with intermediate subcellular structure, ×20,000.

day 21, and later decreased again. The number and size of lysosomes in these cells increase in comparison with cells with signs of initial CC differentiation, as well as the number of mitochondrial cristae and polysomes, while the rough endoplasmic reticulum is less developed. Some mitochondria are elongated or ring-shaped, while others look round and small and probably represent cross-sections of large mitochondria (Fig. 2). The ^{3}H-thymidine label is more often seen over these nuclei and LI of these cells considerably surpassed that of typical CC (Fig. 3).

On day 21 of hyperthermia, LI in the zona reticularis considerably increases (by 20.71%) primarily due to the increase in LI of dark cells (by 26.70%, Table 1). Morphometrical analysis reveals a decrease in the size and shape factor of CC in the zona reticularis, which suggests the formation of small flattened cells during cell population renewal.

On day 30, the number of cells with intermediate subcellular structure considerably decreases; there are solitary cells with signs of initial CC differentiation, degenerative changes in typical CC increases. LI of the zona reticularis markedly decreases primarily due to LI of dark cells (Table 1). The cross-section area and perimeter of blood capillaries decrease by 12.71 and 18.08%, respectively. Capillaries are surrounded by wider and more dense connective tissue layer.

On day 60, only single cells with signs of initial and intermediate CC differentiation are seen. They exhibit moderate degenerative alterations. In typical CC global degenerative changes occur, some of them undergo ballooning degeneration and necrosis. The cross-section area and maximum and minimum diameters decrease by 30.78, 18.93, and 14.74%, respectively, the number of cell organelles and their size also decrease. An essential role in these changes is played by sharp inhibition of RNA synthesis in CC of the zona reticularis [3]. Total LI of the zona reticularis decreases to minimum; this decrease is more pronounced in dark cells in comparison with light cells. Connective tissue fibers around blood capillaries become thicker and more condensed, which results in further decrease in the capillary cross-section area and perimeter (by 23.51 and 40.83%, respectively). This is accompanied by desquamation of endotheliocytes, blood stasis, and sludge.

Autoradiography with ^{3}H-thymidine showed that LI in cells with signs of initial CC differentiation surpassed that of typical CC throughout the observation period. However, hyperthermia reduces the intensity of DNA synthesis in these cells (Table 1). Previous studies with ^{3}H-uridine demonstrated that these cells are characterized by intense RNA synthesis [3].

Our findings suggest the existence of cambial low-differentiated cells, whose proliferation is mar-

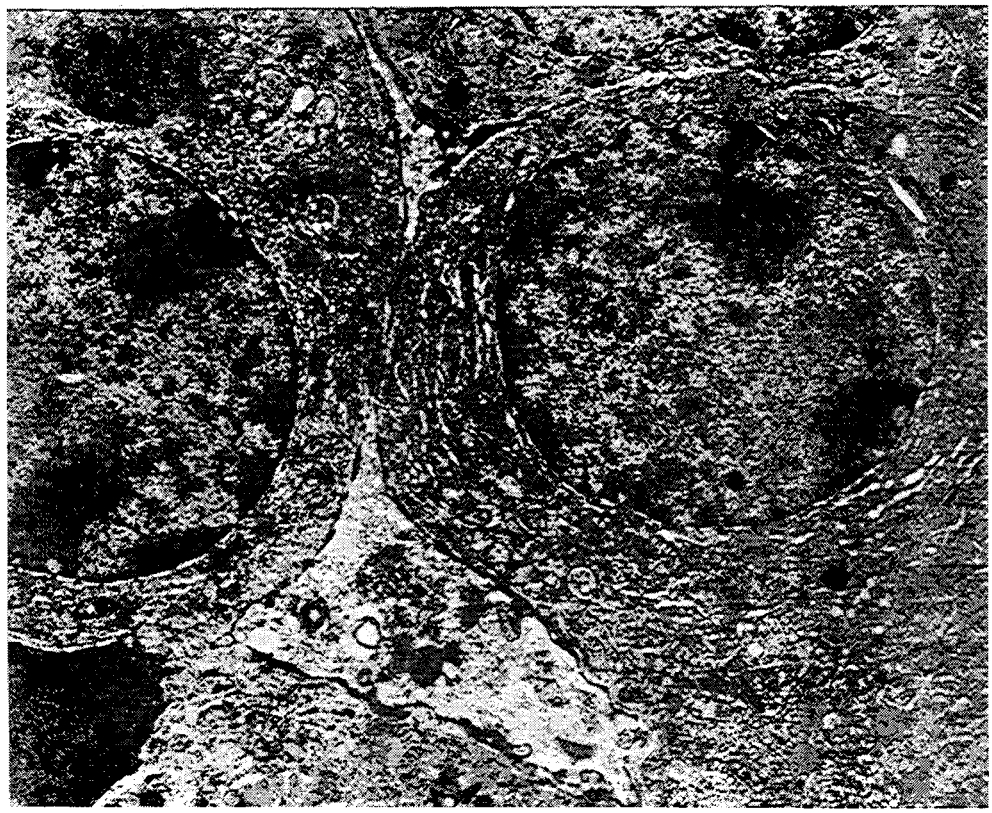

Fig. 3. Electron microscopic autoradiograph of DNA synthesis in a cell with intermediate subcellular structure on day 21 of exogenous hyperthermia, ×20,000.

kedly stimulated by extreme influences yielding a population of cells with signs of initial CC differentiation followed by their transformation to intermediate and then in typical CC of the zona reticularis. These processes promote renewal of CC in the zona reticularis after degeneration and necrosis.

This assumption is confirmed by the fact that physiological regeneration of the zona reticularis is effected through proliferation of typical CC [4]. Moreover, no cells with signs of initial or intermediate differentiation were found in intact animals. The fact that low-differentiated cells are primarily located nearby blood capillaries suggests their close relation with capillaries and the possible migration of these cells along the capillary wall. The excessive formation of connective tissue fibers around blood capillaries and their condensation inhibit migration of low differentiated cells, which in turn inhibits renewal of the CC population in chronic hyperthermia.

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