Scanning Microscopy

Volume 10 | Number 1 Article 21

1-5-1996

Diffuse Neuroendocrine System: Structural and Functional Effects of Radiation Injury to Amine Precursor Uptake and Decarboxylation (APUD) Cells

I. M. Kvetnoy
Russian Academy of Medical Science

V. V. Yuzhakov
Russian Academy of Medical Science

A. O. Molotkov
Russian Academy of Medical Science

L. N. Bandurko
Russian Academy of Medical Science

R. A. Brodsky
Russian Academy of Medical Science

Diffuse Neuroendocrine System: Structural and Functional Effects of Radiation Injury to Amine Precursor Uptake and Decarboxylation (APUD) Cells

Authors

I. M. Kvetnoy, V. V. Yuzhakov, A. O. Molotkov, L. N. Bandurko, R. A. Brodsky, and N. D. Yakovleva

Scanning Microscopy, Vol. 10, No. 1, 1996 (pages 261-278) 1051-6794/96$5.00+.25
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

DIFFUSE NEUROENDOCRINE SYSTEM: STRUCTURAL AND FUNCTIONAL EFFECTS OF RADIATION INJURY TO AMINE PRECURSOR UPTAKE AND DECARBOXYLATION (APUD) CELLS

I.M. Kvetnoy*, V.V. Yuzhakov, A.O. Molotkov, L.N. Bandurko, R.A. Brodsky and N.D. Yakovleva

Laboratory of Experimental Pathomorphology,
Medical Radiological Research Center (MRRC) of Russian Academy of Medical Science (RAMS), Obninsk, Russia

(Received for publication February 13, 1995 and in revised form January 5, 1996)

Abstract

The paper presents a review of the results obtained by the authors on the study of external (gamma) and internal (I-131) radiation effects on the functional morphology and linkage of the diffuse neuroendocrine system (DNES) and amine precursor uptake and decarboxylation (APUD) cells of the stomach and duodenum. The investigations performed enabled us to determine that the morphological changes noted in APUD cells had a dose and time dependency. The present study supports the point of view that the radiation initiates serotonin release from APUD cells, which appears to initiate the mechanism of early postirradiation dysfunctions of the gastrointestinal tract and the subsequent adaptive response of DNES. Analysis of our results, together with a review of the literature, indicates that APUD cells actively participate both in pathogenesis of radiation injury and development of organ and tissue radiosensitivity.

Key Words: Diffuse neuroendocrine system, amine precursor uptake and decarboxylation (APUD) cells, enterochromaffin cells, gut, radiation injury.

*Address for correspondence:
Igor M. Kvetnoy
Laboratory of Experimental Pathomorphology,
Medical Radiological Research Center,
4 Korolev Street,
249020 Obninsk, Russia
Telephone number: (08439) 264-48
FAX number: (095) 956-1440

Introduction

Analysis of systems that control and integrate adaptation processes associated with ionizing radiation stress is of great interest. One of these systems appears to be the diffuse neuroendocrine system (DNES). The current concept is that DNES appears to functionally integrate chemically similar substances, such as neurotransmitters acting as information transfer agents within the nervous system and hormones within the endocrine system that act either locally or distally [30]. Current morpho-functional views on DNES are based on the amine precursor uptake and decarboxylation-concept suggested and developed in detail by A. Pearse, an English histochemist and pathologist [26, 27, 28, 29], as well as on the fundamental work of B. Falck at Lund University (Sweden) in which the "formaldehyde histofluorescence technique for biogenic amines" was developed and used to define several peripheral endocrine systems, based on the identification of amine-accumulating cells following precursor loading [8, 9, 24, 34, 35].

In 1966-69, Pearse suggested that a specialized, highly organized cell system exists in organisms whose main feature was the capability of component cells to produce peptide hormones and biogenic amines. The concept was based on the extensive series of experiments on distinguishing endocrine cells in different organs by a thorough cytochemical and ultrastructural identification of endocrine cell-generated products. Different types of cells, widely dispersed in the organism, have a common ability to take up and decarboxylate monoamine precursors, thus producing biogenic amines. This ability accounts for the term "APUD," an acronym for "amine precursor uptake and decarboxylation" used by Pearse to designate the cell series.

Recent investigations have shown that biogenic amines and active peptides, i.e., regulatory peptides, are present both in neurons of the central and peripheral nervous system and in APUD cells located in different organs [12, 31]. The data on the identification and location of monoamines and identical regulatory peptides both in neural and endocrine cells of different organs

suggests that these elements are incorporated into a common, but diffuse regulating system, namely the DNES. Located in practically all organs and producing vitally important biological active substances, DNES cells fulfill the role of tissue regulators of homeostasis, controlling a multitude of physiological processes via neurocrine, endocrine and paracrine mechanisms of messenger molecule-effects on target cells.

The analysis of the biological features of many physiologically active substances produced by DNES cells suggests an important role of this system in the mechanism of radiation injury. On the one hand, regulatory peptides and biogenic amines are able to serve as radiomodifiers, on the other hand, these substances participate, to a certain extent, in pathogenesis of those disorders which are described as typical ones for the effects of even low and sublethal doses of ionizing radiation, viz., changes in the vascular tonus and permeability; vegetative alterations in arterial pressure rates, contraction frequency and breath rhythm; desynchronization of biological rhythms; abnormalities of mediators exchange and transmitter reception by neurons; abnormalities of the proliferative activity and others.

Having the above features, regulatory peptides and biogenic amines are able to influence various pathological processes resulting from radiation injury, via either the direct potentiation, or reduction, of the radiation effect, or indirectly via their participation in the mechanisms mentioned above.

Thus, the study of the role and significance of the diffuse neuroendocrine system and, in particular, its endocrine part, APUD cells, in pathogenesis of radiation injury lends a new perspective in the interpretation of endogenous mechanisms of ionizing radiation-induced responses of several organ and tissue systems, as well as the organism as a whole.

Recent investigations indicate that together with DNES cells, some non-endocrine cells, i.e., natural killer cells, mast cells, eosinophilic leukocytes and endothelial cells, are able to synthesize, or accumulate, biogenic amines and active peptides [20]. The study of the functional morphology and behavior of these cells in malignant tumors suggests their direct participation in the endogenous mechanisms of carcinogenesis regulation and ionizing radiation effect on tumor cells. This fact opens new prospects for hormonal modification of antitumor radiation therapy.

Many investigations, papers and reviews [1, 2, 5, 11, 14, 15, 16, 21, 22, 33, 36, 41, 45] are dedicated to the biological effect of ionizing radiation on healthy tissues and neoplasms, molecular and cellular targets of radiation injury, radiation histopathology of the nervous and endocrine systems, hormonal dysfunctions, late effects of radiation injuries both due to external sources and incorporated radioactive substances. By contrast, there are few reports on DNES responses following radiation exposure. This is most unfortunate, because the induced responses of the DNES, either as a whole or from its composite cell types, could well account for the often noted, varied and disorganized functions of the organism as a whole.

The purpose of this paper, therefore, is to present a summary of results of long-term research carried out at our laboratory on the role of the DNES in modulating radiation injury. It should be noted, however, that even in a review paper, it is impossible to summarize all material obtained by our team, relative to the influence of ionizing radiation on the functional morphology of APUD cells of different endocrine and non-endocrine organs. Taking into account the fact that most APUD cells were located in the gastro-intestinal tract, we considered it prudent to focus on gut endocrine cells, with the special relevance to enterochromaffin (EC) cells, as well as to assess the possible significance of the presence of biogenic amines and peptide hormones in endocrine and non-endocrine cells for oncoradiobiology.

We lay no claim to the complete review of all results available in the literature and making some proposals, sometimes rather disputable, we would like to call attention to some features of the pathological development of enteroendocrine cells following exposure to ionizing radiation.

Modeling of Radiation Effects, Methods of Studies and Terminology

DNES injury under external and internal irradiation was examined and modelled using both C57Bl/6 mice (F1 hybrids; CBA x C57Bl/6 cross) and Wistar rats. Male F1 mice were exposed to single whole-body doses up to 20 Gy of gamma-rays from a Co-60 source, at a dose rate of 2.25 Gy/min. Internal irradiation effects were achieved by oral I-131 administration at a dose level of 1.0 μCi per gram of body weight (b.w.). Female Wistar rats were used in these studies.

Average cumulative radiation doses and dynamics of their accumulation following I-131 introduction were calculated under radiometry data for individual organs and the whole body according to the automatic system of dosimetric control [38] developed at MRRC RAMS (Obninsk, Russia). The contribution of the entire spectrum of radionuclide irradiation to the absorbed dose was taken into account. To calculate "probable absorbed shares" of energy in organs and tissues an animal phantom for dosimetric calculations was used. The universal dose functions of point sources of electron, quantum and β-irradiation were applied. The calculations were performed by the method of superposition of dose functions

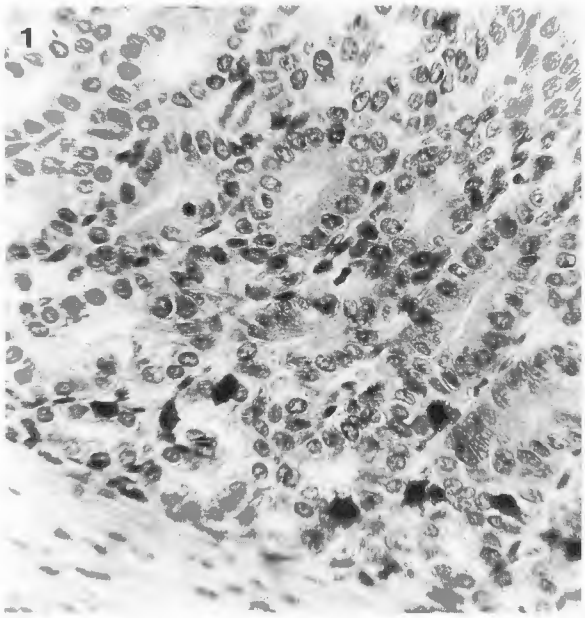


Figure 1. Pyloric mucosa of the control mouse showing serotonin-containing EC cells in the basal areas of the glands; photo width (P.W.) = 275 μm. Immunoperoxidase avidin-biotin method for the localization of serotonin in Bouin's-fixed, paraffin-embedded tissue; polyclonal rabbit antibody to serotonin (Dianova); SINtm universal anti-rabbit kit (Sigma diagnostics); 3-amino-9-ethylcarbazole, haematoxylin.

---

of point sources. The description of the procedure, parameters and gamma-irradiation doses, calculation of the absorbed doses and dynamics of their accumulation in internal irradiation as well as animal series, details of experiments, and preparation of the material for histological investigations were published previously [45].

To study the structural and functional organization of DNES at a given radiation exposure level, a complex approach based on a stage analysis of APUD cell population was used [19]. We used general silver staining methods in the study of endocrine cells [10], and specific immunocytochemistry methods for the detection of select types of regulatory peptides and biogenic amines [39, 40]. For the latter, we applied immunoperoxidase techniques (PAP and ABC) using polyclonal rabbit's antibodies to serotonin (Dianova, Hamburg, Germany) (Fig. 1), melatonin (CIDtech Research Inc., Mississauga, Ontario, Canada), beta-endorphin, insulin, glucagon, somatostatin (all Dako, Glostrup, Denmark) and kits for immunocytochemical analysis {Amersham (Slough, U.K.), Sigma (St. Louis, MO), Dianova}.

The various radioautographic and electron microscopic techniques applied were performed as described previously [43].

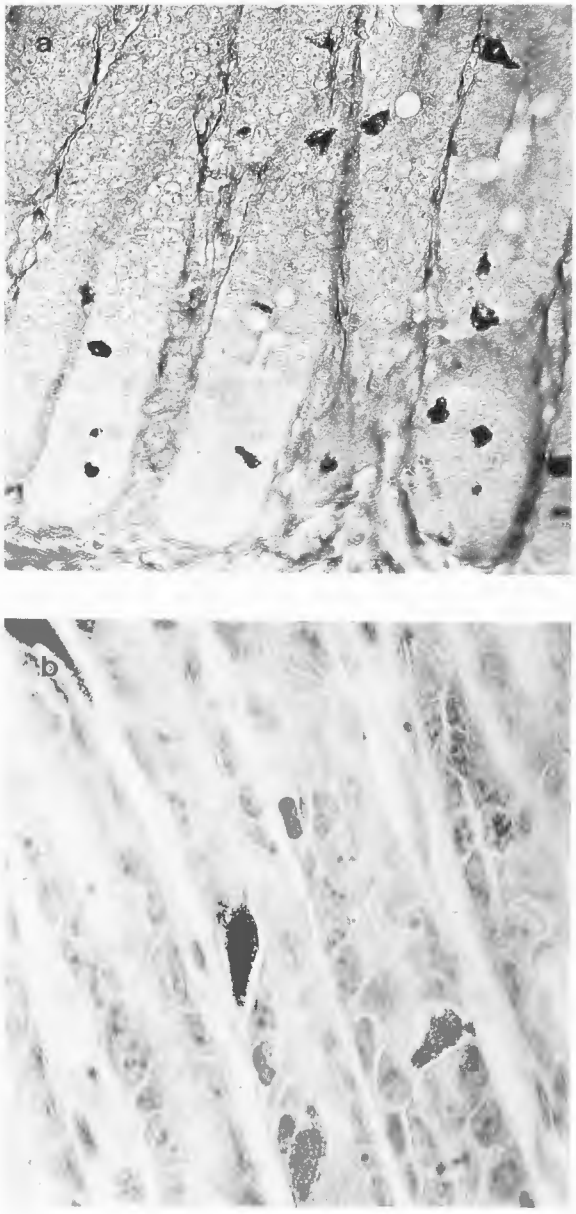


Figure 2. Silver stains in the study of endocrine cells. (a) Argyrophil cells in the mucosa of duodenum of a control rat in Bouin's-fixed, paraffin-embedded tissue. Grimelius stain; P.W. = 282 μm. (b) Argentaffin cells in the duodenal mucosa of a control rat in 4% buffered formaldehyde-fixed, paraffin-embedded tissue. Masson-Hamperl stain; P.W. = 121 μm.

For quantitative studies each control and experimental group consisted of 5-7 animals. Rats were killed with Nembutal (Pharma Fact., St. Petersburg, Russia) anesthesia. Mice were sacrificed by rapid cervical dislocation. Tissues were immediately removed, fixed both in Bouin's fluid with 5% acetic acid and 4% buffered

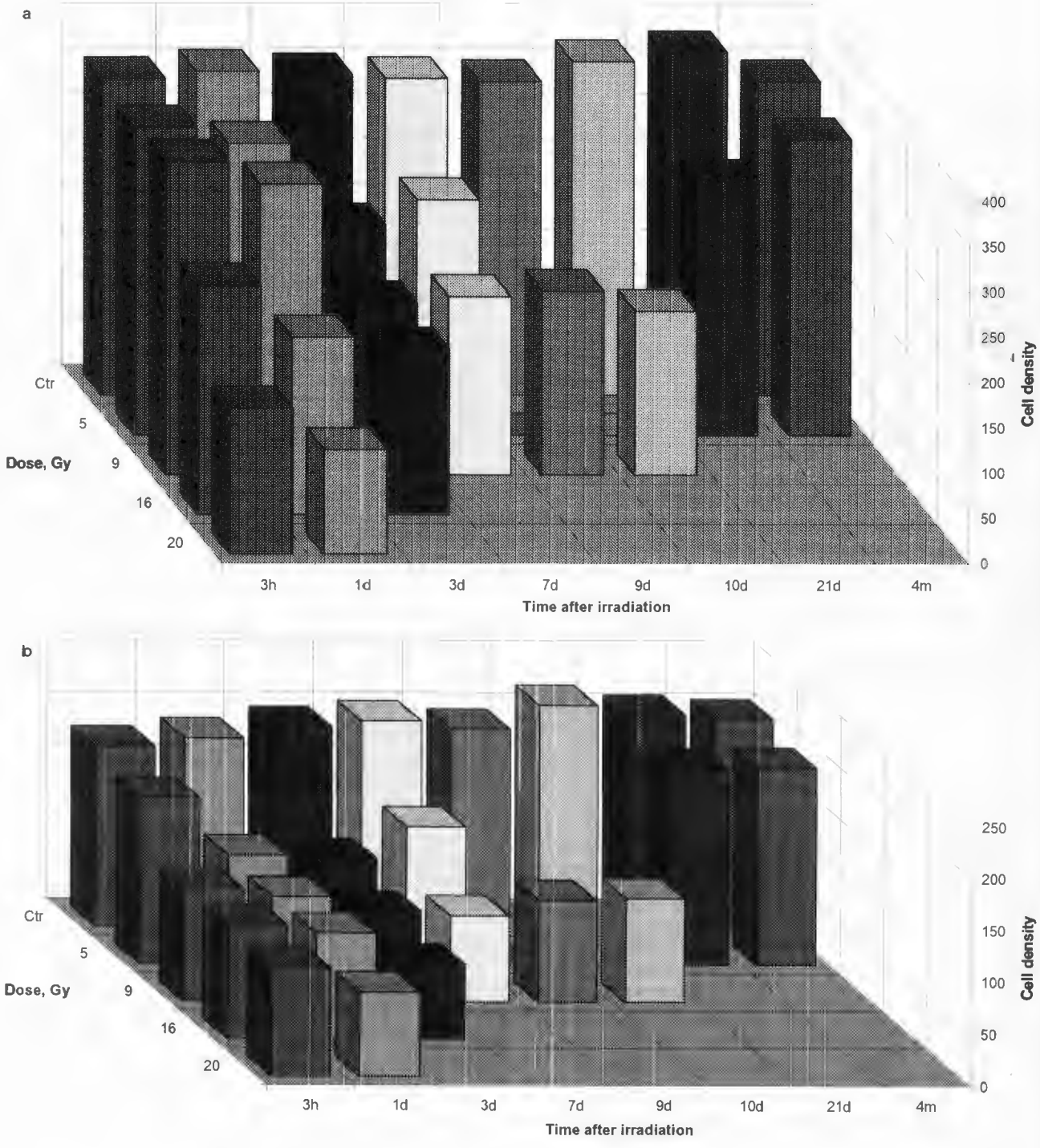


Figure 3. Argyrophil (a) and argentaffin (b) cell density, expressed as the number of cells per 1 mm^2, in pyloric area of gastric mucosa in F1 mice at various intervals after single whole-body exposure to different doses of gamma-rays. Each value represents the mean of 5-7 animals per group. Asterisks indicate statistically significant difference from controls, which were not irradiated, at $p < 0.05$ according to the Mann-Whitney U test.

---

formaldehyde (pH 7.2) for 24 hours, rinsed, dehydrated, embedded in paraffin and then sectioned at 7-μm thickness. Endocrine cell population as a whole was stained using the Grimelius argyrophil technique with Bouin's fixative (Fig. 2a). Serotonin-producing EC cells were stained by Masson-Hamperl argentaffin method using

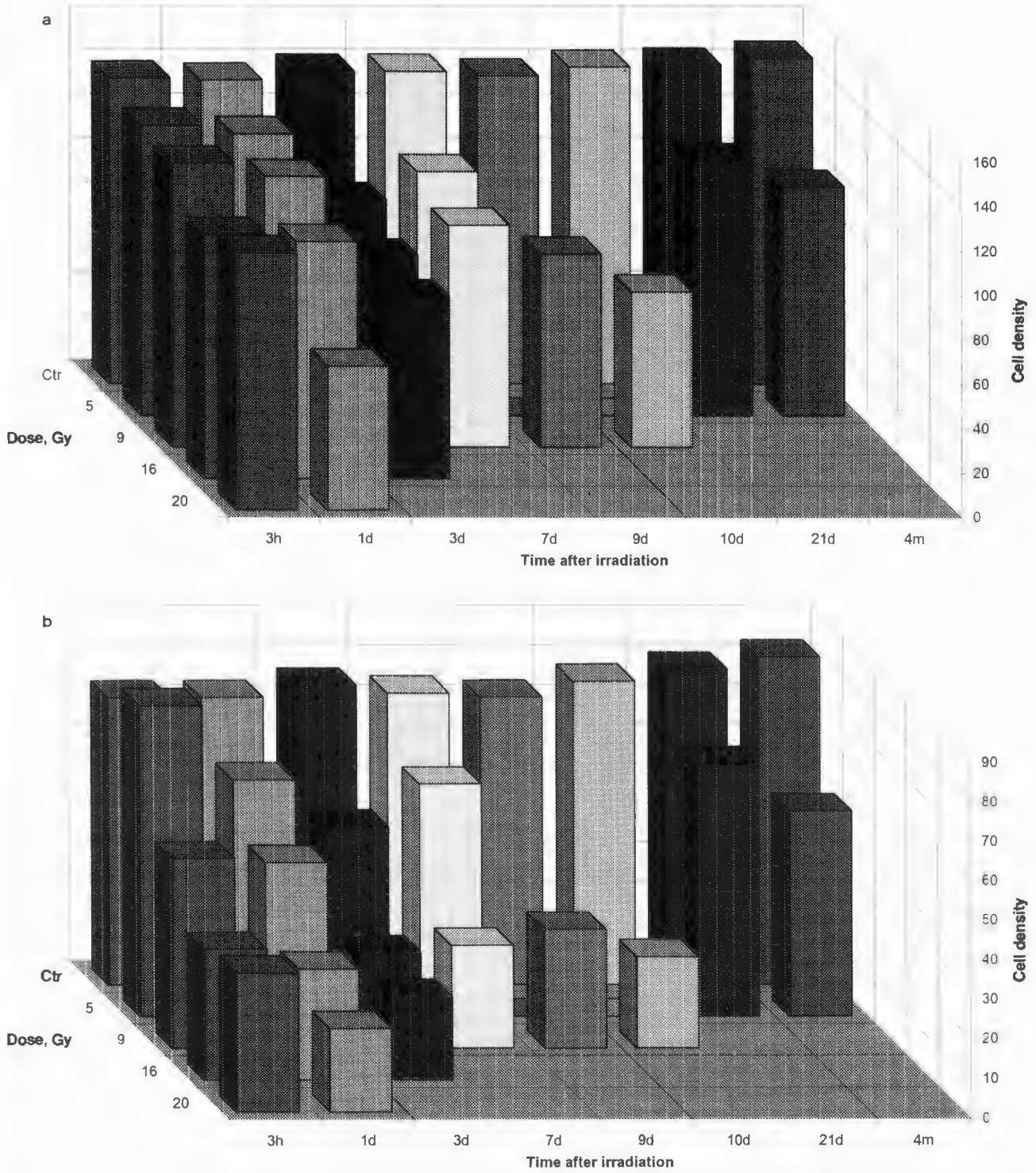


Figure 4. Argyrophil (a) and argentaffin (b) cell content, expressed as the number of cells per 1 cross-section of the organ, in duodenal mucosa in F1 mice at various intervals after single whole-body exposure to different doses of gamma-rays. Each value represents the mean of 5-7 animals per group. Asterisks indicate significant difference from unirradiated controls, at $p < 0.05$ according to the Mann-Whitney U test.

---

buffered formaldehyde as a fixative (Fig. 2b). Morphometric analysis of cell content was performed using some principles according to Weibel *et al.* [42]. Numerical density indicating the number of cells per unit of

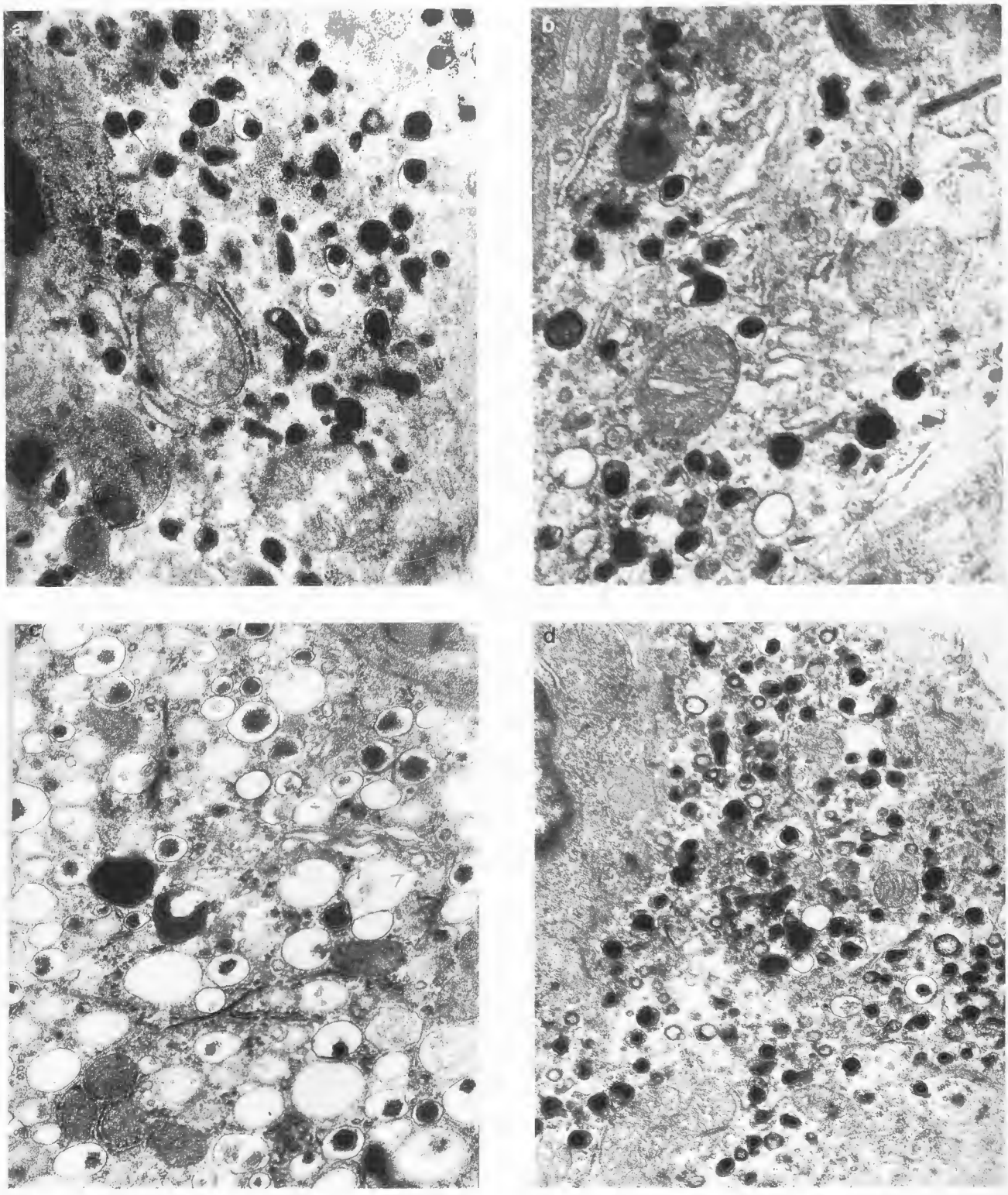


Figure 5. Electron micrographs showing gastric endocrine cells of mice after exposure to a dose of 5 Gy of gamma-rays. (a) Edema of cytoplasm and mitochondrions in endocrine cell, 1 day; P.W. = 2.4 μm. (b) Decrease in number of the secretory granules in endocrine cell, 1 day; P.W. = 2.5 μm. (c) Excess accumulation of the characteristic cytoplasmic vesiculus in ECL cell, 1 day; P.W. = 3.1 μm. (d) The cytoplasmic granules in G cell are numerous, 3 days; P.W. = 4.3 μm.

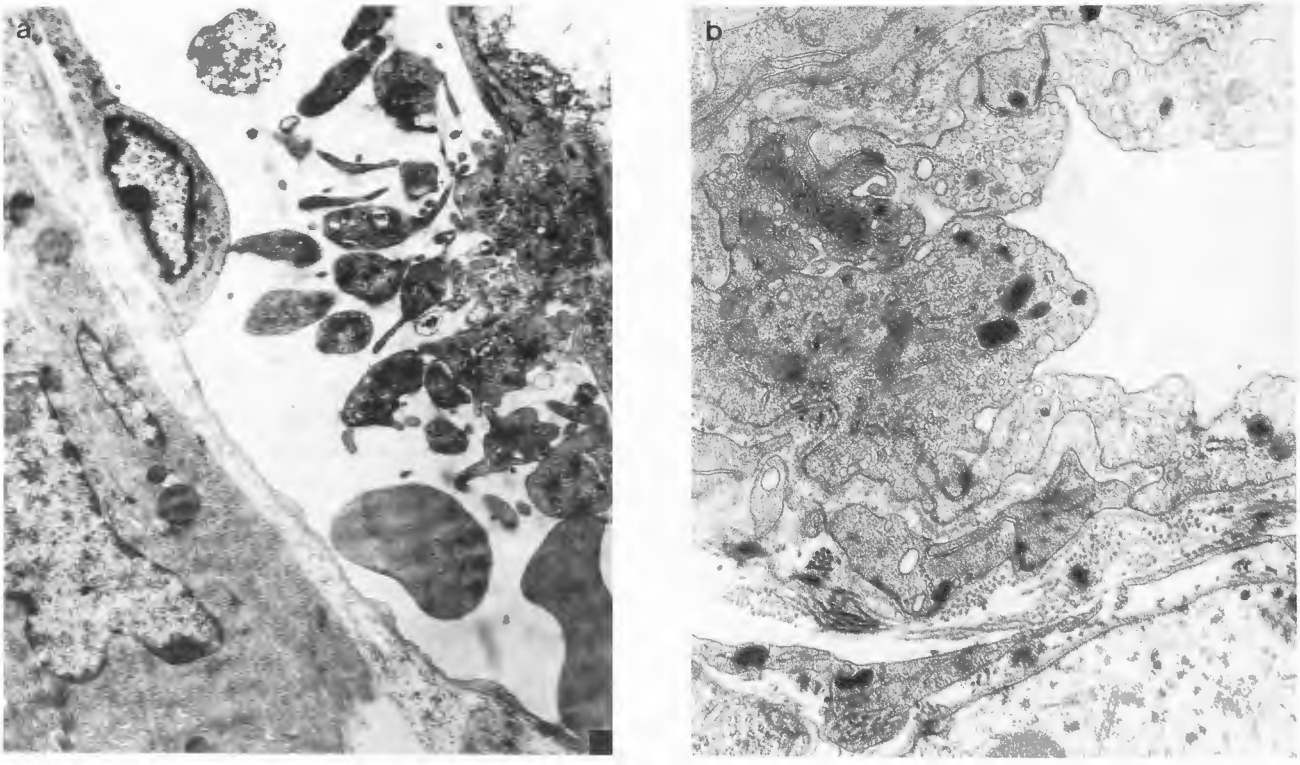


Figure 6. Destruction of hemato-cellular barrier in gastric mucosa of mice after gamma-irradiation to a dose of 5 Gy. (a) Distension of venule, marginal location of platelets and formation of perivascular edema (arrows), 1 day; P.W. = 13.3 μm. (b) Endothelial swelling and some granules in the cytoplasm of endothelial cell, 3 days; P.W. = 4.8 μm.

---

section area was calculated using a multipurpose test system. The examinations were performed with 10X eye-piece and 40X objective (visual field of the test system 170 μm x 140 μm; 100 test points). The cells were counted in 50-100 randomly selected visual fields, e.g., transverse sections of the stomach and cross sections of duodenum, from each section. At least 3 sections from each specimen were examined. The cell counts were expressed as the number of cells per 1 mm^2. The results were statistically evaluated. Non-parametric Mann-Whitney U-test was used to determine significant differences between control and irradiated animals; $p < 0.05$ was considered significant.

To assess the functional activity of APUD cells, radioautography was applied, using tritium-labeled precursor of dopamine. Accordingly, both control and irradiated (5 Gy gamma-rays) male C57Bl/6 mice were injected intraperitoneally with isotopically labelled D,L-3,4-dihydroxy (2,5,6-3H3) phenylalanine (10 μCi/g b.w.; 1.1-1.15 Ci/mmol specific activity; Isotope firm, Moscow, Russia). All animals were killed 2 hours after the injections. Small (1 mm^3) specimens of stomachs and duodenums were fixed by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 hours at 4°C and then rinsed in 0.1 M phosphate buffer (three changes, 2 hours each) at 4°C, dehydrated and embedded in epoxy resin. We applied semithin-thin technique: radioautography on semithin resin sections (1 μm) and comparison of labeled cells with the adjacent ultrathin section. Ultrathin sections were mounted on copper grids and stained with uranyl acetate and lead citrate.

Ultrastructural study of cells was carried out using a JEM-100S (JEOL, Tokyo, Japan) electron microscope.

Some comment on the terminology used in this review is warranted. Currently, the interpretation of terms associated with Pearse's APUD concept and DNES cannot be considered simple [32]. In particular, since it was stated that amine handling was not a basic feature of cells incorporated into APUD System, and furthermore, some non-endocrine cells such as mast, Paneth, gastric chief and pancreatic exocrine cells appeared to show amine handling, the term "APUD" was replaced by "neuroendocrine" [3]. However, the widespread term "neuroendocrine" cells, which has been used in recent years by many authors to designate the relation of endocrine cells to DNES, to our mind, is more acceptable for the group of specific neurosecretory cells. So, in this review the term "APUD cells" is kept as a synonym of endocrine cells producing peptide hormones and biogenic amines and forming endocrine part of DNES.

Functional Morphology of Gut APUD Cells at Radiation Influence

Most commonly identified types of DNES endocrine cells are located in the mucosa of the digestive tract both in animals and man [32]. In the pyloric and fundic parts of stomach there are 6 types of APUD cells: EC (which produce serotonin and melatonin), G (gastrin), D (somatostatin), ECL (histamine), D1 and AL cells. In addition to the above cells, other cells are identified in the small intestine, for instance, I, K, L, N, and S cells which produce cholecystokinin, gastro-inhibiting peptide, enteroglucagon, neurotensin, and secretin, respectively.

The time-dependent pathomorphological picture of radiation injury to gut organs has been fairly well documented and is largely determined by the inherently high radiosensitivity of processes governing cellular renewal [5, 14], However, data on the role of APUD cells in postradiation syndrome are sparse. In particular, there is little information on the physiologically active substances produced by these cells and on the diagnostic significance of the change in the functional activity of the several types of endocrine cells, needed to forecast developing postradiation dysfunctions.

Influence of total single external gamma-irradiation

Quantitative and qualitative changes in APUD cells were studied after gamma-irradiation with a sublethal dose of 5 Gy and lethal doses of 9, 16 and 20 Gy. The dose of 9 Gy caused death due to the hematopoietic form of radiation sickness. The intestinal form of radiation sickness occurred with doses of 16 Gy and 20 Gy.

Dynamics of endocrine cell content in the pyloric part of stomach and duodenum in mice after total irradiation of different doses is given on Figures 3 and 4. For the stomach, the calculation of quantitative cell density was made per 1 mm^2 of the basal part, i.e., at the localization of gastric glands, 140 μm from their bottom. In the duodenum, endocrine cell number was calculated per cross-section of the organ.

The total content of endocrine cells in the stomach tended to decline at early stages after irradiation with a dose of 5 Gy. On the third day, the number of endocrine cells reached the minimal level with the same dose. Subsequently, the density of endocrine cell content increased and by 4 months this cell density did not differ significantly from control levels. With a dose of 9 Gy, the number of argyrophil cells markedly decreased ($\sim$50% control levels) by the third day after irradiation and remained at this level up to the time of death. Already by 3 hours after irradiation, with doses of 16 Gy and 20 Gy, the number of APUD cells appeared significantly reduced, as indicated by the argyrophil method and the capacity of endocrine cells to accumulate reducible silver ions from the solution into secretory granules [10].

Argyrophil cells with the duodenum exhibited the following quantitative characteristics. With a dose of 5 Gy, the maximum reduction in number of endocrine cells occurred on the third day postirradiation; 3 weeks later, recovery of endocrine cell number occurred, but did not achieve control levels even up to periods as late as 4 months later. During early postirradiation periods following lethal dose exposures, the decline in tissue density of APUD cells was dose dependent.

Results of our morphometric analyses showed that about 50% of endocrine cell population, both in the pyloric part of stomach and in the duodenum, appeared to be serotonin-producing EC cells and showed the positive argentaffin reaction; EC cell argentaffin reaction was shown as a condensation product between serotonin and formaldehyde-b-carboline derivative. The change in the number of argentaffin cells after irradiation with different doses indicated that the response dynamics of APUD cell population was determined, to a large extent, by the reaction of EC cells. Further analysis, however, revealed differences in the response dynamics of the argyrophil and argentaffin cell populations in the pyloroduodenal area at early stages after irradiation. The statistically significant decrease of the number of argentaffin cells, with obvious lower staining intensity, was observed earlier (one day later with a dose of 5 Gy and in 3 hours with a dose of 9 Gy) than the comparable response noted in argyrophil cell population, i.e., the argyrophil cells exhibited a peculiar "delayed reaction." These results, to our mind, indicate that ionizing radiation initially causes the release of biogenic amines from APUD cells (in this case, serotonin) that, in turn, serves to initiate the early postradiation processes and transformations in tissues and adaptive response of cells to homeostatic alterations.

Electron microscopic studies showed clearly defined changes in APUD cells 1-3 days after gamma-irradiation exposure to a dose of 5 Gy (Figs. 5a and 5b). These changes included: increased structural heterogeneity of intracellular organelles, development of the focal swelling of mitochondria, extension of Golgi complex and channels of endoplasmic reticulum. However, there was little evidence of such structural damage to many endocrine cells. The noted variability of ultrastructural organization corresponded to normal variation and was largely attributed to differences in degrees of differentiation and the stages of the functional cell cycle. Concomitant to these changes, we noted an "excess" accumulation of the secretory granules in some APUD cells (Figs. 5c and 5d). Greater effects were expressed by stromal elements at early stages of postradiation pathology; e.g., the paretic vascular distensions and formation of the local parts of perivascular and interstitial edema

(Fig. 6). These changes were poorly marked in the early periods following sublethal irradiation, but with extended postexposure times following lethal doses, they were more clearly marked; e.g., small foci of damage to plasma membranes and lamellar complexes progressed into large defects. In APUD cell's cytoplasm, the content of myelin structures and cytolysosomes increased, along with foci of damage. These alterations developed in concert with a growing mucosal edema.

Cytoplasmic degranulation is a common but specific response of the APUD cell during early postirradiation periods following lethal exposures. This response correlates with the decrease in the tissue density of endocrine cells identified by argyrophil method. Despite the extent of degranulation, a fraction of cytoplasmic granules appeared resistant and retained typical morphology and tinctorial features. That latter was the case even in dead cells.

The complex of cytochemical and ultrastructural changes noted within APUD cells following radiation exposure reflects serious functional abnormalities of these cells and their activities at early stages of postradiation pathology. The noted changes are likely the combined result of both direct cellular damage, as well as indirect, cell-damaging responses brought about developing, intracellular edema.

The capacity of APUD cells to absorb amine precursors such as dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophane, to decarboxylate and to accumulate the generated biogenic amines in the secretory granules is a fundamental feature of these cells. As L-DOPA is more intensively incorporated into endocrine cells during differentiation, some authors have suggested that DOPA accumulation reflects the degree of active, amino acid transport, and in turn, the metabolic activity of these cells [25].

Our studies showed that ^{3}H-DOPA administration results in a highly selective, accumulation of label over gut APUD cells (Fig. 7a). Analysis of serial (semithin to ultrathin) sections indicated that labelled DOPA was incorporated into practically all types of gastric APUD cells and most endocrine cells of intestine in animals that were not irradiated. By comparing the ultrastructural features of gut cells with their ability to accumulate ^{3}H-DOPA, we showed that cells with small number of the secretory granules are minimally labelled. However, by contrast, heavily labelled cells have cytoplasms filled with mature secretory granules. It is rather difficult to draw a conclusion on the considerable discrepancies in precursor accumulation by different types of endocrine cells in the marked heterogenic distribution of a label over APUD cells.

One day later after gamma-irradiation exposure with a dose of 5 Gy, a decrease in label concentration over

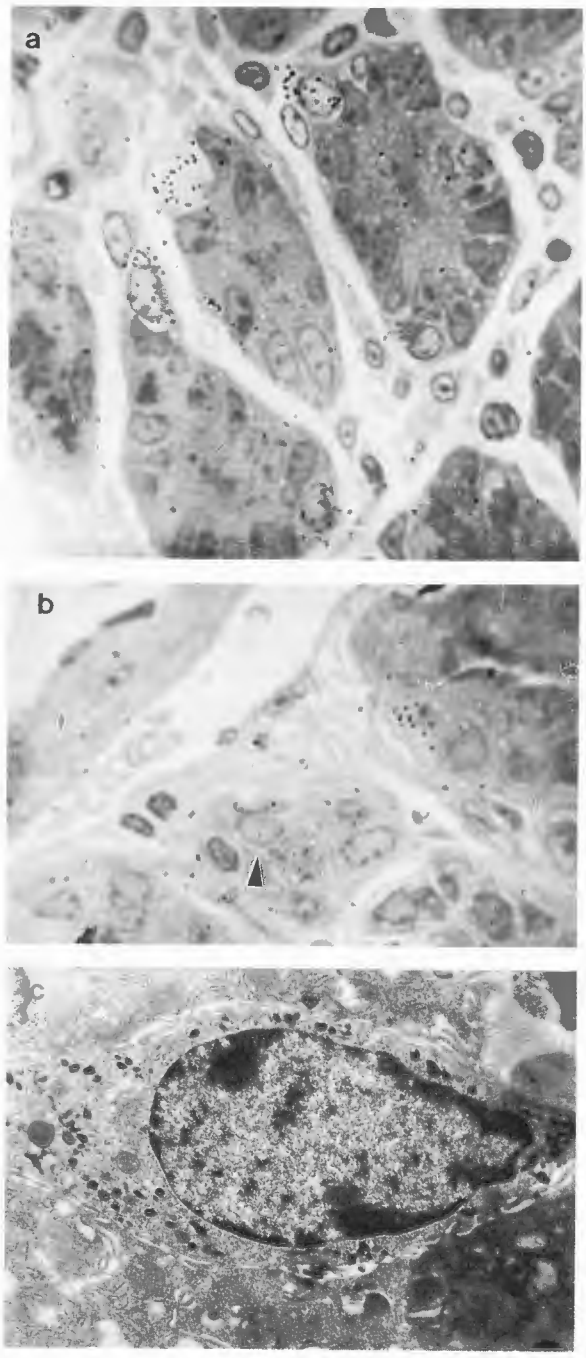


Figure 7. Radioautographs of ^{3}H-DOPA incorporation into APUD cells. Semithin sections (1 μm) of mouse's pylorus. (a) High selectivity of label accumulation over endocrine cells is observed in unirradiated mice; P.W. = 130 μm. (b) Label concentration over cells decreases in area of the developing mucosal edema 1 day after gamma-irradiation to a dose of 5 Gy; P.W. = 121 μm. (c) The same EC cell (arrowhead, b) contents the typical secretory granules; P.W. = 7.3 μm.

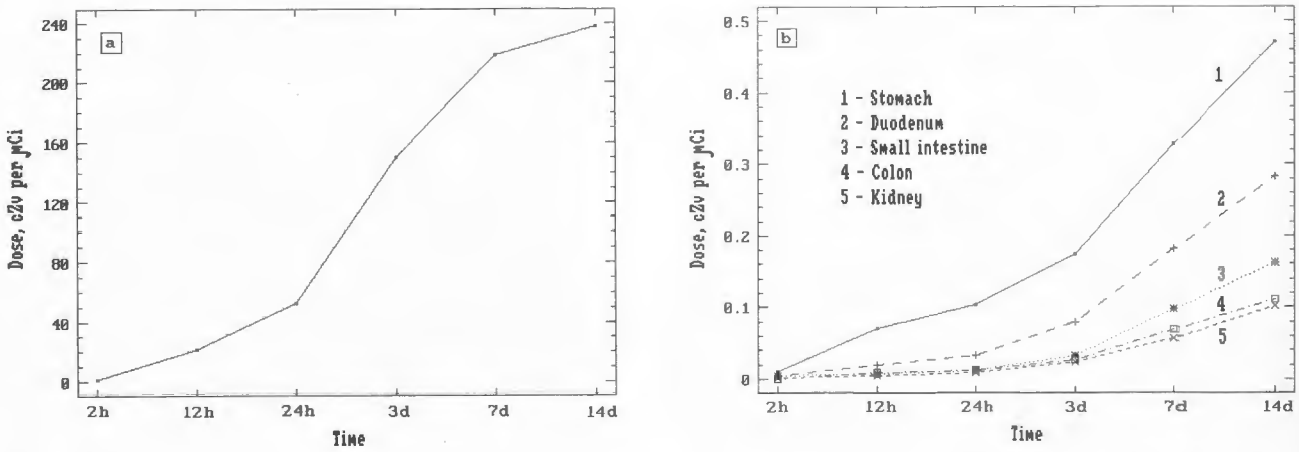


Figure 8. Average cumulative radiation dose to the thyroid gland (a) and other organs (b) as a function of time after oral administration of I-131.

endocrine cells was noted in the pyloro-duodenal area (Fig. 7b). In contrast to the tests of control animals, which were not irradiated, it was not uncommon to find that the concentration of label (silver grains) over APUD cells did not always correlate with the number of the secretory granules within the APUD cell's cytoplasm. Furthermore, we had the impression that the decrease in label concentration was mostly marked in areas of the developing mucosal edema. Some endocrine cells identified in ultrathin sections also appeared to be unlabelled (Fig. 7c).

Results of our electron microscopic analyses suggests that a primary effect of ionizing radiation is on development of intracellular edema, with the specific damage to membrane apparatus. Such intracellular edema could result in vital subcellular organelles being damaged and functionally altered; e.g., pathologic modification of vital mitochondrial structures, and in turn suppression of system energetics.

In summary, disorders of transport, ion and energy intracellular mechanisms in endocrine cells could well lead to the noted decrease in precursor transport and abnormalities of synthesis and accumulation of biogenic amines within the secretory granules of the targeted APUD cell. This proposed process might well account for the fact that the gut APUD cells have a reduced capacity to bind exogenous dihydroxyphenylalanine after gamma-radiation exposure.

One should note that ionizing radiation in the sublethal dose range does indeed affect endocrine cells. During late postirradiation periods, APUD cells showed dystrophic changes in mitochondria and endoplasmic reticulum, higher content of myelin structures and lipofuscin granules. Such changes to a certain extent may account for the functional incarnations of the post irradiated APUD cells.

Internal irradiation influence

The active application of radionuclide I-131 in the clinical treatment of patients with thyroid cancer necessitates a continued evaluation of its long-term medical consequences. The major fraction of radionuclide administered repeatedly to patients is known to accumulate in the thyroid, in associated primary tumors and in tumor metastases. However, the administered I-131 damages not only tumor cells but also intact tissues. In regard to the latter, the kidneys are considered as critical organs in the thyroid blockade, due to the passage and release of the administered radionuclide. Further, the gastrointestinal system appears sensitive as well. There are data in literature suggesting that radioiodine constantly recirculates by way of the walls of stomach and intestine. Clearly, this cannot happen without affecting the structures of these organs, and indeed, this is what appears to happen. A number of clinical studies have indicated that after radionuclide administration, particularly in several courses of treatment, patients often show symptoms of a dyspeptic character and develop the progressing chronic gastritis.

Based on the above, it is clear that further study of I-131 distribution and its pathologic consequences to gut tissues is needed. Taking into account the fact that serotonin plays a specific role in the regulation of selected functions within the stomach and intestine, the dynamics of enterochromaffin cell response within these tissues following I-131 administration provided the focus for further study.

Animals were given 1.0 μCi/g body weight of I-131 orally by esophageal intubation. The material to be studied was taken in 3, 12 and 24 hours, 3 days, 1, 2 and 3 weeks following radionuclide administration.

The results of investigations and calculations (Figs. 8a and 8b) showed that the thyroid gland incurred the

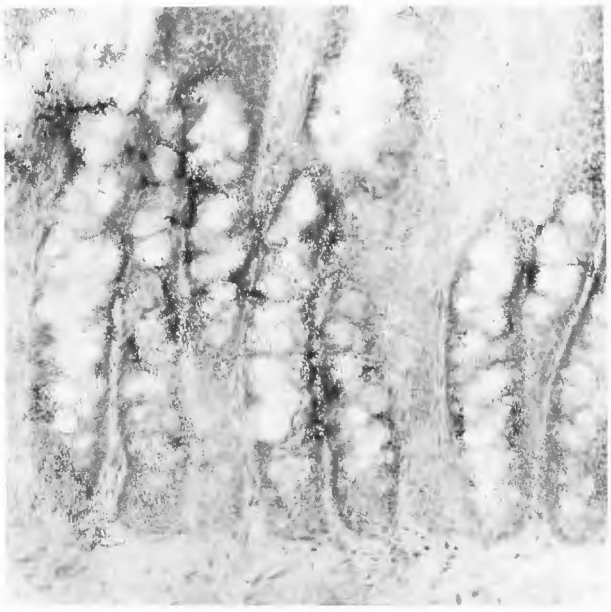


Figure 9. Accumulation of radioautographic label in the intestinal epithelium 1 day after oral administration of I-131 at a 1.0 μCi/g b.w. dose level; P.W. = 400 μm.

largest absorbed dose, as was expected. The levels of absorbed radiation doses within individual organs of the gut were less than those found in the thyroid. The levels noted, in descending order, are as follows: stomach, duodenum, small intestine and colon. However, when the various organs of the gut were considered in total, the radiation levels appeared to be actually higher than those levels found in the kidneys. Therefore, the gut together with the kidneys need to be considered as critical organs in oral I-131 treatment protocols.

Radioautographic study of stomach and intestine showed the accumulation of silver grains mainly over surface capillaries of the gastric mucosa. One day later, label incorporation occurred over the epithelium of stomach, as well as in crypt epithelium of small intestine (Fig. 9) and mucous layers of these organs. In the colon, the label was distributed diffusely with a slight accumulation in the bottom of the crypt.

These radionuclide studies mentioned above, enabled us to consider the stomach and intestine as radiation-sensitive organs for I-131 therapeutic administrations. Furthermore, radioautographic analyses suggest specific structural sites within these tissues for primary radioiodine incorporation. These tissues sites included the surfaces of the gastric mucosa and crypt epithelia of the small intestine.

The cell density of argyrophil and argentaffin cells within the pyloro-duodenal intestinal region of mice markedly changed shortly following I-131 administration

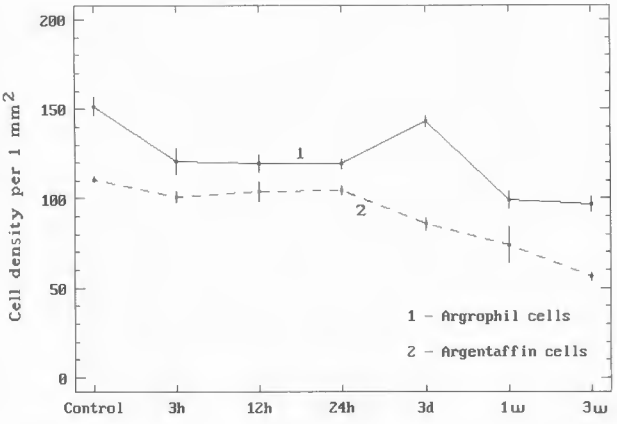


Figure 10. Argyrophil (full line) and argentaffin (broken line) cell density (expressed as the number of cells per 1 mm^2) in pyloric glands in Wistar rats at various intervals after oral administration of I-131 (1.0 μCi/g b.w.). Each value represents the mean + standard error of mean (SEM) of 5-7 animals per group.

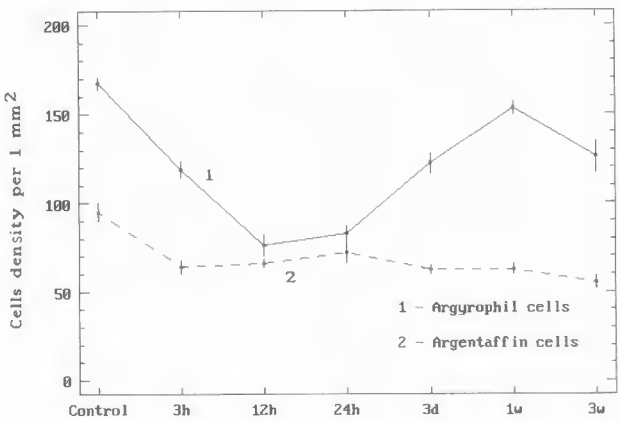


Figure 11. Argyrophil (full line) and argentaffin (broken line) cell content in rat duodenal mucosa at various intervals after oral administration of I-131 (1.0 μCi/g b.w.). Each value represents the mean + SEM of 5-7 animals per group.

(Figs. 10 and 11). Within the first few hours, a considerable decrease in both the common APUD cell population and in EC cell population was noted. However, later an imbalance in the density of endocrine cells as a whole and the enterochromaffin cells occurs.

Our results indicates that within the first few hours after I-131 radionuclide treatment, I-131 recirculates through the walls of the stomach and intestine, exposing these tissues to ionizing radiation and causing the release of serotonin from EC cells, particularly, in the intestine. Considering, the relatively high density of EC cells within gut mucosa and the expected serotonin-release response, one can readily expect pathology to ensue, and

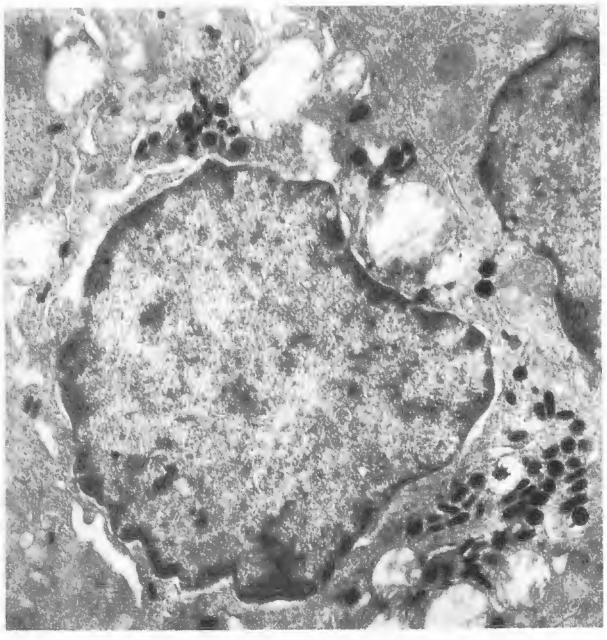


Figure 12. Electron micrograph of EC cell of the rat duodenum 12 hours later after oral administration of I-131 (1.0 μCi/g). Note the reduction in the number of the secretory granules in cytoplasm, edema and mitochondrion destruction; P.W. = 5.7 μm.

---

indeed, this is what happens. Through our ultrastructural analyses, we showed that the APUD cells of the pyloro-duodenal mucosa were damaged following parenteral I-131 administration (Fig. 12).

Serotonin is known to promote mucin formation by epithelium and pyloric glands of stomach, increase pepsinogen formation by chief cells and lead to the reduction of the number of parietal cells by blocking of ECL cells degranulation. Therefore, one cannot discount the strong possibility that serotonin plays a active role in the pathogenesis of chronic gastritis in patients with thyroid cancer following repeated radioiodine therapy.

The prophylaxis and treatment of radiation-stimulated gut dysfunctions require the application of pharmacological drugs. However, often in the oral administrative route, an optimal, slow rate of drug absorption from the enteral medium to gut mucosa is not obtained, thus restricting a gradual accumulation and prolongation the drugs' effects.

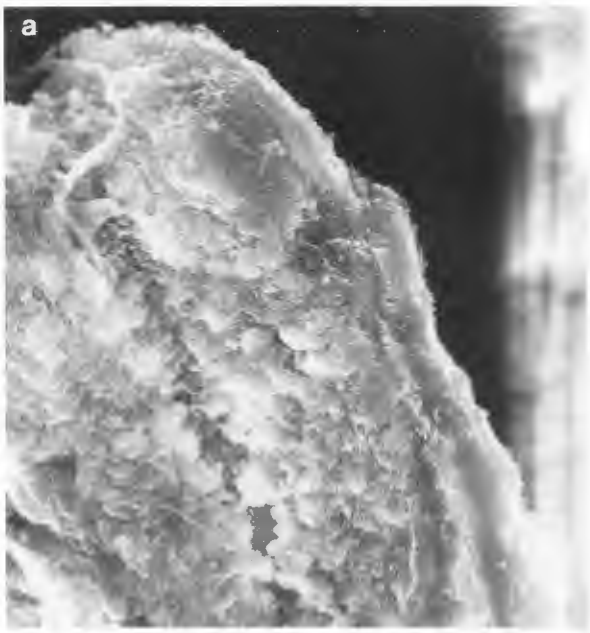
One of the major factors regulating drug deposition and absorption appears to be a layer of mucous lining the luminal surface of the intestine (Fig. 13). Hydrolytic enzymes embedded into the mucosal layer, along with its very heterogenous chemical nature, often times tends to inactive and block transport of many drugs.

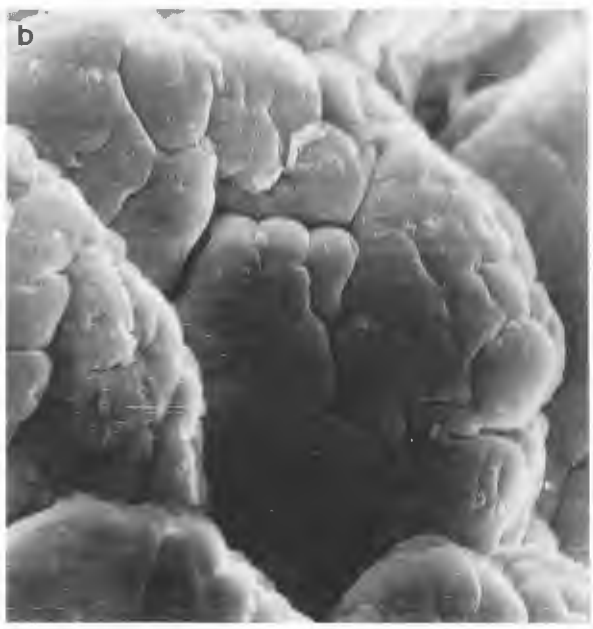
Using a combination of microscopic techniques [4, 23], we showed that we could selectively accumulate and transport specific drugs to the specific tissue sites within the gastrointestinal tract. In one study, we demonstrated the process by which liposome encapsulated drugs were transported from a gel-like, mucosal layer of the luminal surface of the intestine (Fig. 14) to the glycocalyx of microvilli, partially stratified and gradually released. In sum, these results indicated it might be possible to transport drugs following the parenteral introduction and to achieve effective levels of absorption while still maintaining biological activity.

Discussion

There are strong arguments for the endodermal origin of gut APUD cells [6, 7, 32]. Common histogenesis perhaps might explain the common postirradiation kinetics of the seemingly diverse epithelial and endocrine cells. Ionizing radiation is well known to inhibit the proliferative capacity of epithelial stem cells, and as a result, elicits subsequent reduction in more mature epithelial lining populations. Our observations suggest that the noted quantitative changes within APUD cells of the stomach and duodenum are the result of a sequence of radiation injury and repair of a progenitorial APUD progenitor population. Endocrine-determined stem cells stressed with sublethal doses of ionizing radiation probably fosters the increase in APUD cells populations within the duodenum and stomach.

The duodenum is known to be a more radiosensitive tissue than the stomach [14]. This difference is accounted for by the peculiarities of the tissue kinetics. However, the underlying reason for such differences, especially in terms of differences in radiosensitivities of the various stem cell species, remains unclear. For example, the speed of renewal of the intestinal epithelium is higher than that of hemopoietic system, and the time of radiation exhaustion of cellular renewal system is shorter for it than for bone marrow [17]. However, the intestinal epithelium exhibits greater resistance than hemopoietic tissues, in terms of the radiation exposure levels that limit tissue function; lethal doses causing the death of the organism due to the hematopoietic form of radiation sickness are smaller than those determining the death from the intestinal form. Stem cells of the gastric epithelium appear to be more resistant than those of the intestinal epithelium. It is quite possible that radiosensitivity is affected by natural radioprotective agents; for example, the vasoactive hormones, serotonin and histamine. These biogenic amines are able to limit blood supply of the sensitive organs and to reduce radiosensitivity by hypoxia. In this connection, EC and ECL cells are of specific interest. Serotonin-producing cells make up a remarkable part of APUD cell population both in





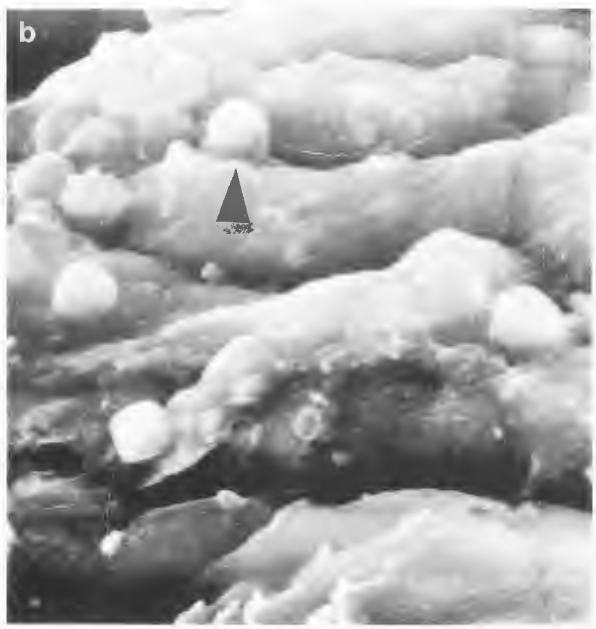


Figure 13 (at left). Scanning electron micrographs of villi of rat small intestine. (a) Isolated villus coated with a thick layer of mucus; P.W. = 30.8 μm. (b) Villi after mechanical removal of mucus; P.W. = 10.5 μm.

Figure 14 (at right). Scanning electron micrographs of liposomes. (a) Liposomes located in mucus coated villi of rat intestine, 5 minutes after their introduction in the digestive cavity; P.W. = 13.2 μm. (b) Fragment of Figure 13a: early stage of liposome immersion in mucus (arrowhead); P.W. = 5.4 μm.

stomach and duodenum. However, the bulk of histamine-producing ECL cells are located in the stomach.

It is of interest to note the early development of the vascular response in pyloro-duodenal mucosa at gamma-irradiation exposure. Pathomorphological pictures of disordered hemodynamics and the development of hemorrhagic diathesis, typical for the primary period and early reactions of radiation sickness, has been well studied [5, 14]. During these early post-irradiation periods, there are distinct relationships between the accumulation of vasoactive substances in organs, the change in the sensitivity of arterioles, venules and capillaries to adrenaline, histamine, serotonin and acetylcholine, the increase in the permeability of vascular wall, and the development of the interstitial edema [13, 16, 37, 41]. It is shown that one of the links of pathogenesis of postirradiation vascular abnormalities appear to be degranulation of mast cells and the release of histamine and serotonin from them. Some clinical manifestations of the syndrome of the primary reaction to irradiation are associated with the excess release of biogenic amines, vasoactive and other peptide hormones from APUD cells. However, the role of the latter substances in the development of these postirradiation dysfunctions remains practically unknown.

To date, the problem of the correlating the type and degree of pathology associated with direct versus indirect (via microcirculation disorder) radiation exposures has remained undetermined. It is not unexpected that the quickly developing interstitial and intracellular edema results from release of substances with different vasoactive mechanism and leads to the decrease in the intracellular oxygen concentration, thus creating an hypoxic, survival-promoting tissue situation for cells under select conditions of irradiation. This mechanism, might promote an increase in radioresistance of gut stem cells; the additional protective effect in the stomach seems to be accounted for by histamine release from ECL cells. Also, the mechanism may in part account for the radiosensitivity of the critical composite organ system or, clearly in the case of radiation therapy of malignant tumors, the development of the intracellular edema and hypoxia often contributes to the survival of tumor cells [44].

In this connection, identification of biogenic amines in non-endocrine cells is of great interest [18, 20]. The data on localization of serotonin and melatonin in natural killer cells, mast cells, eosinophilic leukocytes and some endothelial cells which may play an important oncoradiobiological role. In particular, the results of our investigations showed that mast cell accumulation and the associated release of biogenic amines within tumors results in a "radioprotective shield" which needs to be taken into account in radiation therapy [20, 44]. Taking into consideration the biological function typical for the above non-endocrine cells and their role in homeostasis regulation, these cells could serve, to a certain extent, as possible endogenous radiomodifying factors. It should be that the early morpho-functional vascular responses that promote the formation of the local hypoxic tissue sites and trophic abnormalities may represent an important pathogenic process, as well.

The results found in the open literature, as well as the results of our studies, strongly indicate that DNES cells play an important role in the mechanism of pathogenesis resulting from ionizing radiation exposure. The release of vasoactive substances from APUD cells and their local accumulation in tissues, even short-term, inevitably leads to microcirculation disorders, hypoxia, development of metabolic acidosis and other abnormalities of metabolic processes in different cells, including homeostatic regulators. The ultimate result may be disorganization of neuro-humoral regulation of the functional parameters of the vascular system and formation of vicious circle increasing the primary injuries [41].

The structural and functional effects of radiation injury to DNES cells highly favor risk evaluation of early postradiation dysfunctions, namely, the development of aplastic, hypoplastic and sclerotic states of visceral organs, as well as dyshormonal, dysimmune and other disorders. The development of therapeutic approaches to the early treatment of radiation injury to the DNES and its associated dysfunctions is equally important.

To date, the problem of physiological significance of monoamine synthesis by APUD cells and many non-endocrine cells has remained unclear. A number of indirect results obtained by us in the study of ionizing radiation effect on cells indicate the possible role of biogenic amines in the initiation of exocytosis processes. It is possible that the detected excess accumulation of the secretory granules in some endocrine cells is associated with the disorder of APUD mechanism.

General Conclusion and Future Perspectives

The wide spread tissue distribution of APUD cells, in the organism together with neurons and non-endocrine cells capable of the synthesis, accumulation, separate usage and intertissual transport of regulatory peptide and biogenic amines suggests an urgency and significance of future DNES studies in radiobiology and oncoradiology. The analysis of the experimental results described here shows the direct participation and active role of APUD cells in both the pathogenesis of radiation injury and formation of radiosensitivity of those organs and tissues in which they are located.

Taking into account the fact that some hormones synthesizing and producing APUD cells have a radiomodifying effect and are also able to activate and sup-

press cell proliferation, we consider it important to carry out thorough studies on the development of organ radiosensitivity by changing the functional activity of APUD cells *in situ*.

The success of future investigations may determine an effective means for the protection, prophylactics and treatment of radiation injuries.

For practical reasons, the pharmacological inhibition non-endocrine and DNES cell activities in tumor growth promotion may appear helpful for modification and optimization in radiation therapy of malignant tumors.

In conclusion, it should be noted that the available data on the structural and functional DNES organization, role and significance of biogenic amines and peptide hormones in homeostasis regulation indicate an exclusively important role which APUD cells play in pathogenesis of different dysfunctions including the mechanisms of radiation injury. We consider the performance of such studies to be one of the fundamental directions in modern radiobiology.

Acknowledgements

The authors are grateful to Drs. E.S. Kuriletz and R.P. Manokhina for giving some microphotographs. We also thank Mrs. M. Kopnova for preparation photographs. We acknowledge Drs. L. Tkacheva, I. Ingel and T. Garbuzova for technical assistance in preparing this manuscript for publication.

References

[1] Ahlersova E, Molcanova A, Ahlers I (1988) Thyroid hormones in gamma irradiated rats. Radiobiol Radiother **4**: 507-512.

[2] Ahlersova E, Molcanova A, Ahlers I, Kassayova M (1993) Hormonal response to lethal γ-irradiation in rats of various age. In: Radiation Biology and Its Application in Space Research. Kozubek S, Horneck G (eds.). Univ. Press, Brno, Czech Rep. pp. 94-97.

[3] Andrew A (1982) The APUD-concept: Where has it led us? Brit Med Bull **38**: 221-225.

[4] Brodsky RA, Galperin YM, Lazarev PI, Nadtochy VV, Popov GA (1983) Transport and destruction of liposomes in gut mucous liner. Dokl Acad Nauk USSR **273**: 464-466 (in Russian).

[5] Casarett GW (1980) Radiation Histopathology. Vol. 1-2. CRC Press, Boca Raton, FL. pp. 24-36.

[6] Cheng H, Leblond CP (1974) Differentiation and renewal of the four main epithelial cell types in the mouse small intestine. III. Enteroendocrine cells. Am J Anat **141**: 503-520.

[7] Cheng H, Leblond CP (1974) Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelial cell types. Am J Anat **141**: 537-562.

[8] Falck B, Hellman B (1963) Evidence for the presence of biogenic amines in pancreatic islets. Experientia **19**: 139-140.

[9] Falck B, Owman Ch (1968) 5-Hydroxytryptamine and related amines in endocrine cell systems. Adv Pharm **6**: 211-231.

[10] Grimelius L, Wilander E (1980) Silver stains in the study of endocrine cells of the gut and pancreas. Invest Cell Pathol **3**: 3-12.

[11] Gutin PH, Leibel SA, Sheline GE (eds.) (1991) Radiation Injury to the Nervous System. Raven Press Ltd, New York. pp. 64-69.

[12] Hakanson R, Bottcher G, Ekblad E, Grunditz T, Sundler F (1990) Functional implications of messenger coexpression in neurons and endocrine cells. In: Neuropeptides and Their Receptors. Alfred Benzon Symposium 29. Schwartz TW, Hilsted LM, Rehfeld JF (eds.). Munksgaard, Copenhagen. pp. 211-232.

[13] Hopewell JW, Young CMA (1978) Changes in the microcirculation of normal tissues after irradiation. Int J Rad Oncol **4**: 53-59.

[14] Ivanov AE, Kurshakova NN, Shikhodirov VV (1981) Pathological Anatomy of Radiation Disease. Meditzina, Moscow. pp. 93-97 (in Russian).

[15] Kassayova M, Ahlersova E, Ahlers I (1993) Changes of pineal N-acetyltransferase activity in gamma irradiated rats. Physiol Res **42**: 167-169.

[16] Kobal J, Baum SJ, Parkhurst LJ (1972) Canine intestinal vasoactivity during the development of the gastrointestinal radiation syndrome. Rad Res **50**: 528-538.

[17] Konopljannikov AG (1984) Radiobiology of Stem Cells. Energoatomizdat, Moscow. pp. 15-21 (in Russian).

[18] Kvetnoy IM, Reiter RJ (eds.) (1994) Melatonin: General Biological and Oncoradiological Aspects. MRRC Press, Obninsk, Russia. pp. 66-72.

[19] Kvetnoy IM, Yuzhakov VV (1993) Extrapineal melatonin: Advances in microscopical identification of hormones in endocrine and non-endocrine cells. Microsc Anal **21**: 19-21.

[20] Kvetnoy IM, Yuzhakov VV (1993) Extrapineal melatonin: Non-traditional localization and possible significance for oncology. In: Advances in Pineal Research. Vol. 7. Maestroni J, Conti A, Reiter R (eds.). John Libbey and Company, London. pp. 199-212.

[21] Matsuoka O, Tsuchiya T, Kashima M, Eto H (1966) Serotonin and radiation effects on intestine. In: Gastrointestinal Radiation Injury. Sullivan MF (ed.). Excepta Medica, Dordrecht, Netherlands. pp. 313-326.

[22] Moskalev YI (1991) Latest After-Effects of

Ionizing Irradiation. Meditzina, Moscow. pp. 48-53 (in Russian).

[23] Nadtochy VV, Popov GA, Podgorodnichenko VK, Poverenny AM, Brodsky RA (1984) The transport of 125I-poly(I):poly(C) incorporated into liposomes from the enteric cavity to the small intestine mucosa. Investigation by electron microscopic autoradiography. Cytology (Moscow) **26**: 783-787 (in Russian).

[24] Owman CH, Hakanson R, Sundler F (1973) Occurrence and function of amines in endocrine cells producing polypeptide hormones. Fed Proc **32**: 1785-1791.

[25] Partanen S, Rechardt L, Back N (1975) Histochemical observations on uptake of L-DOPA into endocrine cells of the rat pituitary gland during the postnatal development. Cell Tiss Res **156**: 451-465.

[26] Pearse AGE (1966) Common cytochemical properties of cells producing polypeptide hormones, with particular reference to calcitonin and C cells. Vet Rec **79**: 587-590.

[27] Pearse AGE (1969) The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J Histochem Cytochem **17**: 303-313.

[28] Pearse AGE (1981) The diffuse neuroendocrine system: Falsification and verification of a concept. In: Cellular Basis of Chemical Messengers in the Digestive System. Grossman MI, Brasier MA, Lechargo J (eds.). Academic Press, New York. pp. 13-19.

[29] Pearse AGE (1986) The diffuse neuroendocrine system: Peptides, amines, placodes and the APUD theory. Progr Brain Res **68**: 25-31.

[30] Polak JM, Bloom SR (1979) The diffuse neuroendocrine system. Studies of this newly discovered controlling system in health and disease. J Histochem Cytochem **27**: 1398-1400.

[31] Polak JM, Bloom SR (1986) Immunocytochemistry of the diffuse neuroendocrine system. In: Immunocytochemistry: Modern Methods and Applications. 2nd ed. Polak JM, Van Noorden S (eds.). John Wright and Sons, Bristol, U.K. pp. 328-348.

[32] Raikhlin NT, Kvetnoy IM (1994) The APUD system (Diffuse Endocrine System) in normal and pathological states. Sov Sci Rev F Physiol Gen Biol **8**: 1-44.

[33] Saiton K, Toyooka M, Fujita K, Hashimoto J, Kunikata M, Mori M (1992) Neuron-specific enolase reduction in irradiated salivary glands of the rat: An immunohistochemical study. Acta Histochem Jena **93**: 277-281.

[34] Sundler F, Hakanson R (1982) Fluorescence histochemical methods for the study of peptide hormone-producing cells. Brain Res Bull **9**: 107-116.

[35] Sundler F, Hakanson R, Loren I, Lundquist I (1980) Amine storage and function in peptide hormone-producing cells. Invest Cell Pathol **3**: 87-103.

[36] Thomlinson RH (1973) Biological basis of radiotherapy. Radiation and the vascularity of tumors. Brit Med Bull **29**: 29-32.

[37] Timmermans R, Gerber GB (1980) Reaction of blood pressure and mesenteric blood flow to infusion of biogenic amines in normal and supralethally X-irradiated rats. Rad Res **82**: 81-92.

[38] Tstepanenko VS, Dedenkov AN, Yaskova EK (1985) Automatic system of dosimetric control in nuclear medicine. In: Fourth International Symposium of Application of Mathematic Methods in Medical and Biological Investigations. Nauka, Moscow. pp. 308-310 (in Russian).

[39] Van Noorden S, Polak JM (1983) Immunocytochemistry today: Techniques and practice. In: Immunocytochemistry: Practical Applications in Pathology and Biology. Polak JM, Van Noorden S (eds.). John Wright and Sons, Bristol. pp. 11-42.

[40] Verhofstad AAJ, Steinbusch HWM, Joosten HWJ, Penke B, Varga J, Goldstein M (1983) Immunocytochemical localization of noradrenaline, adrenaline and serotonin. *ibid.* pp. 143-168.

[41] Vorobjev EI, Stepanov RP (1985) Ionizing Irradiation and Blood Vessels. Energoatomizdat, Moscow (in Russian). pp. 13-17.

[42] Weibel ER, Kistler GS, Scherle WF (1966) Practical stereological methods for morphometric cytology. J Cell Biol **30**: 23-38.

[43] Yuzhakov VV (1983) Electron autoradiographic analysis of ^{3}H-thymidine distribution in the nuclei of Chinese hamster cells. Cytology (Moscow) **25**: 1013-1018 (in Russian).

[44] Yuzhakov VV, Kaplan MA, Kvetnoy IM (1993) Functional morphology of tumors exposed to laser and ionizing radiation. Prospects of the low level laser applications in complex antitumor therapy. Phys Med (Obninsk) **3**: 5-13.

[45] Yuzhakov VV, Yakovleva ND, Kvetnoy IM, Ulitina YD (1994) Radiation pathomorphology of the gastrointestinal endocrine cells. Med Radiol (Moscow), **39**: 39-45 (in Russian).

Discussion with Reviewers

Z. Somosy: Are there any differences in the magnitude of the radiation-induced reductions in endocrine cells found in crypt versus those found in the villus?

Authors: We have quantitative results received in rats after gamma-radiation with a dose of 5 Gy [45], but not included in this review, that such differences are available and start to manifest 1 day later after total irradiation. In 3 days, the number of endocrine cells in duo-

denal crypts is reliably decreasing by half while their quantitative density in the villus is higher for this period of time; however, the difference between irradiated and rats that were not irradiated was not statistically significant, $p > 0.05$. During the next period of time, the quantitative dynamics of endocrine cells in crypts and villus has a distinct phase character.

Z. Somosy: Is there any relationship between the two results: that is (a) the number of cells at the 3rd postirradiation day nadir and (b) the turnover time of the so-called enteroendocrine cells, i.e., 3.9 days in the duodenum and 4.0 days in the jejunum [6]?
Authors: We think that there is a direct relationship between these results. In 3 days, stem cells damage, which has a dose dependence seems to develop as much as possible. It should be noted that in radiation damage turnover time of enteroendocrine cells would probably differ from the data received by Cheng and Leblond.

Reviewer II: The use of 5-hydroxytryptophane (5-HTP) as a precursor instead of DOPA is indicated when studying serotonin-containing APUD cells. Why did the authors not use this approach?
Authors: Unfortunately, we had no opportunity to use tritium-labeled 5-HTP to study serotonin-containing APUD cells. As known, such research has been carried out previously [i.e., Rubin W, Gershon MD, Ross LL (1971) Electron microscope radioautographic identification of serotonin-synthesizing cell in the mouth gastric mucosa. J Cell Biol **50**: 399-415].

H.L. Waldum: What is the role of the neuroendocrine cells in postradiation fibrosis?
Authors: We think that tissue hypoxia develops as a result of the simultaneous release of vasoactive substances from neuroendocrine cells. This response appears to be one of the reasons for the noted late arising pathologic effects, including postradiation fibrosis. To our mind, this problem should be a subject of thorough study and discussions among specialists in radiation medicine.

H.L. Waldum: Can the authors discuss the impact of new knowledge that the neuroendocrine cells are able to self-replicate?
Authors: We are in the process of carrying out the specific studies to address this question, i.e., the capability of endocrine cells to self-replicate, especially following radiation insult.

Reviewer V: One of the major problems is that relatively unspecific methods for identification of endocrine cells have been used. Thus, e.g., argyrophil cells comprise several distinct types of endocrine cells with differing functional properties.
Authors: Methods of silver stains, argentaffin/Masson and argyrophil/Grimelius, appear to be rather sensitive in the study of endocrine cells, easily applicable and inexpensive when it is necessary to analyze and count hundreds of samples for the quantitative analysis. There is an evidence that argyrophilia of endocrine cells correlates with immunohistochemical identification of chromogranin A in them (see reference [29]).

Reviewer V: The illustrations provided include some questionable transmission electron micrographs, e.g., the Figure 5d legend the cell shown is stated to be a G cell; this is rather an EC cell. G cell granules are clearly different from those shown in the figure. Figure 5c is supposed to show accumulation of ECL cell granules. This is impossible to judge without an accompanying control cell. In Figures 9 and 10, how where these cells identified?
Authors: The types of endocrine cells were identified under the specific character of secretory granules ultrastructure.

Despite the fact that secretory granules are relatively resistant to gamma-irradiation, the developing edema of cytoplasm influences their ultrastructure. Cell granules showed in Figure 5d have mainly a round form and variable electron-density which are typical of G-cells, as mentioned in the literature.

Figure 5c shows the excess accumulation of ECL cell granules which is found only in some cells in the sites of the developing edema of tissues. The figures presented in the paper of Bottcher *et al.* (1989) Cell Tissue Res **256**: 247-257 show a similar picture for daily treatment with large doses of antisecretory agent omeprazole.

Figures 9 and 10 demonstrate the dynamics of argentaffin and argyrophil endocrine cells.

Reviewer V: Do the EC cells along the gastrointestinal tract show a uniform reaction pattern upon radiation?
Authors: In the early periods following gamma-radiation, the decrease in argentaffin reaction and numerical density (ND) of EC cells is observed in pylorus, duodenum and large intestine. However, ND recovery of these cells and phase character of dynamics with the same radiation doses differ.

Reviewer V: How is the "reaction delay" of argyrophil cells defined?
Authors: At the same doses of radiation, the decrease in argyrophil reaction and, respectively, statistically significant reduction of ND argyrophil cells occurs later than the decrease in argentaffin reaction. We think that ionizing radiation primarily initiates serotonin release

from secretory granules, maybe by molecular diffusion due to the disturbance of ion links in granules, and that cell degranulation is an adaptive response to homeostasis change.

Reviewer V: Are there chemical data supporting a "massive release" of serotonin from EC cells upon exposure to ionizing radiation?
Authors: The results of the changes in the content of endogenous serotonin in GI tract were obtained in the sixties [for example, 21]. Results of those experiments showed that intestinal 5-HTP decreased after X-irradiation. According to literature data, 80% to 95% of endogenous serotonin in the organism are stored in EC cells of GI tract. The reduction of argentaffin reaction and immunostaining of EC cells to serotonin for the first hours after radiation exposure enables "massive release" of serotonin in these organs to be supposed.

Reviewer V: What are the practical implications of liposome drug delivery?
Authors: The opportunity to transport drugs through the layer of mucous linings by liposomes is shown in the paper.