BIOGERONTOLOGY

Age-Specific Ultrastructural Postradiation Changes in Intestinal Endocrine Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 132, No. 11, pp. 579-585, November, 2001
Original article submitted September 10, 2001

Age-specific ultrastructural postradiation changes in the main types of duodenal endocrine cells (apudocytes) were studied in rats 6 and 12 months after single whole-body irradiation in doses of 7 and 7.5 Gy. Ultrastructural disorganization of different severity was detected, which depended on apudocyte type and term postradiation. Degranulation was the basic mechanism of hormone secretion in delayed periods after the exposure.

Key Words: *endocrine cells; apudocytes; ultrastructure; ionizing radiation; rats*

Molecular and cellular mechanisms of radiation damage are an important problem of modern radiobiology. Recent findings indicate that bioactive substances (peptide hormones and biogenic amines) produced by the diffuse endocrine system cells (apudocytes) present in many organs, tissues, and systems [5,10,12] are an important key factor in the development of adaptive, compensatory-adaptive, and pathological reactions. Many peptide hormones and biogenic amines are involved in the mechanisms regulating cell proliferation and can even act as endogenic radiomodifiers [2].

The majority of apudocytes are situated in the gastroduodenal mucosa, mainly in the antral part of the stomach and in the duodenum [7,13,15]. On the other hand, it is well known that gastrointestinal organs are very sensitive to ionizing radiation, the duodenum, specifically its proximal portion, being more radiosensitive than the stomach [1].

Though the morphology and physiology of apudocytes has been amply studied, their structure and function during radiation exposure are little known, and the age-specific characteristics of their ultrastructure after exposure to ionizing radiation virtually have never been described.

We therefore investigated the age-specific changes in the ultrastructure of duodenal endocrine cells after exposure to ionizing radiation.

MATERIALS AND METHODS

Ultrastructure of endocrine cells of the duodenal proximal portion was studied in male Wistar rats surviving for 6 and 12 months after single whole-body γ-irradiation in doses of 7 and 7.5 Gy, respectively ($LD_{50/30}$). Single whole-body γ-exposure of 180-200 g animals was carried out on a GUB 20000 cobalt device at dose power of 1.60 and 1.77 Gy/min, respectively.

The material was dehydrated and embedded in blocks. Ultrathin (150-50 nm) sections were contrasted with uranyl acetate and lead citrate. The preparations were examined and photographed under a JEM-100S electron microscope (Jeol) at ×16,000, which allowed examination of the largest area of cell with clearly seen ultrastructural signs of secretory granules.

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0007-4888/01/1325-1110$25.00

RESULTS

Ultrastructural analysis of duodenal apudocytes showed that mainly mitochondria, endoplasmatic reticulum (EPR), and all membrane structures were damaged in delayed periods after irradiation in lethal doses. The mitochondria swelled, their cristae shortened and were destroyed, the outer (rarely inner) membrane was damaged. EPR were vacuolated, cisterns dilated and filled with electron-transparent matter of. Focal destruction of cell membranes resulted in appearance of solitary myelin structures in the apudocyte cytoplasm.

Lipofuscin granules of different size were sometimes seen in some endocrine cells (mainly D cells), which should be regarded as a result of protein-carbohydrate dismetabolism. It is noteworthy that 12 months after irradiation these lipid droplets in the cytoplasm of endocrine cells were more abundant than after 6 months.

Comparative electron microscopic analysis of ultrastructural lesions in the main types of duodenal apudocytes in delayed periods after irradiation in lethal doses showed the following.

Similar ultrastructural lesions of the same severity were observed 6 and 12 months after irradiation in EC cells producing serotonin, melatonin, substance P, and motilin. In later periods different number of secretory granules retaining mainly homogeneous electron density were identified in the cytoplasm of EC cells. Degranulation of some EC cells was observed.

Six months after irradiation, the duodenal mucosa contained gastrin-producing (G) cells with ultrastructure similar to the control and with pronounced disorders. After 12 months ultrastructural changes in G cells were less pronounced than after 6 months. Prolongation of the post-irradiation period led to a decrease in the total number of secretory granules, while their homogeneity increased: the granules were characterized by higher homogeneous electron density. Some G cells were degranulated.

In somatostatin-producing D cells cytoplasm vacuolation after 12 months was less pronounced than after 6 months. In contrast to intact animals, in rats exposed to ionizing radiation in lethal doses the number of secretory granules in D cells sharply increased in later post-irradiation periods. Granules with different electron density were identified, which indicated active secretion by molecular extrusion (Fig. 1, *a*; Fig. 2, *c*; Fig. 3, *a*).

In later periods after single whole-body irradiation, histamine-producing (ECL) cells were characterized by good ultrastructural organization, sufficiently close to the control. The formation of myelin structures in the cytoplasm 1 month after the exposure is worthy of note, as it was not observed 6 months post-exposure. The total number of granules in ECL cells of irradiated animals notably increased in comparison with the control (Fig. 1, c; Fig. 2, *a*; Fig. 3, *c*).

Ultrastructural organization of N cells producing neurotensin was similar to the control by many parameters after 6 and 12 months; the population of N cells is small. Their cytoplasm contained few homogeneous electron-dense secretory granules (Fig. 1, *e*; Fig. 2, *e*; Fig. 3, *d*).

Ultrastructure of K cells containing gastroinhibitory peptide was preserved 6 months after irradiation, but after 12 months the majority of K cells were edematous and their cytoplasm was clarified. In later period after irradiation the cytoplasm of these cells contained numerous secretory granules with high electron density.

Enteroglucagon-producing (L) cells were rarely seen in the duodenal mucosa in delayed periods after irradiation, but their ultrastructure had virtually no signs of disorganization. The number of secretory granules in L cells was decreased in comparison with L cells of intact animals, the granules were larger and characterized by high homogeneous electron density (Fig. 1, *d*; Fig. 2, *d*; Fig. 3, *b*).

Ultrastructure of D1 cells producing vasoactive intestinal peptide were characterized by more pronounced destruction after 12 months than after 6 months: intensive detachment of the external nuclear membrane, dilatation of EPR cisterns and Golgi complex were seen. Vacuolation of the cytoplasm was pronounced, the content of individual lysosomes increased. In later periods after irradiation the total number of secretory granules in D1 cells did not change in comparison with intact animals. This was paralleled by appearance of granules with heterogeneous electron density in the cytoplasm. D1 cell degranulation was more expressed 12 months after irradiation (Fig. 1, *b*; Fig 2, *b*; Fig. 3, *e*).

Bombesin-producing (P) cells in delayed periods after exposure to ionizing radiation were characterized by good functional organization. Moderate number of partially and completely degranulated P cells were identified in the duodenum after irradiation.

Ultrastructural changes were seen in secretin-producing (S) cells 6 months after the exposure, which were partially restored after 12 months. The number of secretory granules in S cells of irradiated animals decreased 6 months after the exposure in comparison with the control and remained at the same level after 12 months. High electron density of secretory granules did not change.

Hence, ultrastructural disorganization of different severity appeared in apudocytes in delayed periods after irradiation in lethal doses. Pronounced ultrastructural injuries were seen in EC, G, D, and S cells, but

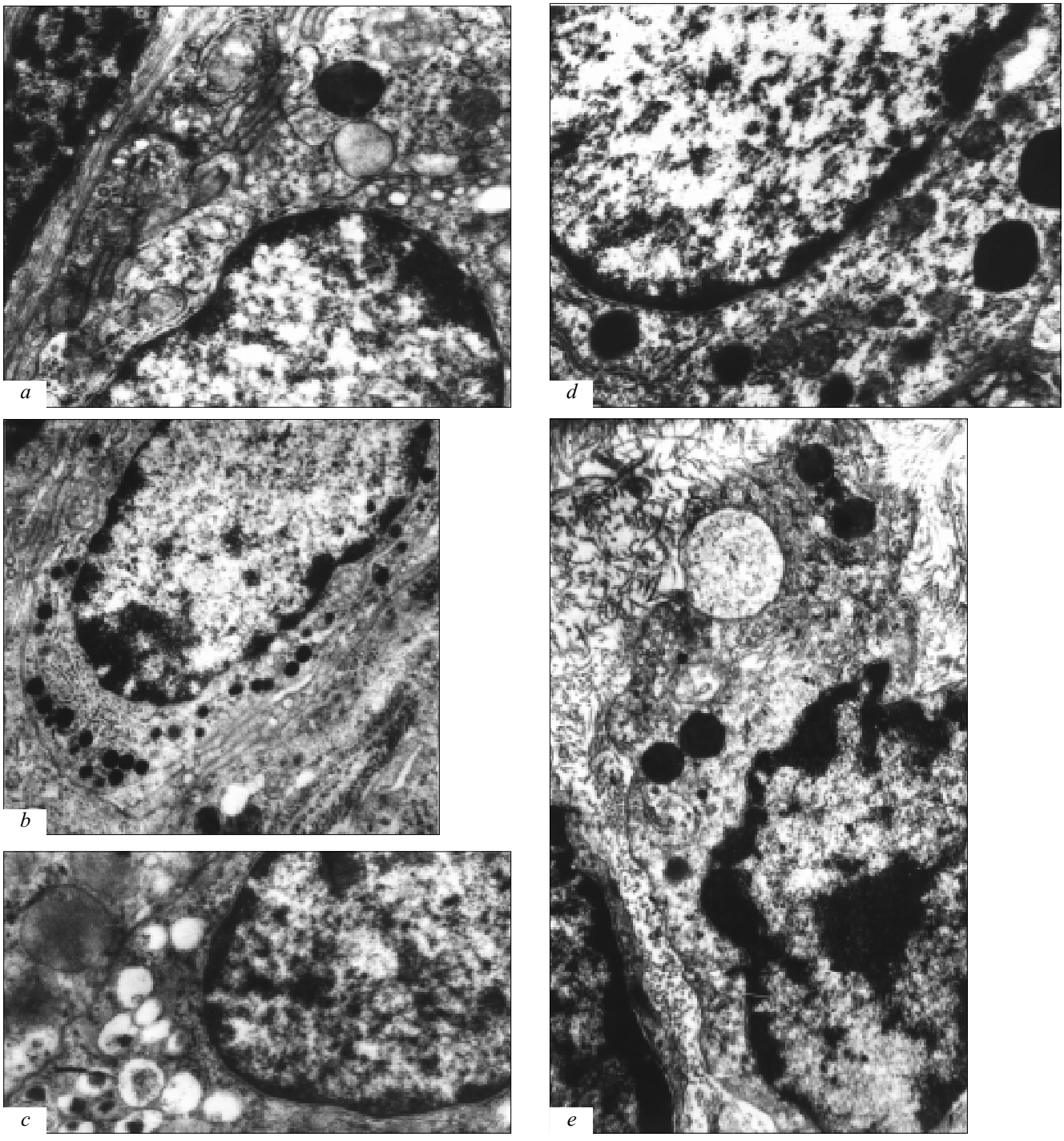


Fig. 1. Duodenal endocrine cells of intact rats, ×16,000. *a*) D cell: few characteristic round secretory granules with finely granular osmiophilic matrix of different electron density; *b*) D1 cell: small round secretory granules with high electron density; *c*) ECL cells: vesicular secretory granules with eccentric large slightly elongated core, hard or varying in density; *d*) L cells: large round secretory granules with high electron density; *e*) N cell: round electron-dense medium-sized secretory granules.

cell ultrastructure was partially restored after 12 months. In K and D1 cells the shifts augmented after 12 months in comparison with 6 months post-irradiation, while ultrastructure of other endocrine cells (ECL, N, L, and P cells) was radioresistant.

These data indicate that the doses of 7 and 7.5 Gy damaged the endocrine cells, which manifested in delayed periods after irradiation. It seems that exposure to ionizing irradiation in lethal doses led to damage of a certain part of duodenal stem cells, while endo-

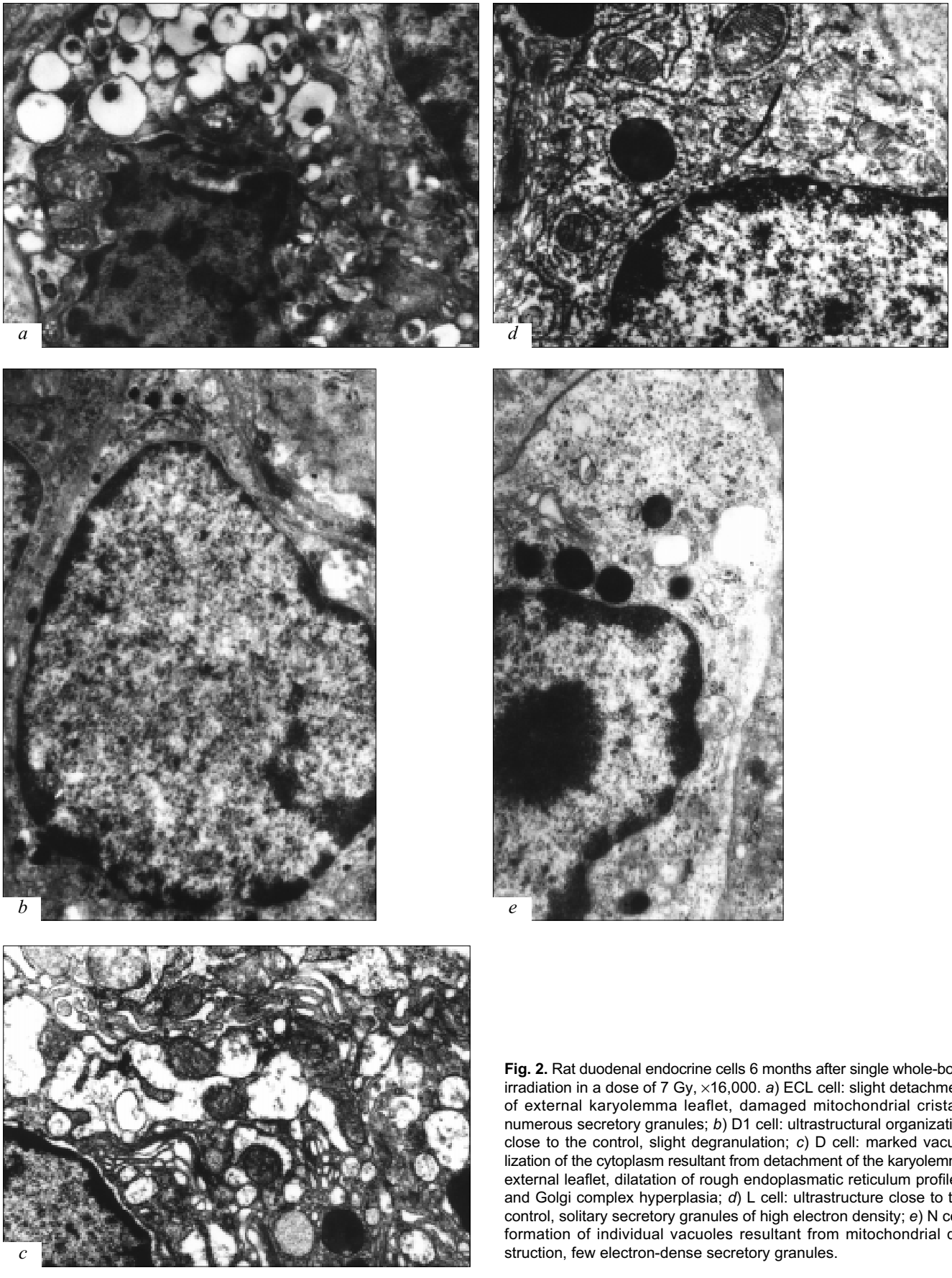
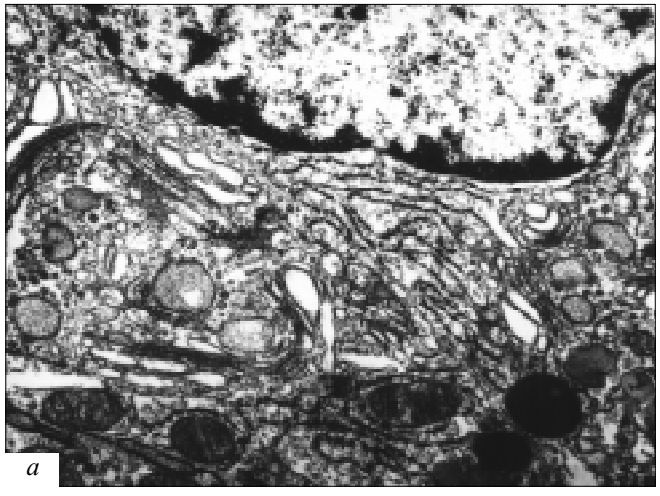
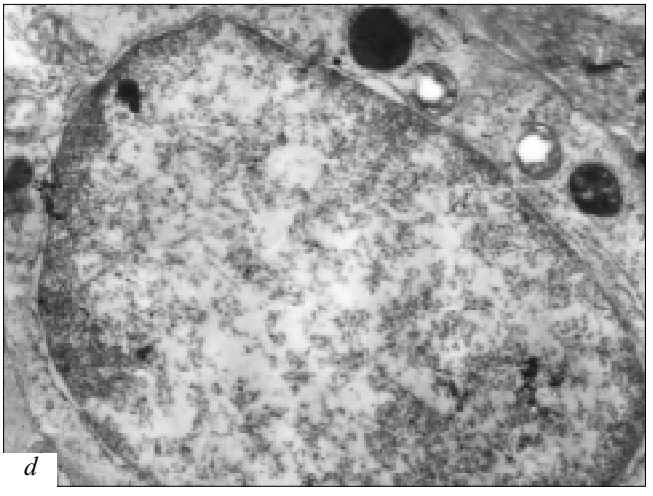
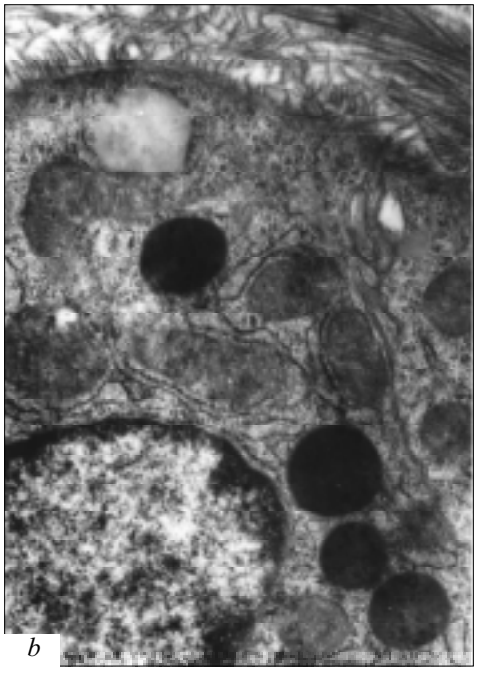
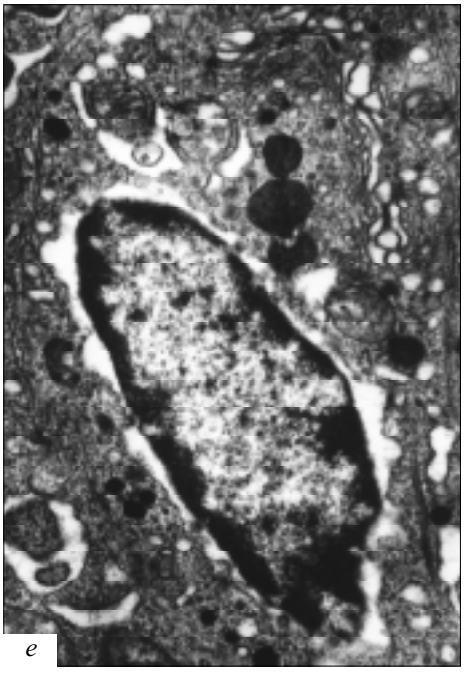


Fig. 2. Rat duodenal endocrine cells 6 months after single whole-body irradiation in a dose of 7 Gy, ×16,000. *a*) ECL cell: slight detachment of external karyolemma leaflet, damaged mitochondrial cristae, numerous secretory granules; *b*) D1 cell: ultrastructural organization close to the control, slight degranulation; *c*) D cell: marked vacuolization of the cytoplasm resultant from detachment of the karyolemma external leaflet, dilatation of rough endoplasmatic reticulum profiles, and Golgi complex hyperplasia; *d*) L cell: ultrastructure close to the control, solitary secretory granules of high electron density; *e*) N cell: formation of individual vacuoles resultant from mitochondrial destruction, few electron-dense secretory granules.









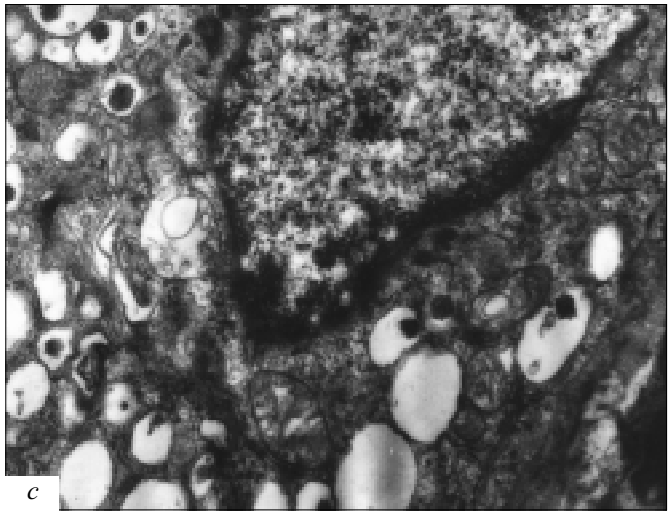


Fig. 3. Rat duodenal endocrine cells 12 months after single whole-body irradiation in a dose of 7.5 Gy, ×16,000. *a*) D cell: slight detachment of the karyolemma external leaflet, pronounced hyperplasia of the Golgi complex, numerous secretory granules with finely granular matrix; *b*) L cell: good ultrastructural organization, numerous active mitochondria, secretory granules of high electron density; *c*) ECL cell: individual mitochondria with partial disorganization of cristae, formation of myelin structures, numerous secretory granules; *d*) N cell: slight detachment of external nuclear membrane, starting vacuolization of damaged mitochondria, few electron-dense secretory granules; *e*) D1 cell: intensive detachment of external nuclear membrane, dilatation of endoplasmatic reticulum profiles and Golgi complex, moderate number of secretory granules, located round the nucleus.

crine-determined cells surviving exposure in these doses allowed partial recovery of the apudocyte population. On the other hand, it is noteworthy that secretory granules in endocrine cells possess sufficient morphological stability and retain typical signs and tinctorial properties even in dead cells.

Among other late ultrastructural reactions of endocrine cells to irradiation are cytoplasm vacuolation resultant from destruction of cell organelles and degranulation. Some features of degranulation and vacuolation of the endocrine cell cytoplasm are worthy of note: sometimes these processes are parallel, and in this case secretory granules are usually located along the periphery of vacuoles and tightly adhere to them. Sometimes the cytoplasm of vacuolated cells contains only solitary secretory granules. It seems that these phenomena can be regarded as a method for releasing the secretory material.

In is believed that products accumulated by endocrine cells in secretory granules are released mainly by molecular dispersion: the secretory product is released from granules into the cytosol and then onto cell surface through the cytoplasmatic membrane, which becomes permeable for the hormone under certain conditions [11]. Another form of secretion is exocytosis on the basoteral surface of cells, though it is well known for some endocrine organs [14]. Our findings indicate that massive release of the secretory products can be realized by their lysis in numerous vacuoles under conditions of adaptation of the organism to extreme factors (in our case irradiation).

Intensive destruction of EPR observed in late periods after irradiation leads to disorders in the transport mechanisms involved in exocytosis of the apudocyte secretory granules [4,8]. Irradiation in doses of 7 and 7.5 Gy causes destruction of the tubular-filamentous system in endocrine cells, thus preventing the secretory granule exocytosis even in delayed periods after irradiation [9].

The following characteristics of secretion were observed in apudocytes. Degranulation of different degree was observed in EC, G, D, K, D1, and P cells, molecular extrusion being intensified in D and D1 cells. Both modes of secretion were activated in the descendants of endocrine cells exposed to single whole-body irradiation in lethal doses. By contrast, no secretion was detected in some endocrine cells (N, L, S cells), which can also be explained by impairment of the mechanisms of secretion in descendant cells because of exposure of the precursor cells.

Many peptide hormones and biogenic amines possess radiomodifying properties. For example, serotonin, melatonin, and histamine are characterized by pronounced radioprotector effects, while motilin, substance P, gastrin, and vasoactive intestinal peptide are radiosensitizers [3,6]. Our studies confirm it, to a certain degree. After single whole-body irradiation in lethal doses the number of secretory granules in the cytoplasm increased in ECL cells, while in EC, G, and D1 cells degranulation was observed.

EC cells simultaneously secrete hormones with radioprotective properties (serotonin and melatonin) and hormones with radiosensitizing effects (motilin and substance P). However the duodenal EC2 cells contain mainly motilin and substance P and far lower amounts of serotonin, hence, the hormones produced by EC2 cells are characterized mainly by radiosensitizing effects. And since this cell type predominates in the duodenal mucosa apudocyte population, it is the EC2 cells that make an essential contribution to the formation of duodenal radiosensitivity.

Hence, electron microscopic examination of the ultrastructure of endocrine cells after irradiation opens new vistas in the study of mechanisms of radiation injury. Presumably the substances released by apudocytes actively modify the type and degree of radiation injury, and, considering their radiomodifying effects, contribute to further course of the pathological process caused by radiation exposure.

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