ACID PHOSPHATASE IN THE MUCOSA OF THE SMALL INTESTINE AFTER EXPOSURE TO VARIOUS DOSES OF γ-RAYS

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It was shown by electrophoresis in agar gel and electron microscopy that acid phosphatase activity and the structure of the cells synthesizing the enzyme depend on the dose of γ-irradiation and the time elapsing thereafter. KEY WORDS: irradiation; lysosomes; acid phosphatase.

Postradiation degradation in the cells of organs and tissues develops through the participation of lysosomal hydrolases and, in particular, of acid phosphatase (AP) [1]. Investigations of this last enzyme as a rule have been confined to its histochemical study [3, 4, 10] or the biochemical determination of its activity in tissue extracts [6, 7, 9, 11]. Meanwhile information has been obtained that the tissue cells contain many different molecular forms of AP, which differ in certain properties and in their reaction to irradiation [2, 5, 11].

The object of this investigation was to study the effect of various doses of γ-rays on the activity of the various molecular forms of AP and the structure of the cells of the mucosa of the rat small intestine.

EXPERIMENTAL METHOD

Sexually mature male August rats were irradiated with ^{60}Co γ-rays on the GUB-20,000 apparatus in doses of between 100 and 15,000 rad and at a dose rate of 72-85 rad/min. Laparotomy was performed on the control and irradiated animals under pentobarbital anesthesia and the organs were washed free from blood by perfusion with 0.85% NaCl solution. Pieces of jejunum for electron-microscopic investigation were fixed in Palade's solution, taken through alcohols of increasing concentration, and embedded in a mixture of Epons. Ultrathin sections were obtained on the LKB-4800 Ultrotome, stained with lead and uranyl acetates by Reynolds' method in the writers' own modification, and studied in the JEM-5y electron microscope. For biochemical investigation scrapings of mucosa of the small intestine were homogenized in Tris-sucrose buffer and centrifuged at 105,000 g. The supernatant was subjected to electrophoresis in agar gel. The AP fractions were detected by incubation of the agar gels in β-naphthyl phosphate solution with Fast Blue B in acetate buffer at pH 4.8. Activity of the fractions was determined by densitometry on an ERI-10 integrating extinctiometer. Details of the method of electrophoretic investigation of AP were described previously [2].

EXPERIMENTAL RESULTS

Up to four fractions of AP were identified in the mucosa of the small intestine (Fig. 1), and activity of three of them (1, 2, and 4), although relatively high in intact animals, was considerably disturbed after irradiation (Figs. 1 and 2). Irradiation in a dose of 350 rad caused a sharp rise in the activity of fraction 1 of AP on the 1st and 15th days, and of fractions 2 and 4 of AP on the first and seventh days of observation (Fig. 2). Irradiation in a dose of 700 rad lowered the activity of fraction 1 of AP on the first to seventh days but increased it toward the 15th day (Fig. 2). Activity of fraction 4 was significantly reduced on the fourth and seventh days after irradiation. At other times of observation AP activity in the mucosa after irradiation in this dose did not differ significantly from normal.

Irradiation in a dose of 1000 rad was accompanied by an increase in the activity of fraction 1 of AP after 3 h, followed by a decrease to the normal level. The activity of fraction 2 of AP fell on the first and at the be-

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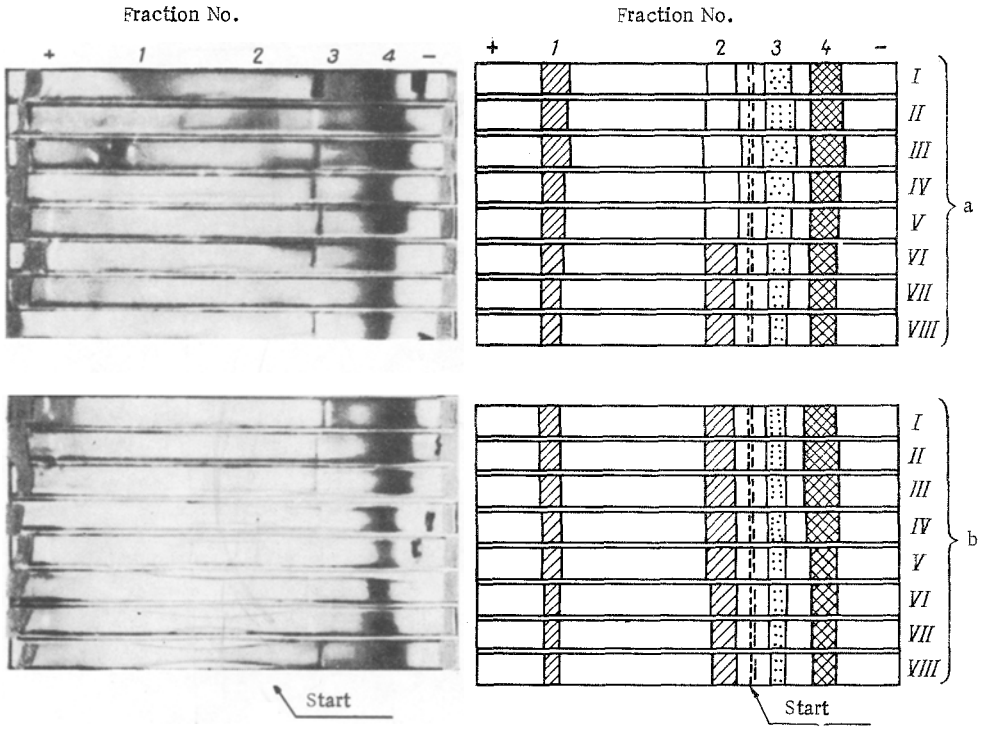

Fig. 1. Electrophoresis (actual and schematic) of AP in mucosa of rat small intestine 1 day (a) and 3 days (b) after γ-irradiation. I) Control; II-VIII) after irradiation with 100 (II), 350 (III), 700 (IV), 1000 (V), 3000 (VI), 7000 (VII), and 15,000 rad (VIII). Intensity of shading of fractions on scheme corresponds to their activity.

ginning on the fourth day, whereas activity of fraction 4 of AP fell only on the third day and rose again toward 76 h after irradiation (Fig. 2).

Disturbances of AP activity after irradiation in doses of 3000, 7000, and 15,000 rad were similar, although they depended to a certain degree on the dose of irradiation: They consisted of a sharp rise in the activity of all AP fractions 3 h after irradiation, especially in a dose of 15,000 rad, a marked fall in their activity on the first to second days, some increase again toward the end of the third day, and returned to the normal level or higher by 76 h (Fig. 2).

Electron-microscopic investigation of the mucosa of the small intestine 3 h after irradiation of the rats in doses of 1000 rad or more showed the accumulation of cytolysosomes in the proliferating cells of the crypts. Their quantitative and qualitative composition was virtually independent of the dose of irradiation, but with an increase in the dose there was an increase in the number of lysosomes and multivesicular bodies in the enterocytes of the rugae of the mucosa and also in the number of destroyed stromal cells (plasma cells, lymphocytes, mast cells), together with activation of the macrophages (Fig. 3).

Toward the end of the first day after irradiation most cells of the epidermis contained no cytolysosomes, and only after large doses (7000 and 15,000 rad) were they still present as an electron-dense mass, bounded by a single membrane. Structures of this type occurred more frequently in wandering cells of the stroma and also in the fibroblasts and capillary endothelium.

In later observations the cells were completely freed from destroyed matter. Toward the end of the third and beginning of the fourth days the number of lysosomes and multivesicular structures increased.

Changes in the activity of individual AP fractions after irradiation, as these observations show, develop according to a similar pattern. Only a tendency was noticed for wider fluctuations in the content of the anodal AP fractions – 1 and 2. Disturbances of the AP content in the mucosa of the small intestine described in the literature relate mainly to changes in the total activity of the enzyme. For instance, an increase in AP activity was found during the first to second days after irradiation in a dose of 500 rad [6] or there was a brief rise followed by a fall in AP activity on the fourth to sixth days after irradiation in a dose of 600 rad [7], which corresponds to the character of the disturbances in the content of AP fractions observed in the present experiments in animals irradiated with sublethal (350 rad) or lethal (700 rad) doses of γ-rays.

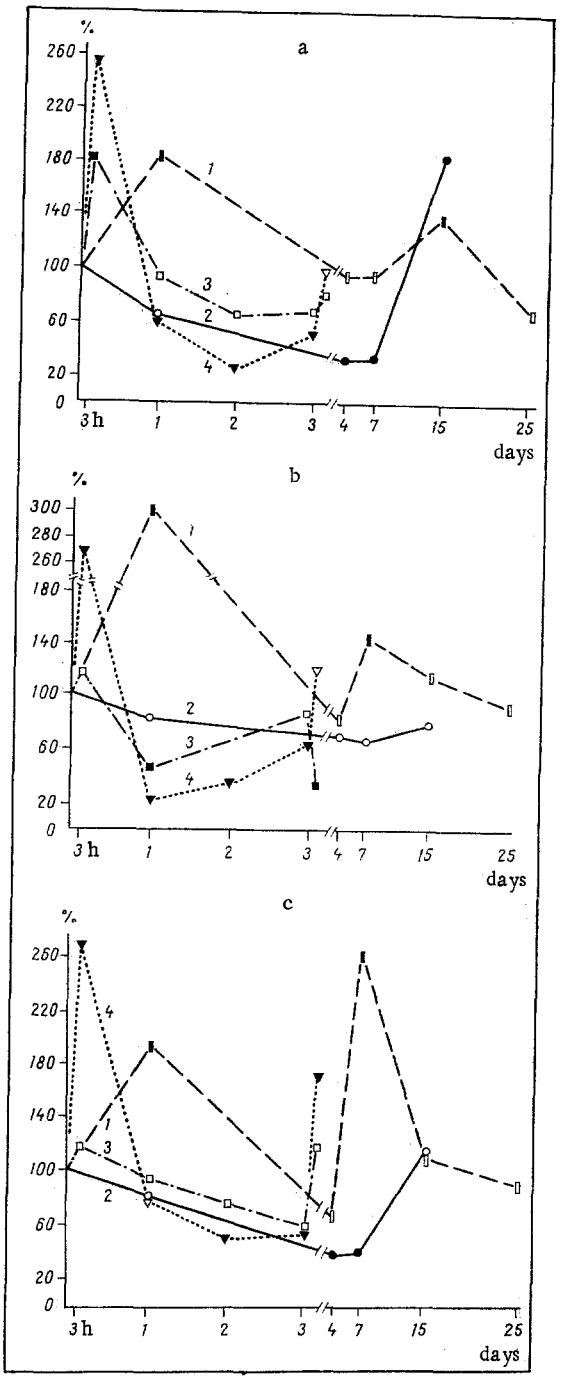

Fig. 2. Changes in activity of fractions 1 (a), 2 (b), and 4 (c) of AP at various times after γ-irradiation. 1) 350 rad, 2) 700 rad, 3) 1000 rad, 4) 15,000 rad. Ordinate, activity (in % of control); abscissa, time after irradiation. Black symbols indicate $P < 0.05$.

The sharp rise in activity of the AP fractions discovered 3 h after irradiation in an absolutely lethal dose (1000 rad) and, in particular, in superlethal doses (3000, 7000, and 15,000 rad) was undoubtedly connected with enlargement of the cytolysomes in the mucosal cells, as other workers have observed at the same times [3].

Degradation and desquamation of mature epithelial cells of the mucosa were evidently responsible for the sharp decline in activity of AP isozymes which developed during the first and second days after irradiation with absolutely lethal and superlethal doses of γ-rays. Under these circumstances giant cells, functionally im-

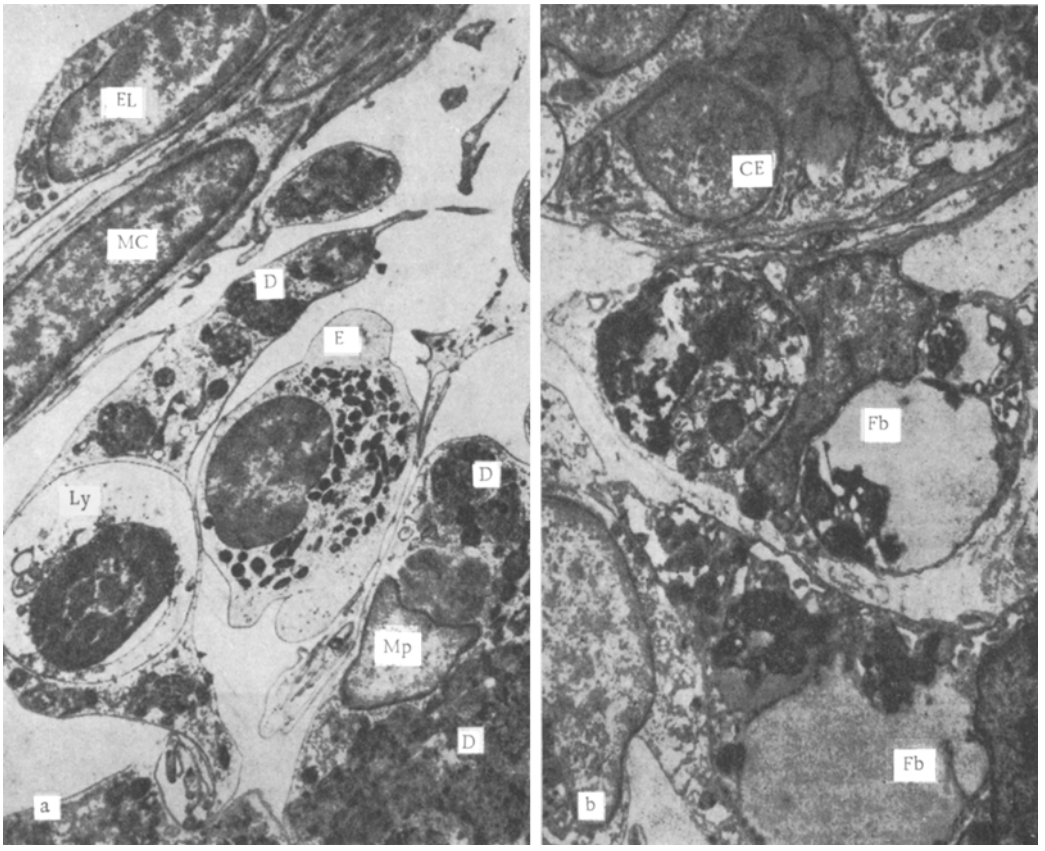

Fig. 3. Tunica propria of villus (a) and cryptal region (b) of mucosa of rat small intestine 3 and 24 h respectively after γ-irradiation in dose of 15,000 rad (5500 times). Mp) Macrophage, D) debris in Mp, Ly) lymphocyte in Mp, E) eosinophil, MC) muscle cell, EL) endothelium of lymphatic, CE) crystal epithelium, Fb) fibroblast performing role of macrophage.

perfect and poor in enzymes, begin to predominate in the cell population of the mucosa toward the third day [8]. By the fourth day processes of repair and regeneration are intensified [10], and in the present experiments this was manifested as an increase in activity of the AP isozymes.

As the results show, differences were found between the effects of irradiation in absolutely lethal and superlethal doses, despite the fact that the dominant feature of all these doses was an intestinal syndrome. The fall in AP activity 1-2 days after irradiation in a dose of 1000 rad was less marked than after superlethal doses (3000-15,000 rad), but in the last case the rise of AP activity was higher in the early periods (3 h) of observation. The early sharp response of the lysosomal apparatus to superlethal doses of irradiation was evidently accompanied by their more rapid exhaustion and by inhibition of the elimination of injured components of the tissue cells. It is these processes which develop actively after irradiation with sublethal doses of γ-rays, as reflected in the prolonged increase of AP activity. This inhibition of elimination of injured components of the mucosa may explain the relatively minor morphological disturbances in the epithelium after exposure to superlethal doses, despite the considerable reaction of the connective-tissue elements.

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ANABOLIC EFFECTIVENESS OF NITROGEN PREPARATIONS FOR PARENTERAL FEEDING IN TOXIC HEPATITIS

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Assimilation of the nitrogen preparations moriamine S-2 and improved casein hydrolysate when given parenterally to 100 albino rats was studied. Both healthy animals and animals with toxic hepatitis induced by CCl_4 were used. Administration of the nitrogen preparations to healthy animals converted the negative nitrogen balance to positive and restored the normal content of amino nitrogen in the blood and tissues when disturbed as a result of protein deprivation. In toxic hepatitis the rate of assimilation of the preparations was considerably reduced. Parenteral feeding for eight days did not convert the negative nitrogen balance into positive and did not abolish the hypoproteinemia, although it restored the normal amino-nitrogen concentration in the blood and tissues. KEY WORDS: parenteral feeding; protein hydrolysates; amino acid mixtures; assimilability of administered nitrogen; toxic hepatitis.

Parenteral feeding in clinical practice is used in the treatment of patients with considerable disturbances of tissue metabolism due to disturbances of the functions of the nervous and endocrine systems, pancreas, liver, and so on [1, 5, 6]. The writers have shown both experimentally and clinically that disturbances of function of the endocrine part of the pancreas and of the thyroid gland considerably limit the assimilability of parenterally administered nitrogen preparations [3, 4].

The object of this investigation was to study the role of the initial state of liver function in the assimilability of parenterally administered nitrogen preparations.

EXPERIMENTAL METHOD

Experiments were carried out on 100 albino rats of both sexes weighing 180-250 g, some of which were healthy, whereas the rest had parenchymatous hepatitis. Toxic hepatitis was induced by three subcutaneous injections of a 50% oily solution of CCl_4 in a dose of 0.5 ml/100 g body weight in the course of one week. The presence of hepatitis was confirmed histologically. The animals as a whole were divided into eight groups; the rats of six groups were kept throughout the experimental period on a synthetic protein-free diet [2], and those of the other two groups on the ordinary animal house diet. Daily for 8 days the protein-deprived rats received subcutaneous injections of physiological saline and of nitrogen preparations: moriamine S-2 (Japan) and improved casein hydrolysate (USSR) [7] in a dose of 0.3 g conventional protein/100 g body weight. Glucose solution (0.5 ml of a 40% solution/100 g body weight) was injected at the same time. The volume of fluid injected was equivalent to 7 ml/100 g body weight. Periodically the nitrogen balance of the animals and the excretion of amino nitrogen in their urine were determined. At the end of the experimental period (8 days) animals of all groups were decapitated and the concentrations of amino nitrogen and total protein were determined in their blood and the concentration of amino nitrogen in the tissues (muscle, heart, liver). Total nitrogen in the urine and feces was determined by the micro-Kjeldahl method and amino nitrogen by the ninhydrin method [8]. The nitrogen balance was judged from the difference between the nitrogen administered and excreted in the urine and feces.

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