BIOGERONTOLOGY

Reparative Effect of Epithalon on Pineal Gland Ultrastructure in γ-Irradiated Rats

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Electron microscopy of the pineal gland in γ-irradiated rats treated with epithalon revealed ultrastructural signs attesting to enhancement of its functional activity.

Key Words: *pineal gland; ultrastructure; epithalon; irradiation*

Pineal gland (PG) is one of the main organs controlling aging. An important role in this process is played by pineal peptide complexes (cytomedines), informational molecules with specific amino acid sequence and conformation modifications that mediate the interaction between various ultrastructural elements [4,5]. Disturbed transport of information molecules triggers pathological processes and aging, while administration of exogenous peptides eliminates the pathology and promotes recovery of the lost functions. Cytomedines are most efficient in tissues of the original organ [3,5].

PG plays an important role in the regulation of physiological processes by releasing hormonal and peptide factors, which affect hypothalamic and hypophyseal functions, modify metabolic processes, and neutralize the effect of gonadotropic hormones [10]. Administration of epithalamin, a polypeptide complex isolated from PG normalizes function of various organs and systems [2,7,8]. Epithalamin corrects the action of γ-radiation and produces a pronounced effect on radiation-induced carcinogenesis by decreasing the number of neoplasms [1,6]. A tetrapeptide epithalon (Ala-Glu-Asp-Gly) synthesized on the basis of the amino acid sequence of epithalamin possesses even higher activity [9].

Our aim was to study the effect of epithalon on the ultrastructure of PG in rats subjected to fractional γ-irradiation.

MATERIALS AND METHODS

Experiments were carried out on 30 male Wistar rats (body weight 180-200 g) divided into 3 equal groups. Two groups were γ-irradiated in an Luch-1 apparatus (^{60}Co) during 5 days (daily dose 1.25 Gy). Experimental rats were treated with 5 μg epithalon subcutaneously for 10 days after the beginning of irradiation. Controls were injected with physiological saline according to the same scheme. The rats were decapitated at 10.00-12.00 a.m. under nembutal anesthesia on experimental days 14 and 21. PG of experimental, control, and intact rats fixed in Carnoy fluid were routinely dehydrated and embedded in epon mixture. Ultrathin sections (100 nm) were cut on a LKB-7A ultratome (LKB), contrasted with uranyl acetate and lead citrate, and examined under a JEM-100S microscope (Jeol).

RESULTS

In intact rats PG had regular ultrastructure (Fig. 1, *a-c*). It consisted primarily of pinealocytes with irregular shape and long cytoplasmic processes. Their nuclei

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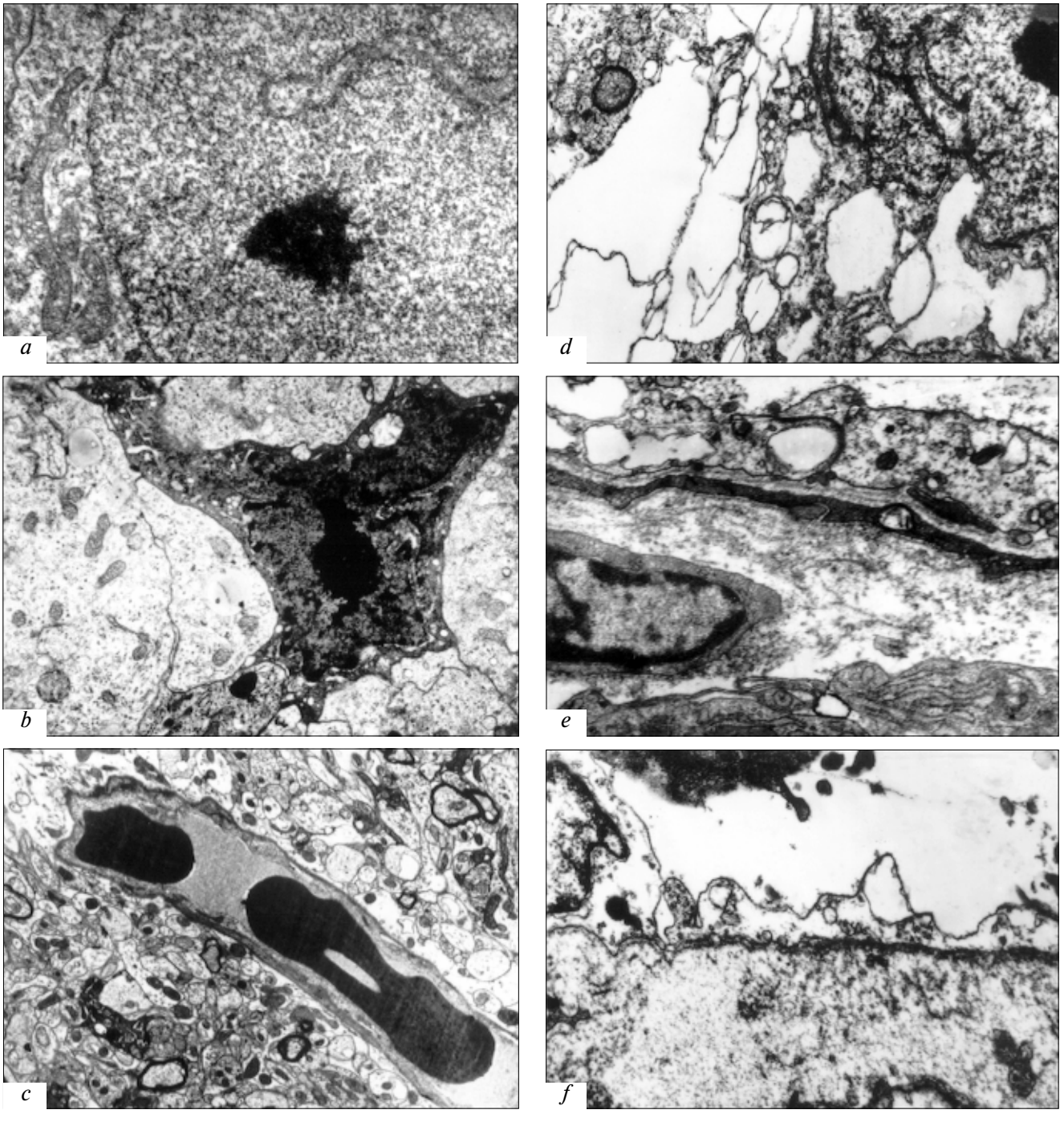

Fig. 1. Ultrastructure of pineal gland in intact (*a-c*) and irradiated rats on day 14 after the beginning of irradiation (*d-f*). *a*) pinealocyte with the nucleus having poorly expressed invaginations of nuclear membrane and dense nucleolus, ×10,500; *b*) glial cell with numerous processes and dense nucleus, ×5600; *c*) fenestrated blood vessel: wide perivascular space contains numerous myelinated and unmyelinated nerve fibers, ×3500; *d*) vacuolation and edema of pinealocyte cytoplasm, ×10,500; *e*) vacuolation and edema of endothelial cells and loosening of the perivascular space, ×10,500; *f*) edema of endothelial cell, complete absence of organelles in cytoplasm, ×10,500.

had oval or irregular shape and low electron density. Karyolemma often formed invaginations. There was one nucleolus in the nucleus, although some nuclei contained 2 nucleoli. Pinealocyte cytoplasm was partially occupied by granular endoplasmic reticulum (EPR). Golgi complex was well developed and consisted of a system of flat compartments connected with light vesicles. Pinealocyte cytoplasm was enriched with mitochondria of medium electron density. Their shape varied from round and oval to elongated (Fig. 1, *a*).

The glial cells in PG were less abundant and differed from pinealocytes. They were characterized by

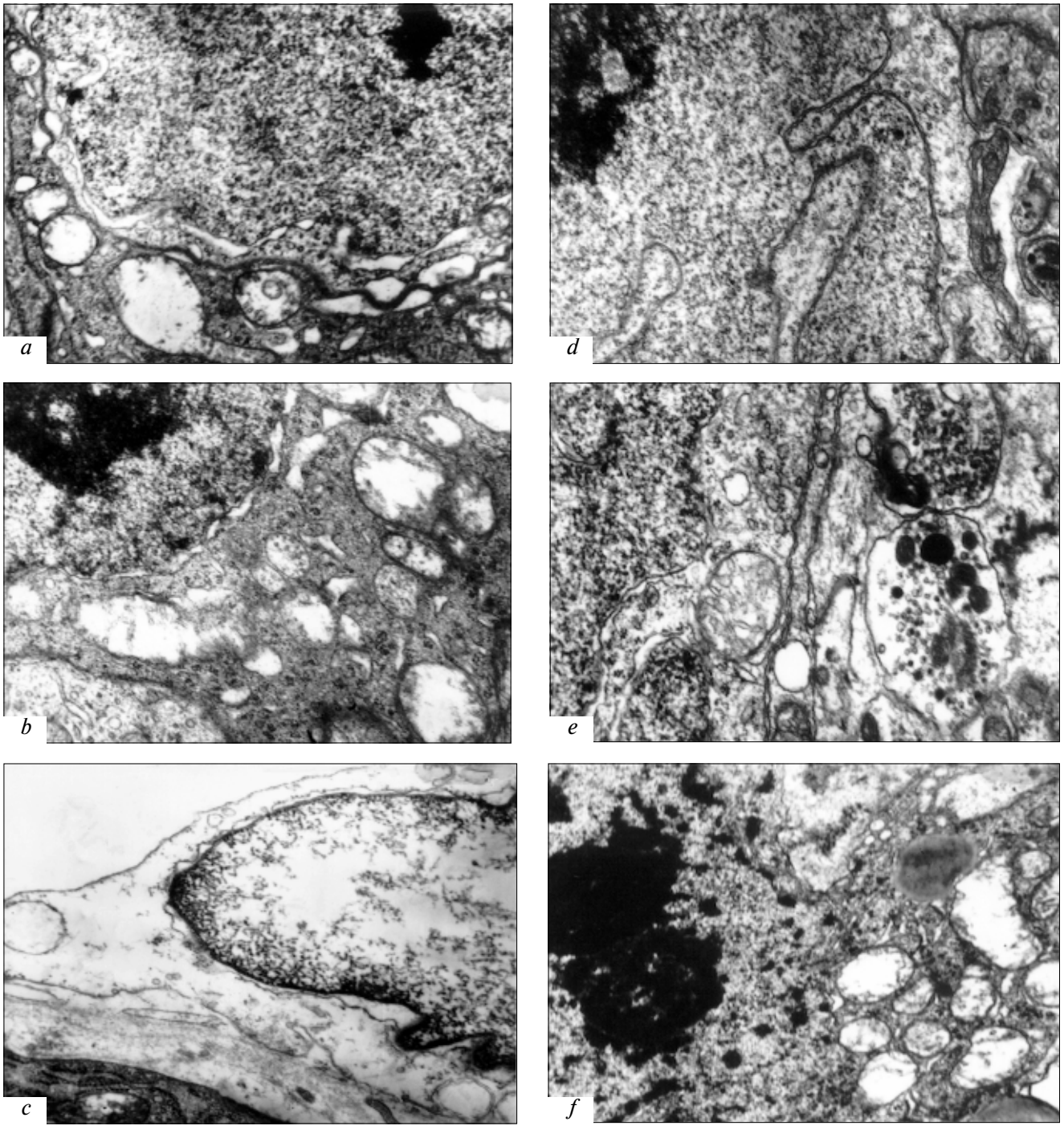

Fig. 2. Ultrastructure of pineal gland on days 21 (*a-c*) and 14 (*d-f*) after the beginning of irradiation. *a*) pinealocyte nucleus with pronounced desquamation of the outer nuclear membrane, ×10,500; *b*) glial cell with detached external nuclear membrane and lysed cristae in mitochondria, ×14,000; *c*) blood vessel with edematous endothelium, in which chromatin is loosened and cytoplasmic organelles are absent, ×14,000; *d*) pinealocyte nucleus with deep invaginations of nuclear membrane, ×14,000; *e*) electron dense and electron light vesicles in contacts of nerve terminals with pinealocytes, ×17,500; *f*) restoration of nuclear structure and enlargement of nucleolus in a glial cell. Cytoplasm contains mitochondria with light matrix, ×10,500.

high electron density of the cytoplasm, irregular shape of the nucleus with pronounced marginal chromatin condensation (although in some cells the chromatin lumps were distributed throughout the nucleus). These cells had long cytoplasmic processes, which frequently formed close contacts with adjacent pinealocytes. They had well-developed Golgi complex, numerous oval or round large mitochondria, whose matrix was frequently light in the central part. Lysosomes and lipid droplets were regularly observed in the cyto-

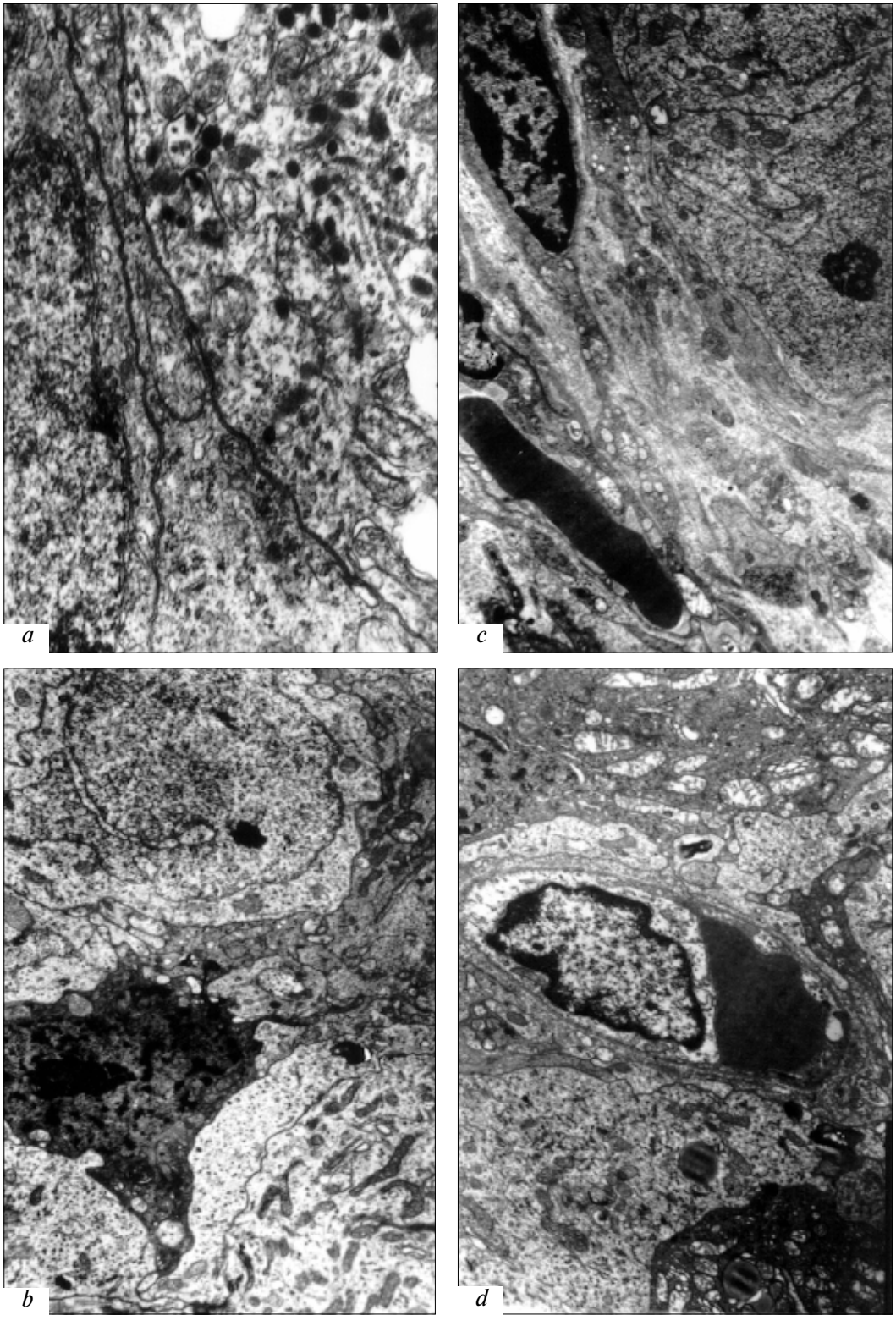

Fig. 3. Ultrastructure of pineal gland in epithalon-treated rats on days 14 (*a*) and 21 (*b-d*) after the beginning of irradiation. *a*) neurosecrete granules in pinealocyte cytoplasm, ×17,500; *b*) glial cell under a pinealocyte, whose nucleus has numerous invaginations of the nuclear membrane, ×5600; *c*) fenestrated blood vessel: glial cells processes in perivascular space, ×5600; *d*) blood vessel making close contacts with glial cells, their processes, and pinealocytes, ×5600.

plasm (Fig. 1, *b*). PG contained Schwann cells (a component of unmyelinated nerve fibers). Sometimes they were located in the parenchyma, but more frequently they could be observed in wide perivascular space around the thin-walled porous vessels (fenestrated capillaries). In the epiphysis, the unmyelinated fibers formed close contacts with pinealocytes and glial cells (or their processes). Myelinated nerve fibers are also seen in the perivascular space (Fig. 1, *c*).

On day 14 after the beginning of irradiation, the most pronounced ultrastructural changes were found in the vascular bed of the control rats. They were manifested by swelling, edema, and vacuolation of fenestrated epithelium, disappearance of cytoplasmic organelles and pinocytic vesicles, widening and loosening of the perivascular space (Fig. 1, *e, f*). Desquamation of the outer nuclear membrane was observed in some pinealocytes, which was accompanied by swelling and destruction of central mitochondria crystae with appearance of myelin figures and large vacuoles in the cytoplasm (probably, formed due to widening of EPR membranes). In addition, we observed appearance of lysosomes and enlargement of lipid drops. As a rule, pinealocytes of the control rats had single nucleolus with homogenous structure (Fig. 1, *d*).

The observed alterations persisted on day 21: invaginations and desquamation of the outer nuclear membrane and decreased cytoplasmic volume. There were pinealocytes with a very narrow cytoplasmic rim (Fig. 2, *a*). The most pronounced alterations were found in the cytoplasm (vacuolation due to widening of EPR membranes). Some pinealocytes contained swollen mitochondria with light matrix or completely lysed crystae (Fig. 2, *b*). The glial cells demonstrated desquamation of the outer nuclear membrane, widening of the granular EPR, and hypertrophy of the Golgi complex. Both glial cells and pinealocytes contained lipid drops, often near the Golgi complex, and myelin figures. In many blood capillaries endotheliocytes were edematous and contained few cytoplasmic organelles. Nuclear chromatin was loosened, and in many cases the outer nuclear membrane was desquamated along the entire nucleus perimeter. The perivascular space was also loosened and edematous (Fig. 2, *c*).

In epithalon-treated rats, the nuclear surface increased on day 14 after the beginning of radiation due to numerous deep invaginations of the nuclear membrane (Fig. 2, *d*). The cytoplasm had no large vacuoles, but contained numerous membranes of the granular EPR and ribosomes. Some pinealocytes contained dense bodies ultrastructurally similar to neurosecretion granules (Fig. 2, *e*). In the glial cells, EPR membranes were widened and the outer nuclear membrane was desquamated. This phenomenon was much more pronounced in glial cells, but in some glial cells nuclear membrane was preserved. The contacts between nucleus and cytoplasm were extended due to multiple invaginations. Condensed chromatin was located not only along the nuclear membrane, but occupied the entire nucleus area. The number of nucleoli increased. Some glial cells demonstrated partial recovery of mitochondrial ultrastructure (crystae), while their matrix remained light and edematous (Fig. 2, *f*). Similarly to pinealocytes, Golgi complex in glial cells was enlarged, while the number of lipid drops in the cytoplasm decreased. On day 21, the ultrastructure of pinealocytes and glial cells did not differ from that in intact rats, although the nucleus-cytoplasm boundary was not easily revealed in dense glial cells (Fig. 3, *b*). Blood capillaries also had normal ultrastructure (Fig. 3, *c*, *d*).

The present electron microscopy study of PG showed that fractional γ-irradiation produces extensive structural alterations in the vascular bed, pinealocytes, and glial cells. In epithalon-treated rats, ultrastructural signs reflect functional activation of cells, which indicates pronounced reparative properties of epithalon in PG, the tropic organ for this peptide.

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