Ultrastructure of the Rat Duodenal Endocrine Cells after Prolonged Irradiation

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We propose classification of duodenal endocrine cells of intact rats based on ultrastructural signs of secretory granules and subdivided these cells into 10 basic types. The effect of long-term irradiation in a total dose of 2.5 Gy on ultrastructural organization of duodenal apudocytes was studied. Irradiation induced nonspecific changes of cell organelles in apudocytes. Differences in the ultrastructural disorganization were detected between different types of apudocyte populations and between different types of endocrine cells. Under conditions of adaptation to radiation apudocytes released the secretory product not only through molecular extrusion and exocytosis, but also via degranulation.

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

The gastrointestinal tract is an important endocrine organ, presented by APUD cells. Apudocytes accumulate biogenic amine precursors, decarboxylate them, and synthesize biogenic amines and peptide hormones. Endocrine system of the gastrointestinal tract (gastroenteropancreatic system) is the most important component of the APUD system. The number of endocrine cells in this system far surpasses the total number of cells in all endocrine glands [13].

More than 20 types of apudocytes are now identified in the stomach and intestine, the greatest number being located in the duodenum [1,14]. The morphology and physiology of individual cells of the APUD system and biochemistry of hormones synthesized by them have been extensively studied, but the ultrastructural organization of apudocytes of different types located in the duodenum remains little known.

The duodenum is a radiosensitive organ; radiomodifying effects of hormones produced by some endocrine cells [2,5] contribute to the formation of radiation sensitivity of the duodenum and to processes of its postradiation reparation.

Postradiation pathology of endocrine organs after a single exposure, particularly during early periods, has been extensively studied [10], while damage to the APUD system induced by long-term irradiation is described in only few reports. The effects of prolonged irradiation are of particular importance from the viewpoint of reparation processes, which are most activated during exposure to ionizing radiation in nonlethal doses [3].

We investigated the ultrastructural organization of duodenal endocrine cells in intact rats, classified these cells into basic types by the ultrastructure of secretory granules, and studied the ultrastructure of endocrine cells immediately after prolonged irradiation in nonlethal dose.

MATERIALS AND METHODS

Male Wistar rats (140-160 g) were exposed to γ-irradiation on an Experiment device (^{127}Cs, 5.32 mGy/h dose power in direct bundle); the animals were daily exposed to 0.125 Gy during 20 days. The proximal portion of the duodenum was isolated under Nem-

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butal narcosis (35 mg/kg) 24 h after prolonged exposure. The material was fixed by Karnovskii's method, dehydrated, and embedded in blocks. Sections were contrasted according to Reynolds and examined under a JEM-100S electron microscope (Jeol). Morphological studies were carried out at magnification 16,000, as this magnification shows the greatest area and shape of the examined cell and the size of its secretory granules.

RESULTS

Electron microscopy showed great apudocyte population and a variety of endocrine cell types in the duodenal mucosa of intact rats. EC, D, D1, and S cells were the most numerous in the total apudocyte population, while some types (G and ECL cells) were only occasionally seen mainly in the gastric mucosa [6].

Apudocytes were situated alone or in groups including several cells among larger oval or round non-endocrine enterocytes. Many endocrine cells (EC, G, N, D1, S cells) had a long apical process reaching the lumen of the crypt and it provided cell contact with the environment and performed receptor function [11].

Apudocytes of all types had polymorphic nuclei with condensed chromatin and well-contoured nucleolus. The cytoplasm contained mitochondria, ribosomes, polysomes, lysosomes, and sometimes vacuo-

TABLE 1. Classification of Normal Rat Duodenal Endocrine Cells

Cell type	Hormones	Granule size, nm	Ultrastructural characteristics of granules
EC1	Serotonin, melatonin	200-300	Polymorphic; round, bud-shaped, dumbbell-like, rocket-shaped; sometimes surrounded with light rim and thin membrane with electron-dense core
EC2	Substance P, motilin, serotonin	300-350	Polymorphic; often round and bean-shaped; high electron density; sometimes surrounded with a light rim and thin membrane
G	Gastrin	150-350	Round; matrix can be homogenous, granular, fibrillar; density varies from electron-transparent to electron-dense; halo between the matrix and membrane
D	Somatostatin	250-350	Round; fine-granular matrix, moderately osmiophilic; enveloped in thin membrane
ECL	Histamine	400	Vesicular granules with eccentrical core, hard or varying in density; round, slightly elongated
N	Neurotensin	300	Round, homogeneous, with high electron density
K	Gastroinhibitory peptide	350	The shape varies from round to irregular, often droplet-like, electron-dense, homogeneous
L	Enteroglucagon	400	Round, slightly elongated, homogeneous, electron-dense
D1	Vasoactive intestinal peptide	150-170	Round, electron-dense, sometimes with a narrow light rim and thin membrane
P	Gastrin-releasing peptide (bombesin)	90-100	Round, homogeneous, electron-dense, surrounded with a light rim of different width and thin membrane
S	Secretin	200	The shape varies from round to irregular, homogeneous, electron-dense

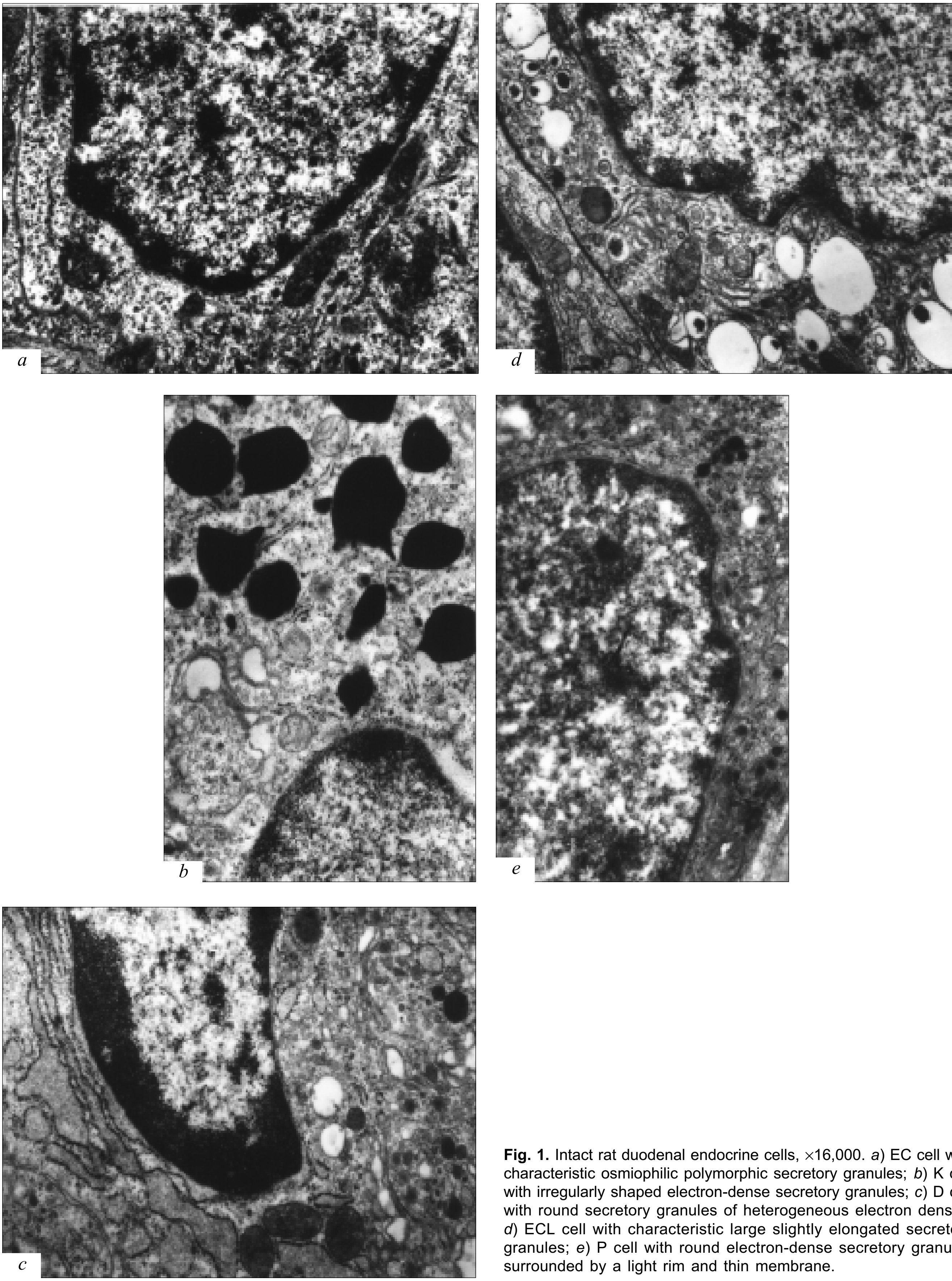
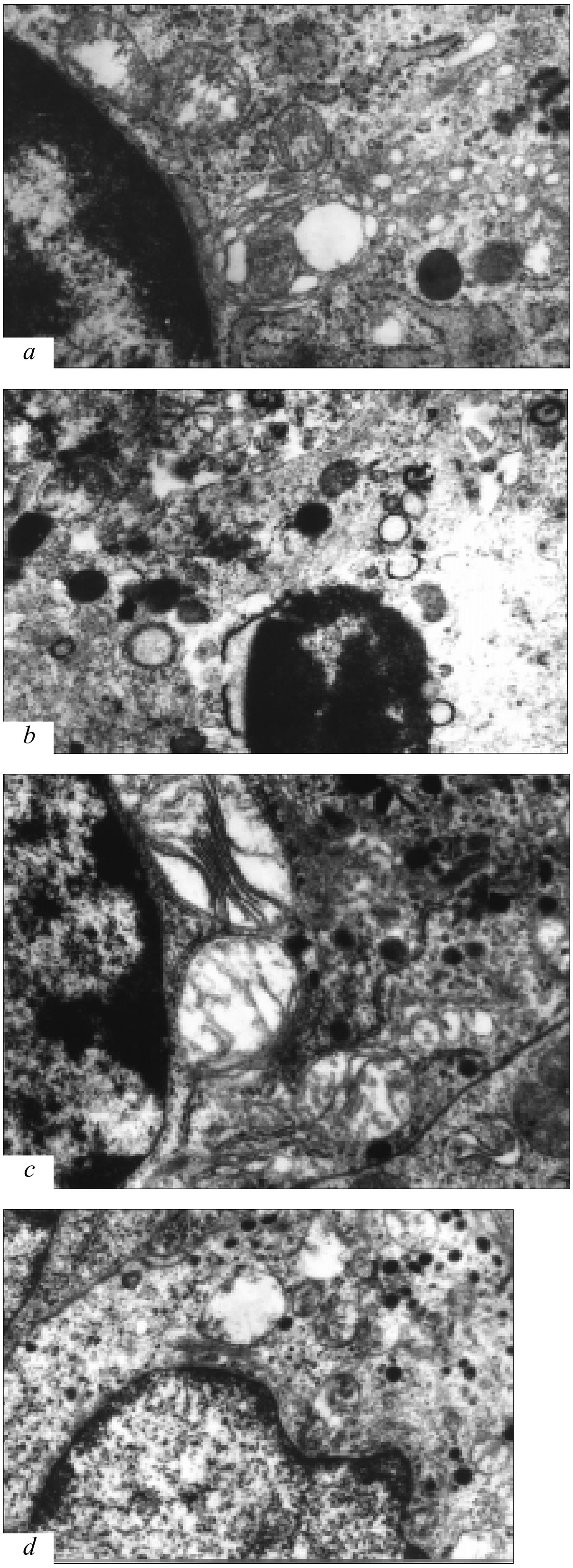


Fig. 1. Intact rat duodenal endocrine cells, ×16,000. *a*) EC cell with characteristic osmiophilic polymorphic secretory granules; *b*) K cell with irregularly shaped electron-dense secretory granules; *c*) D cell with round secretory granules of heterogeneous electron density; *d*) ECL cell with characteristic large slightly elongated secretory granules; *e*) P cell with round electron-dense secretory granules surrounded by a light rim and thin membrane.



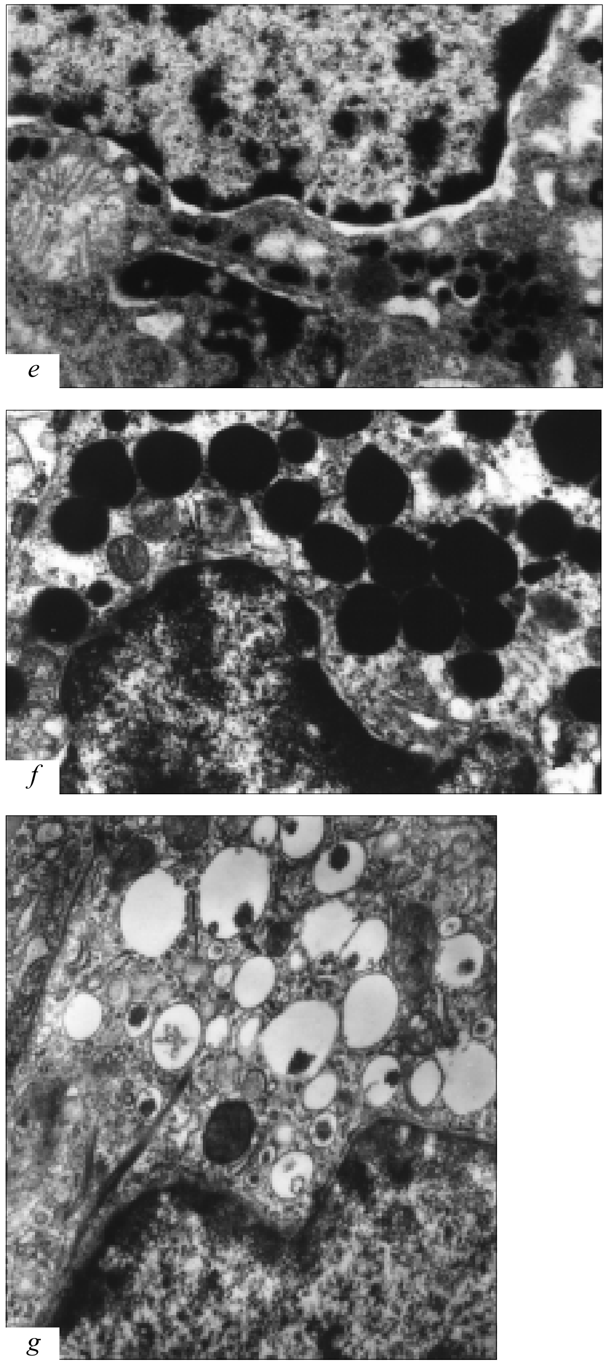


Fig. 2. Duodenal endocrine cells of rats exposed to prolonged irradiation in a total dose of 2.5 Gy, ×16,000. *a*) D cell: slight extension of Golgi complex, swelling of mitochondria with damaged cristae; *b*) D cell: nuclear pyknosis, destroyed limiting cell membrane and cell organelles; *c*) EC cell: edematous mitochondria with pronounced disorganization of cristae; *d*) P cell: formation of individual vacuoles resultant from destruction of mitochondria; *e*) EC cell: detached external karyolemma leaf, cytoplasm vacuolation, mitochondrial injuries; *f*) K cell: ultrastructure close to the control, numerous secretory granules of d ifferent size and shape; *g*) ECL cell: ultrastructural organization close to the control.

les. Rough and smooth endoplasmatic reticulum (EPR) had abundant cisterns in EC, G, D, L, and P cells and was poorly developed in other types of endocrine cells. Golgi complex was also clearly seen and showed signs of different activity depending on the stage of the functional cycle. Secretory granules of different size and shape were seen in the basal part of the cytoplasm under the nucleus, which largely correlated with the type of hormone produced by this or that endocrine cell (Fig. 1).

Study of the intact rat duodenum allowed us to identify 10 main types of endocrine cells located in the duodenal mucosa and to classify them by ultrastructural signs of secretory granules (Table 1).

In rats exposed to irradiation, detachment of the outer karyolemma leaflet, moderate vacuolation, edematous mitochondria with pronounced disorganization of cristae were seen in the cytoplasm of enterochromaffine (EC) cells (Fig. 2, *e*). At the same time, some EC cells had slight ultrastructural changes confined to injuries of enlarged mitochondria (Fig. 2, *c*). Abundant electron dense secretory granules were seen around the nucleus. EC cell secretion was realized either via poorly expressed degranulation (mainly in cells with pronounced injuries) or by exocytosis (in normal cellsm).

Cells producing somatostatin and enteroglucagon (D and L cells) were characterized by ultrastructural disorganization of different degree (Fig. 2, *a*). Some of these cells underwent necrosis, their cytoplasm consisted of edematous foci without organelles. The plasma membrane of these cells was destroyed, and interface between these cells and the adjacent epithelial cells was blurred (Fig. 2, *b*). Partial degranulation of individual D and L cells was observed.

Ultrastructural disorders of different degree were observed after irradiation in G and D1 cells producing gastrin and vasoactive intestinal peptide, respectively. Cells with manifest injuries had notably extended cisterns of the rough EPR and Golgi complex, clarified enlarged mitochondria with partially damaged cristae, which resulted in vacuolation of the cytoplasm. Many G and D1 cells had signs of degranulation.

The ultrastructure of neurotensin (N) and bombesin-producing (P) cells was characterized by good function close to the control, except some mitochondrial injuries. The cytoplasm contained numerous ribosomes, polysomes, vesicular components, and few secretory granules; degranulation was observed in P cells (Fig. 2, *d*).

No apparent impairment of cell structures (except mitochondria) were seen in ECL (Fig. 2, *g*), S and K cells (Fig. 2, *f*) producing histamine, secretin, and gastroinhibitory peptide, respectively. Numerous secretory granules were distributed in the cytoplasm; no manifest degranulation was observed in these cells.

Hence, postradiation examination revealed differences in the ultrastructure of different apudocyte types and even in different types of endocrine cells. There were cells with virtually intact ultrastructure and cells with pronounced impairments among EC, G, D, L, and D1 apudocytes. ECL, N, K, P, and S cells were characterized by virtually normal ultrastructure.

The lifespan of duodenal endocrine cells varies from 100 to 124 h [7], and the duration of cell migration from the crypt to the apex of the villus in rats is 48 h [12]. Hence, after prolonged irradiation we examined the populations of apudocytes with ultrastructural genetic injuries caused by ionizing radiation in precursor cells and at the same time exposed to prolonged irradiation.

Almost all endocrine cells, except ECL and N cells, had signs of degranulation. In addition, secretory granule exocytosis was seen in some cells, which was not observed in cells exposed to irradiation in lethal doses [9], and we therefore conclude that prolonged irradiation under these conditions did not impair the transporting mechanisms responsible for secretory granule transfer to the basal membrane.

On the other hand, intracellular reparation processes were activated, which was due to more intensive metabolism and organoid compensation. The number of lysosomes, free ribosomes and polysomes increased, larger mitochondria with more dense matrix formed, the nucleolar system increased, and the rough EPR tubules with electron-dense contents dilated, which attested to more intense biosynthesis in the cells and indicated that the synthesized products were intended for release into the extracellular space [4].

Results of electron-microscopic studies confirmed different reactions of apudocytes of different types to irradiation, which seems to reflect their different contribution to the realization of irradiation effects. Apudocytes reacted to irradiation by nonspecific changes in common organelles; along with common forms of secretion (molecular extrusion and exocytosis) [8], apudocytes released the secretory product by lysis in numerous vacuoles (degranulation).

REFERENCES

1. L. I. Aruin, I. V. Zverkov, and V. A. Vinogradov, *Klin. Med.*, No. 6, 22-31 (1987).
2. E. N. Goncharenko and Yu. B. Kudryashov, *Chemical Protection from Radiation Injury* [in Russian], Moscow (1985), pp. 58-65.
3. A. M. Kuzin, *The Idea of Radiation Hormesis in the Atomic Era* [in Russian], Moscow (1995), pp. 12-17.

4. R. P. Manokhina, *Regulatory Peptides and Biogenic Amines: Radiobiological and Oncoradiological Aspects* [in Russian], Obninsk (1992), pp. 138-143.
5. V. G. Ovakimov, G. M. Airapetyan, V. N. Ivanov, *et al.*, *Radiobiologiya*, **10**, No. 4, 561-565 (1970).
6. M. A. Osadchuk, V. F. Kirichuk, and I. M. Kvetnoi, *Diffuse Neuroendocrine System: General Biological and Gastroenterological Aspects* [in Russian], Saratov (1996), pp. 26-46.
7. O. A. Smirnova, *Radiobiologiya*, **28**, No. 6, 817-821 (1988).
8. A. M. Ugolev, *Enterine (Intestinal Hormone) System* [in Russian], Leningrad (1978), pp. 51-72.
9. V. V. Yuzhakov, I. M. Kvetnoi, E. D. Ulitina, and V. P. Lepekhin, *Regulatory Peptides and Biogenic Amines: Radiobiological and Oncoradiological Aspects* [in Russian], Obninsk (1992), pp. 67-82.
10. V. V. Yuzhakov, N. D. Yakovleva, I. M. Kvetnoi, and E. D. Ulitina, *Med. Radiol.*, **39**, No. 3, 39-45 (1994).
11. V. V. Yaglov and G. A. Lomonosov, *Uspekhi Sovrem. Biol.*, No. 2, 264-276 (1985).
12. H. Cheng and C. P. Leblond, *Am. J. Anat.*, **141**, 461 (1974).
13. T. Fujita, *Igaku Shoin*, Tokyo (1973).
14. D. Grube, *Anat. Embryol.*, **175**, 151-162 (1986).