Intestinal Enzyme Activities Following Whole-Body Irradiation in Hamsters[1] (35650)

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The sensitivity of the mammalian small intestine to ionizing radiation is reflected in an alteration of crypt cell renewal (1, 2), motility (3), and absorptive functions (4). A number of studies have indicated a decrease in glucose absorption by the small intestine of rats exposed to sublethal doses of ionizing radiation (5, 6). In hamster intestine, it has been reported that absorptive function was elevated on the second and third day and decreased on the fourth and fifth day after 1,000 R γ-irrdaiation (7). In the present report we have examined profiles of intestinal enzyme activities with increasing postirradiation time in the hamster. The conditions of irradiation were similar to those used in the studies that have shown the increase in the intestinal glucose absorption following exposure to 1,000 R γ-irradiation (7). The intestinal enzymes studied were the Mg^{2+} dependent, $(Na^+ + K^+)$-activated adenosine triphosphatase [Mg-(Na + K)-ATPase] and phosphomonoesterase.

Methods. Male and female hamsters weighing 85–90 g were given whole-body exposure of 1,000 rads (^{60}Co) at 160 rads/min from surrounding beams in a cylindrical chamber. Details of procedures and equipment are given in another report (8). Irradiated and sham-irradiated animals were sacrificed by cervical fracture at 8, 20, 32, 50, 60, 73, and 98 hr following exposure. The small intestines were removed and flushed with chilled Kreb's Ringer-bicarbonate solution and divided into three approximately equal segments. Each segment was slit open and blotted with filter paper. The mucosa was then scraped off with a glass slide and homogenized in a 0.4 m*M* tris(hydroxymethyl)aminomethane–HCl (Tris) medium, pH 7.4, using a Teflon glass tissue grinder. The wet tissue concentration in the homogenate was 0.5% (w/v). Similarly prepared homogenates from a group of control animals that were not sham-irradiated were utilized in preliminary enzyme studies. Homogenates from some of the irradiated and the sham-irradiated animals were analyzed for their protein contents using the method of Lowery *et al.* (9). One milliliter of homogenate was incubated at 37° with 2.0 m*M* adenosine triphosphate (ATP) or glucose 1-phosphate (G1P) in a 50 m*M* Tris medium, pH 7.8. The hexose phosphate was used as substrate for the phosphomonoesterase system. In selected experiments, 5 m*M* $MgCl_2$, 25 m*M* KCl and 100 m*M* NaCl were also added to the incubation medium. The total volume of reaction mixture in all experiments was 3 ml. The reactions were stopped by the addition of trichloroacetic acid (TCA) to a final concentration of 5% (w/v), and the amount of phosphorus formed was quantitated in the TCA filtrates. The procedure for phosphorus determination was adapted from the method described by Quinn and Lane (10). The specific activity of both the adenosine triphosphatase and phosphomonoesterase was expressed as microgram P formed/(mg tissue × hour).

Results. The time course of ATP and GlP hydrolysis by jejunal mucosal homogenates of control animals is shown in Fig. 1. The amount of ATP hydrolyzed was higher than the amount of GlP hydrolyzed at each of the

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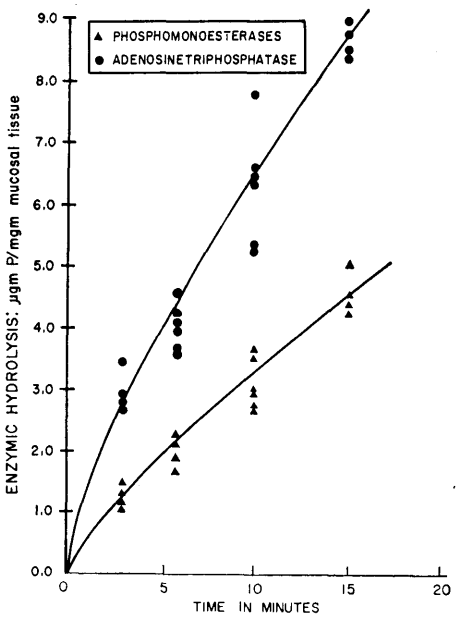

FIG. 1. Time course of enzyme activities. Each point represents an animal.

time periods studied. The ATP hydrolysis was measured in the presence of Mg^{2+}, K^+, and Na^+, and GlP reaction in the absence of ions. The hydrolysis of both substrates increased roughly in proportion to time through 15 min of incubation. The order of enzymic reactions, with respect to time, could not be established because of large variances in the data. All incubations with mucosal homogenates from sham-irradiated and irradiated animals were carried out for 15 min. Figure 2 shows the distribution of the enzyme activities in the three areas of intestine in control animals. There were no apparent differences in the ATPase activity in the three areas. However, phosphomonoesterase activity was higher in the upper two segments (duodenum, jejunum) than in the lower segment (ileum). Only the middle segment (jejunum) was used in experiments with sham-irradiated and irradiated animals.

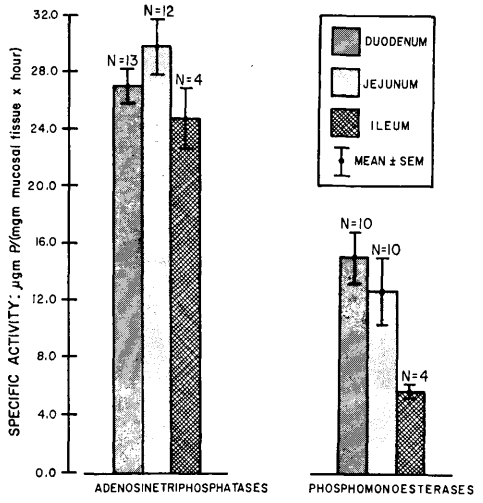

FIG. 2. Enzyme specific activities along the intestinal length. N = number of animals.

The effect of 5 mM Mg^{2+}, 10 mM K^+, and 100 mM Na^+ on the hydrolysis of the two substrates by jejunal homogenates of control animals is shown in Fig. 3. Both substrates were hydrolyzed to the same extent in the absence of ions. The addition of ions, however, enhanced hydrolysis of ATP only. It appears that ATP was hydrolyzed by nonspecific as well as specific ion-requiring enzymes. This specific ion effect on ATPase also explains the higher amounts of ATP than GlP hydrolyzed at various incubation times (shown in Fig. 1). The ATPase in the irradiated and the sham-irradiated animals was measured both with and without the added ions. The increase due to addition of ions was taken to represent the Mg-(Na + K)-ATPase activity. The phosphomonoesterase

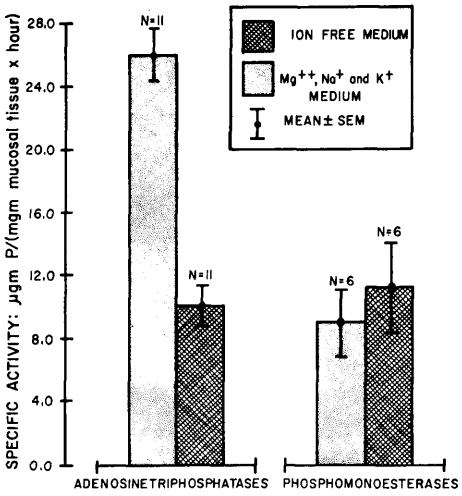

FIG. 3. Effect of ions on enzyme specific activities, paired data. N = number of animals.

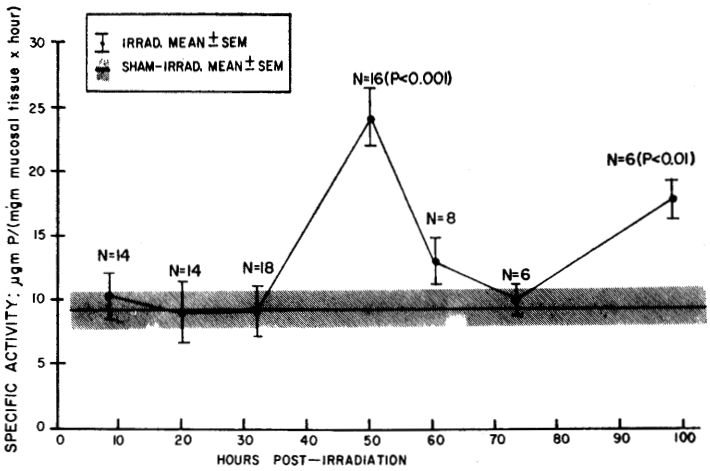

FIG. 4. Phosphomonoesterase activities as a function of postirradiation time. N = number of animals. The sham-irradiation data represents 27 animals (See "*Results*" for further explanation).

activities of the irradiated and the sham-irradiated animals were determined in the absence of ions.

Three or four sham-irradiated animals were used at each postirradiation time. The total number of sham-irradiated animals studied was 27. Since there were no significant differences in either Mg-(Na + K)-ATPase or phosphomonoesterase activity of sham-irradiated animals at varying postirradiation times, the data from the 27 animals were pooled and are shown in Figs. 4 and 5 along with the data obtained from irradiated animals. The phosphomonoesterase activity (Fig. 4) of irradiated animals was unaltered through 32 hr postirradiation, increased about 150% at 50 hr, and at 70 hr it was comparable to activities measured through the initial 32 hr. A second rise in phosphomonoesterase activity was observed at 98 hr following irradiation. The ATPase activity (Fig. 5) of irradiated animals exhibited a somewhat similar pattern except that an increase in activity (about 90%) at 60 hr and a decrease to below sham-irradiation level at 98 hours postirradiation were observed. The protein contents of jejunal homogenates from sham-irradiated and irradiated animals are shown in Table I. There were no differences in the concentration of protein between the mucosal preparations of sham and any of the groups of irradiated animals. Neither were there any significant differences in the protein contents of irradiated animals.

Discussion. This study demonstrated that hamster jejunal mucosal phosphomonoesterase and Mg-(Na + K)-ATPase activity per unit of tissue weight is elevated at approximately 2 days after 1,000 rads single whole-body ^{60}Co exposure. Increase in the specific activity as well as in the total organ activity of acid and alkaline hydrolases has been shown in rat intestinal mucosa following 1,000 R γ-irradiation by Wrigglesworth and Pover (11). These authors have attributed this increase in enzyme activities to an *in situ* increase in enzyme synthesis and/or invasion of damaged cells by enzyme-rich epithelial cells. The increase in the enzymic activities following irradiation was found to be ap-

TABLE I. Protein Contents of Hamster Intestinal Mucosa.

Animal treatment	No. of animals	Protein[a]
Sham-irradiated	13	61.90 ± 1.78
Irradiated:		
Group 1, 10 hr PR[b]	7	63.81 ± 2.95
Group 2, 20 hr PR	8	67.27 ± 4.00
Group 3, 32 hr PR	5	64.11 ± 4.10
Group 4, 50 hr PR	6	61.56 ± 5.41
Group 5, 60 hr PR	4	52.63 ± 4.27
Group 6, 60 hr PR	3	61.51 ± 6.54
Group 7, 98 hr PR	3	59.49 ± 0.32

[a] Mean ± SE values have units of mg/g tissue weight.

[b] PR is postirradiation.

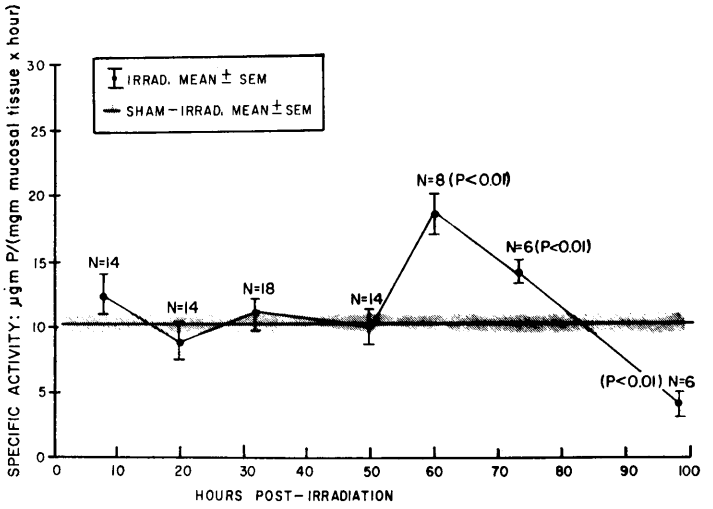

FIG. 5. Mg-(Na + K)-ATPase activities as a function of postirradiation time. N = number of animals. The sham-irradiation data represents 27 animals.

proximately twofold on a unit tissue weight basis in this study. This twofold increase in the specific enzyme activity could be produced by a 50% reduction in intestine weight. Intestinal weight loss in hamster and guinea pig is known to be much less pronounced than in rat and mouse intestines (3). Since the percentage of the loss of weight in rat and mouse intestines after 950 R of x-irradiation has been shown to be about 35 and 20%, respectively (3), it is unlikely that hamster intestinal weight would be reduced to half following 1,000 R γ-irradiation. Thus, the increase in specific enzyme activity in hamster intestine is not entirely due to a loss of intestinal weight. Furthermore, the increase in enzyme activities was not related to changes in protein content per unit tissue weight as protein was constant in sham and irradiated intestines.

The phosphomonoesterase and Mg-(Na + K)-ATPase are known to be located in the microvilli of the mature epithelial cells (12). The epithelial cells harvested from hamster intestine 2 days after irradiation could have been derived in part from irradiated crypt cells which had matured precociously following a prolonged interphase during the cell renewal. A prolongation of interphase in crypt cells exposed to radiation has been shown (2, 13). Such a prolonged interphase could allow an increased synthesis of enzyme protein and may be related to the increased phosphomonoesterase and Mg-(Na + K)-ATPase activities found in this study. The elevation of Mg-(Na + K)-ATPase and phosphomonoesterase activity may be associated with an increased activity of the sodium pump and the absorptive functions of the mature epithelial cells. That the (Na + K)-ATPase plays a key role in the sodium transport process has been shown in frog skin (14), and in red blood cells (15). This enzyme may also be involved in the active glucose absorption by small intestine (16). While the progressive decline in ATPase activity beyond Day 2 may be related to the decrease of glucose absorption (7), the second increase of phosphomonoesterase activity at Day 4 cannot be explained.

In conclusion, these studies showed that the phosphomonoesterase and Mg-(Na + K)-ATPase activities were higher in the intestinal mucosa of hamsters 2 days after irradiation as compared to sham-irradiated animals. Phosphomonoesterase activity was also increased at Day 4 postirradiation. However, ATPase activity was significantly lowered. These modifications, particularly the ATPase activity, at Day 2 were interpreted as corresponding to the increased levels of glucose absorption in an earlier experiment (7) where animals were similarly treated.

Summary. The phosphomonoesterase and

Mg-(Na + K)-adenosine triphosphatase (ATPase) activities were measured in the mucosa of the small intestine of hamsters (*Mesocricetus auratus*) exposed to 1,000 rads ^{60}Co whole-body irradiation. The enzyme assays were made in irradiated and sham-irradiated hamsters sacrificed at intervals through Day 4 postirradiation. The two enzymic activities expressed per unit of tissue weight were significantly higher in the irradiated animals as compared to the sham-irradiated group at Day 2 postirradiation. No changes were found in the protein contents of jejunal mucosa between irradiated and sham-irradiated animals. While the ATPase activity was significantly lowered at Day 4, the phosphomonoesterase activity was observed to be higher at that time. The increase in enzyme activities may represent increased synthesis of these enzymes in the crypt cells that gave origin to the mature cells of the intestinal villi of animals sacrificed at Day 2 postirradiation. The increase in ATPase activity at Day 2 followed by a decrease at Day 4 corresponds to the previously observed alterations in the intestinal glucose absorption by irradiated hamsters.

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