LITERATURE CITED

1. W. Hodgson, P. K. Poddar, E. J. Mencer, et al., Am. J. Gastroenterol., **72**, 133 (1979).
2. C. W. Putnam, Surg. Gynec. Obstet., **157**, 475 (1983),
3. K. Tranberg, P. Rigotti, K. A. Brackett, et al., Am. J. Surg., **151**, 368 (1986).

MORPHOLOGICAL CHARACTERISTICS OF INTESTINAL ENDOCRINE CELLS IN MICE

T. G. Barkhina, Yu. G. Parkhomenko, I. M. Salakhov, and O. E. Bogatyreva

UDC 611.34.018.72 611.43+612.33.014.2.01

KEY WORDS: small and large intestine, endocrine cells, cytotopography, ultrastructure.

Much evidence has now been obtained to show that endocrine cells are present in nonendocrine organs belonging to the extensive APUD-system, or in other words, in the composition of the diffuse endocrine system [2-6]. According to the modern classification the following types of these cells are distinguished: A, B, D, D_1, EC_1, EC_2, ECII, ECL, G, I, K, L, N, P, PP, S, X, IG, TG, MO, YY. Thanks to the widespread use of highly sensitive immunocytochemical methods combined with electron-microscopic analysis, more than 20 types of these cells, containing biogenic amines and peptides, and located in the mucous membrane of organs of the gastrointestinal tract, have been identified in different species of animals [3, 7]. However, there has been no attempt at morphological identification of these cells of the mouse intestine used in the different experiments, and that was accordingly the aim of the investigation described below.

EXPERIMENTAL METHOD

Pieces of small and large intestine from BALB/c mice were fixed in a 10% solution of neutral formalin and embedded in paraffin wax. Histologic sections 5-7 μ thick for quantitative analysis of endocrine cells were stained with silver nitrate by Grimelius' method and the cells in 1 mm^2 of intestinal section were counted. In this way it was possible to determine that the cells belonged to the APUD-system, although it was not possible to identify precisely their types. This defect was largely compensated by electron-microscopic analysis, which enables the types of endocrine cells to be determined not only by the shape and size of the granules, but also with respect to various other ultrastructural data. Student's test was used for statistical analysis. Material for ultrastructural study was fixed in a mixture of 1% glutaraldehyde and 4% formaldehyde in 0.05 M cacodylate buffer, and then in 1% osmium tetroxide solution, and then dehydrated and embedded in Vestopal. Ultrathin sections were stained with lead citrate and examined in the JEM-100C electron microscope.

EXPERIMENTAL RESULTS

Endocrine cells (EC) were found to be most numerous in the duodenal mucosa The number of EC in 1 mm^2 section of the small intestine was: in the duodenum 243 ± 15, jejunum 175 ± 16, ileum 87 ± 7; in the large intestine the distribution was: in the ascending colon 33 ± 4, transverse 39 ± 6, descending 24.4, and in the rectum 53 ± 1 in 1 mm^2. There was a tendency for the number of EC to diminish from the proximal to the distal parts of the intestine.

Laboratory of Infectious Pathology, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. K. Permyakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 3, pp. 314-317, March, 1991. Original article submitted March 22, 1990.

TABLE 1. Distribution of Different Types of Endocrine Cells in Mouse Intestine and Their Characteristics Relative to Granules

Type of cells	Size of granules, nm	Location				Products secreted
		small intestine		large intestine		
		duodenum	jejunum, ileum	colon	rectum	
EC_1	290±49	+	+	+	+	Serotonin, substance P
EC_2	330±63	+	+	—	—	Serotonin, motilin
EC_n	190±38	+	+	—	—	Serotonin
G	280±57	+	—	—	—	Gastrin. peptides
D	320±74	+	+	—	—	Somatostatin, Met-enkephalin
D_1	150±36	+	+	+	+	VIP
L	380±82	+	+	+	+	GLI, glycentin
X	300±25	+	+	+	+	Not precisely established
S	180±31	+	+	—	—	Secretin
I	240±17	+	+	—	—	Cholecystokinin–pancreozymin
K	350±69	+	+	—	—	GIP
N	310±48	+	+	—	—	Neurotensin
PP	170±19	+	+	+	+	Pancreatic polypeptides
P	120±23	+	+	—	+	Bombesin
Mo	220±46	+	+	—	—	Motilin

It will be clear from Table 1 that 15 types of EC were found in the duodenum of the mice, 14 in the jejunum and ileum, five in the colon, and six in the rectum.

Endocrinocytes of the EC type (EC_1, EC_2, ECII) are prismatic in shape, small in size, and contain granules of secretion mainly in the basal part away from the nuclei while the nucleus, itself is in the middle part of the cell; the granules are characteristically bean shaped. The types of these cells differ from one another only in the size of the granules: the diameter of the granules in EC_1 cells is 290 ± 49 nm, EC_2 330 ± 63, and ECII 190 ± 38 nm; in the mature state these granules lack the pale nimbus (Fig. 1). In these cells the rough endoplasmic reticulum (RER) is poorly developed, and the smooth endoplasmic reticulum (SER) is found extremely rarely; elements of the Golgi complex (GC) are sufficiently well developed but few in number. The topography of the EC cells is sufficiently clear both in villi of the small intestine and in crypts of the small and large intestine.

Cells of D-type are large, round or oval in shape, with large round granules, 320 ± 74 nm in diameter, with pale and indistinct outlines, with granular contents, usually without a nimbus, and only rarely with a narrow pale interval near the paragranular membrane. Characteristically the RER, SER, and GC are quite well developed in these cells (Fig. 2a). Cells of this type were quite uniformly distributed along the villus and crypt.

Cells of D_1 type have a round or oval shape, with small (150 ± 36 nm), homogeneous, round secretory granules of average osmiophilia, and with a nimbus (Fig 2b). The cytoplasm of these cells is quite pale and rich in mitochondria and cisterns of the RER; in the small intestine they are mainly distributed in the basal part of the villi and crypts, whereas in the large intestine they are mainly found at the bottom of the crypts.

Cells of L-type are the largest endocrinocytes with large (380 ± 82 nm), dark, round, homogeneous granules, distributed throughout the cytoplasm, and with a nimbus which is more marked in immature granules (Fig. 2c). The quite large number of mitochondria and elements of the RER and SER in these cells is noteworthy; components of GC are located in the supranuclear and paranuclear zone. Their topography is clearly distinguishable in the basal part of the villi of the small intestine and at the bottom of the crypts of the large intestine, more or less the same in all parts, but a little more numerous in the rectum.

Cells of the X-type are large in size and have a pale cytoplasm; their granules are heterogenous, but more often round or oval in shape, they measure 300 ± 25 nm, are of average osmiophilia, and have a pale nimbus (Fig. 2d), The organelles in these cells are poorly developed, and the nucleus is large and located in the middle part of the cell. X-cells were found in all parts of the intestine, but mainly in the crypts.

Cells of the G-type are quite large, round in shape, with round granules 280 ± 57 nm in diameter, and with a more or less visible nimbus; the dark osmiophilic contents of the granule are round, oval, or irregular in shape. The organelles of these cells are more highly developed than those of other types of endocrinocytes. Identification of G-cells is greatly facilitated by the presence of microfilaments in the paranuclear zone, concentrated into a bundle or distributed in the form of separate filaments around the nucleus (Fig. 2e). We found these cells only in the duodenum, in both villi and crypts.

Cells of the S type are prismatic or oval in shape, small in size, with tiny homogeneous round granules, but without a nimbus, and 180 ± 31 nm in diameter (Fig. 2f). The cytoplasm of these cells is of average electron density and contains a few organelles; they are located in the basal part of the villi and crypts of the small intestine.

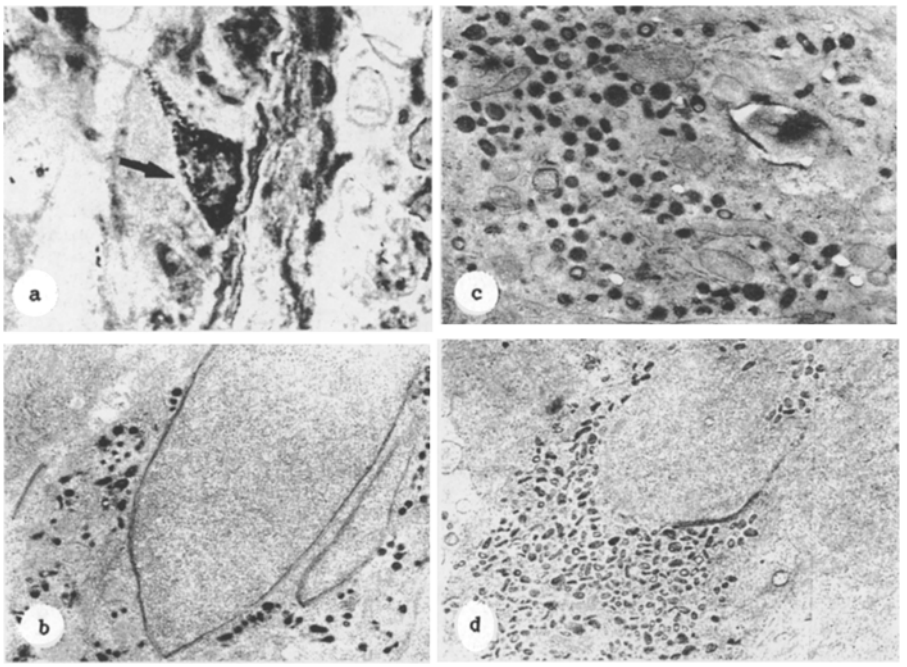

Fig. 1. Different types of EC in mouse intestine: a) EC in duodenal crypt, Grimelius' stain, 400×; b) EC_1 cell in crypt of rectum 10,000×; c) EC_2 cell in villus of duodenum. 10,000×; d) EC cell in duodenal crypt 8000×.

Cells of the Mo-type are round or spherical in shape, different in shape from the other types of cells; they have a small nucleus, numerous granules of secretion, and a fairly large number of organelles; the bounding plasmalemma of these cells consists of multiple interdigitations. The granules of these cells are of average size (220 ± 46 nm), round in shape, nearly always with a nimbus, and uniformly distributed throughout the cytoplasm (Fig. 3a). Mo-cells were found in the villi and crypts of the small intestine only.

Cells of the I-type have cytoplasm of average electron density, and homogeneous, more often round, osmiophilic granules 240 ± 17 nm in diameter mainly without a nimbus (Fig. 3b). Many organelles, especially mitochondria, are observed in these cells. Their topography is equally well defined in both villi and crypts but only of the small intestine.

Cells of K-type have characteristic features distinguishing them from cells of the other types. They contain distinctive, irregularly shaped lumpy granules with average electron density, with granular contents, without a nimbus, and 350 ± 69 nm in diameter (Fig. 3c). These cells also are quite large, round or oval in shape, and have pale cytoplasm, a large nucleus, and a few organelles. Many K-cells are seen in the duodenum and proximal parts of the jejunum, fewer in the ileum; they are equally represented in the villi and crypts.

Cells of the N-type are of average size, with pale cytoplasm, and with homogeneous round osmiophilic granules measuring 310 ± 48 nm; in the immature granules a pale nimbus is present, but not always clearly outlined (Fig. 3d). The cell nucleus is located in the middle or basal part of the cytoplasm, and there are few organelles. The distribution of the cells in the villus and crypt is similar.

Cells of the PP-type are small, which applies both to the cells and to their granules (170 ± 19 nm). Mature granules are homogeneous and osmiophilic, whereas immature are pale, with granular contents, and oval or semilunar in shape (Fig. 3e); not all granules have a nimbus, which if present is narrow. These cells are located in the basal part of the villi and crypts of all parts of the intestine.

Cells of the P type are larger than PP-cells and contain the smallest granules — these are round, osmiophilic, without a nimbus, 120 ± 23 nm in diameter, and scattered throughout the cytoplasm around the large nucleus (Fig. 3f). The cells are of average electron density and contain a few organelles, located in the basal parts of the villi and crypts of the small intestine, but in the large intestine only in the rectum, in very small numbers at the bottom of the crypts.

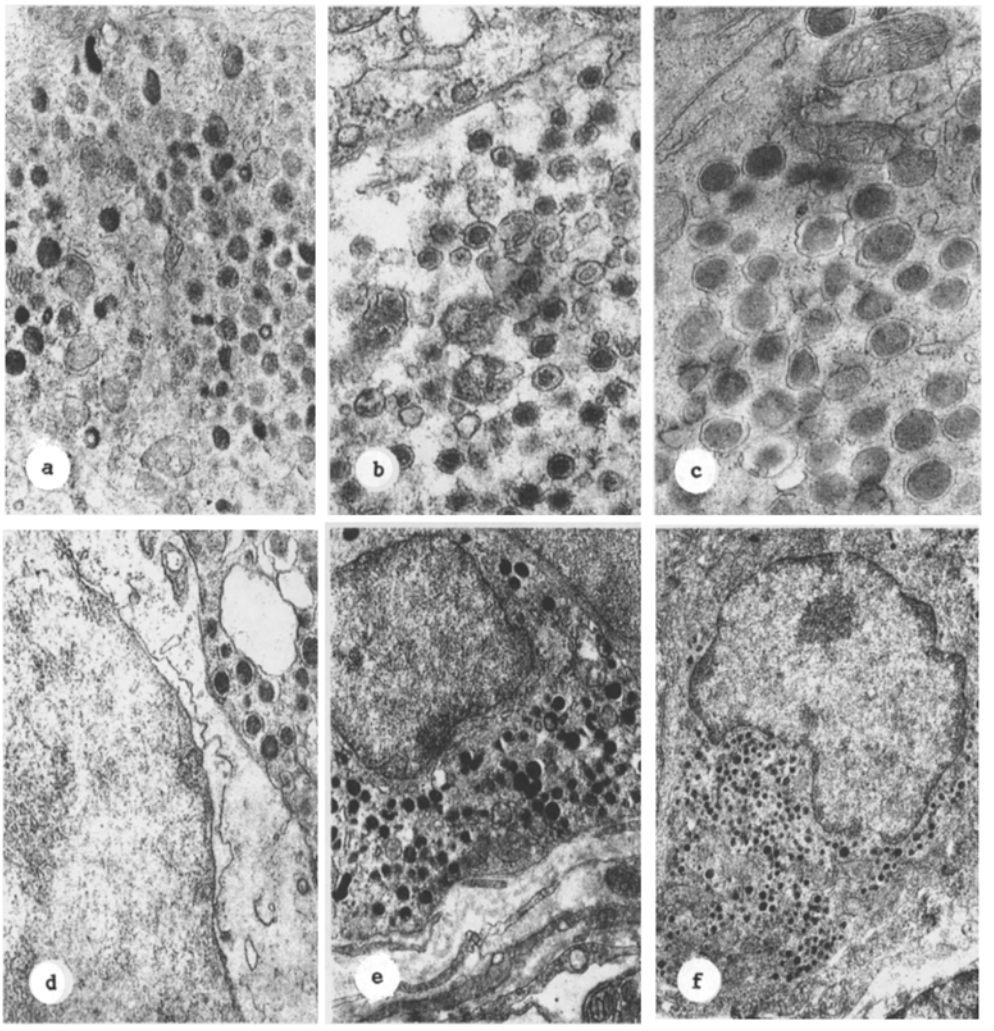

Fig. 2. Various types of endocrine cells in mouse intestine. a) D-cell in duodenal villus, 14,000×; b) D_1 cell in crypt of jejunum. 40,000×; c) L-cell in crypt of jejunum. 30,000×; d) X-cell in crypt of jejunum. 20,000×; e) G-cell in duodenal villus. 10,000×; f) S-cell in duodenal crypt. 10,000×.

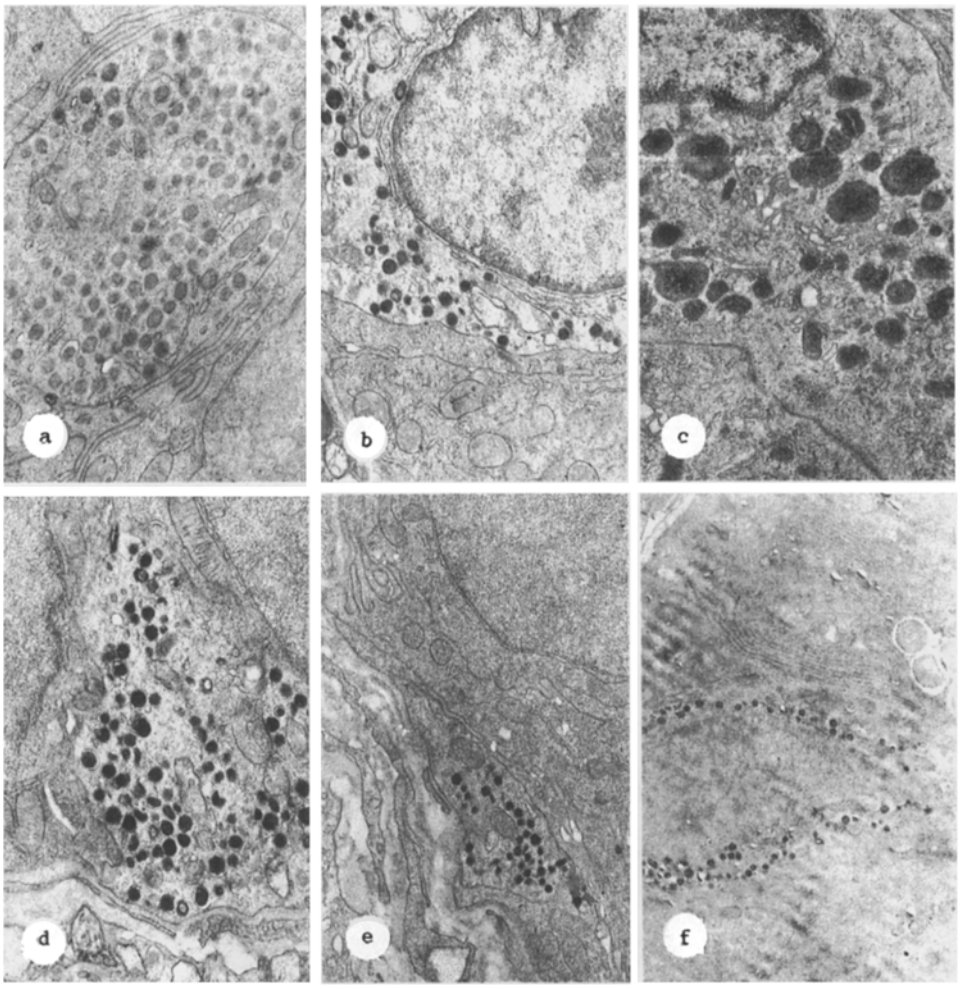

Fig. 3. Different types of endocrine cells of mouse intestine: a) Mo-cell in villus of jejunum. 14,000×; b) I-cell in duodenal villus. 14,000×; c) K-cell in duodenal crypt. 30,000×; d) N-cell in duodenal crypt. 20,000×; e) PP cell in crypt of rectum. 14,000×; f) P-cell in duodenal crypt. 20,000×.

The investigation thus showed 15 types of endocrinocytes in different parts of the intestine of BALB/c mice. Some types of cells and, in particular, G-cells, are found only in the duodenum in mice, just as in other species of animals, and also in man [7-9]. The presence of Mo-cells predominantly in the small intestine is observed not only in mice, but also in man and the dog [10].

The experimental study of different types of endocrinocytes and the demonstration of their characteristic species-specific features in animals and, in particular in mice provides a convenient and economically advantageous model with which to study acute intestinal infections [1], which is essential for the understanding of the role and importance of the APUD-system in the mechanisms maintaining homeostasis under pathological conditions.

LITERATURE CITED

1. A. P. Avtsyn, Yu. G. Parkhomenko, and I. N. Emel'yanenko, Byull. Éksp Biol. Med., No. 9, 371 (1985).
2. S. A. Blinova, Arkh. Anat., No. 2, 55 (1989).
3. N. T. Raikhlin, G. Makhnik, and D. Katenkamp, The APUD-System: Progress and Prospects of Study in Oncoradiology and Pathology [in Russian], Obninsk (1988), pp. 5-19.
4. V. V. Yaglov, Arkh. Anat., No. 1, 14 (1989).
5. M. L. Cristina, T. Lehy, P. Zeitoun, and F. Dufougeray, Gastroenterology, **75**, No. 1, 20 (1978).
6. D. Grube, Anat. Embriol., **175**, No. 2, 151 (1986).

7. D. Grube and W. G. Forssman, Horm. Metab. Res., **11**, 589 (1979).
8. E. Solcia, W. Creutzfeldt, and S. Falkmer, Cellular Basis of Chemical Messengers in the Digestive System, London (1981), pp. 159-165.
9. F. Sundler and R. Hakanson, Evolution of Tumor Pathology of the Neuroendocrine System, Amsterdam (1984), pp. 111-135.
10. L. Usellini, A. M. J. Bucham, and J. M. Polak, Histochemistry, **81**, No. 4, 363 (1984).

EFFECT OF BONE MARROW TRANSPLANTATION ON RESTORATION OF PANCREATIC MORPHOLOGY AND FUNCTION IN IRRADIATED RECIPIENTS

A. A. Tsutsaeva, T. A. Glushko, L. E. Khatilova, and G. S. Tupchienko

UDC 612.45.014.1:612.479-089.845

KEY WORDS: lethal irradiation, transplantation, native and freeze-dried bone marrow, pancreas.

It has now been established that cells either in the cell cycle or in a functionally active state exhibit increased radiosensitivity. An increased number of these cells is observed not only in the hematopoietic, immunocompetent, and reproductive systems, but also in endocrine tissues. Structural and functional changes in the endocrine organs developing under the influence of irradiation and disturbances of neuroendocrine regulation arising under those conditions may play an essential role in the development of secondary pathological processes in the irradiated organism [1, 3]. In particular, many investigators [2, 3, 5, 7, 8] have observed considerable changes in the pancreas in an irradiated animal. Considering the important role of insulin in the regulation of metabolism, and also the absence of any sufficiently profound studies of the structural and functional state of the pancreas in lethally irradiated recipients, protected by bone marrow, we decided to study the state of the pancreas in lethally irradiated recipients at different stages after transplantation of native and freeze-dried hematopoietic cells.

EXPERIMENTAL METHOD

Experiments were carried out on 250 inbred (CBA × C57BL)F_1 hybrid male mice aged 2 months. The animals were divided into four groups. Group 1 consisted of lethally irradiated animals, group 2 of lethally irradiated animals receiving native syngeneic bone marrow, the animals of group 3, after lethal irradiation, were given an injection of syngeneic freeze-dried bone marrow, and group 4 was the control (intact animals). Irradiation was given on the RUM-17 apparatus. The conditions of irradiation were: dose rate 39.5 R/min, U = 200 kV, I = 10 A, filter: 0.5 mm Cu + 1 mm Al. The bone marrow was taken from the femora of mice and preserved by the method in [6]. Syngeneic bone marrow obtained from donors, both native and freeze-dried, was injected intravenously in a dose of $1 \cdot 10^7$ cells/ml. The animals were irradiated and bone marrow transplanted at the same time, between 9 a.m. and 12 noon. Modern methods of determination of the blood insulin level do not always give adequate information, and accordingly in practical work, to assess the function of the endocrine part of the pancreas, several parameters of carbohydrate metabolism are studied, and in particular, glucose. Sugar was determined by the method in [4], 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 40, 60, and 90 days after irradiation and bone marrow transplantation. The histologic structure of the pancreas was studied 1, 3, 7, 10, 30, 40, 60, and 90 days after irradiation and transplantation of the bone marrow. Paraffin

Department of Cryoimmunology, Institute for Problems in Cryobiology and Cryomedicine, Academy of Sciences of the Ukrainian SSR, Khar'kov (Presented by Academician of the Academy of Medical Sciences of the USSR L. T. Malaya.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 3, pp. 318-320, March, 1991. Original article submitted July 17, 1990.