INFLUENCE OF WHOLE-BODY IRRADIATION ON CALCIUM TRANSPORT IN RAT SMALL INTESTINE

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The everted gut-sac technique was used to evaluate radiation effects on calcium transport in isolated duodenal and jejunal segments of rat small intestine. Whole-body irradiation (900 rads) significantly reduced duodenal transport at days 2 through 5 post-irradiation. It was concluded that radiation effects were greatest in segments of intestine which transport calcium most actively. The biological significance of these observations is discussed.

Cells which are immature or possess a high rate of mitotic activity are in general very sensitive to radiation damage (1). Therefore, the rat intestinal epithelium, which completely regenerates every 3 to 5 days, is an extremely radiosensitive tissue (2). Marked alterations in the physiologic function of the gastrointestinal tract are produced by relatively small doses of radiation. Symptoms of whole-body irradiation in the rat are anorexia, dehydration, diarrhea, reduced gastric emptying, and intestinal propulsive motility (3). Further, irradiation has previously been shown to reduce the intestinal absorption of carbohydrate, fatty acids, sodium, and fluid (4-7). Perris has proposed that radiation may disrupt all active transport processes in the small intestine (8).

The object of the present study was to examine the influence of whole-body irradiation on calcium transport, which is known to be an energy requiring-process (9).

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 100-200 g, were used in this study. During time-course irradiation experiments, the animals were fed Wayne Lab Blox and tap water *ad libitum*. In the adaptation-irradiation study, dietary groups were fed either a low calcium diet (LCD) (containing < 0.02% calcium) (10), a normal diet (ND) (LCD supplemented with 1.5% calcium), or a high calcium diet (HCD) (LCD supplemented with 5% calcium) and deionized drinking water *ad libitum*.

Animals were exposed individually to whole-body doses of cobalt-60 radiation by means of a gamma-irradiator (U.S. Nuclear Corp, Model GR-12). The radiation source, which provided a dose rate of approximately 200 rads/min, was arranged so that the radiation flux over the volume of the irradiation chamber varied less than 5% (11). Each animal was placed in a transparent lucite chamber during irradiation and the controls were sham-irradiated under the same conditions. A dose of 900 rads was used throughout this study to produce biologically significant radiation effects. All animals were fasted for 24 hr before irradiation, which was carried out between 10 a.m. and 3 p.m.

The *in vitro* everted gut-sac technique used in this study is a modification (12) of the method of Wiseman (13). Animals which had been fasted for 24 hr were killed by cervical fracture and the small intestine removed, everted, and rinsed thoroughly in isotonic saline. A 5-cm segment of intestine (either duodenum or jejunum) was drained completely and filled with 0.5 ml of a phosphate buffer which contained dibasic sodium phosphate 2.4 mM, primary sodium phosphate 1.6 mM, sodium chloride 150 mM, calcium chloride 0.25 mM, calcium-45, 0.025 μCi, and glucose 5.6 mM, and had been titrated to pH 7.4 with 0.1 N HCl. The gut-sac was ligated securely and placed in a 25-ml Erlenmeyer flask containing 3 ml of the same buffer solution. The flasks were incubated for 1 hr at 37 C under an atmosphere of 95% O_2 and 5% CO_2.

At the end of the incubation period, a 0.1-ml aliquot of mucosal media (external solution bathing the gut-sac) and serosal

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media (internal solution) were solubilized in 10 ml of scintillation cocktail which contained 380 ml of Biosolve BBS-3 (Beckman), 3420 ml of toluene, and 32.2 g of Fluoralloy (Beckman). The samples were counted on a liquid scintillation counter (Picker Nuclear, Model 650), and quenching was determined by means of the counter external standard. Counting times were automatically adjusted to obtain a counting error of less than 5% for each sample. The ratio of calcium-45 concentration in the serosal medium (S) to the concentration of calcium-45 in the mucosal medium (M) (designated S/M) provides an index of active calcium transport.

The term "adaptation" used in this study refers to a statistically significant increase in calcium transport as a result of dietary calcium deprivation.

The 2-tailed Student's t-test was used in statistical comparisons between experimental groups.

RESULTS

The effect of 900 rads whole-body irradiation on duodenal calcium transport at 2, 3, 4, and 5 days post-irradiation is illustrated in Figure 1. Irradiation produced an obvious reduction in active transport at days 2 through 5. The greatest decrease was observed at day 3 ($P < 0.001$); however, there were also significant reductions in transport at days 2 ($P < 0.05$) and 5 ($P < 0.005$).

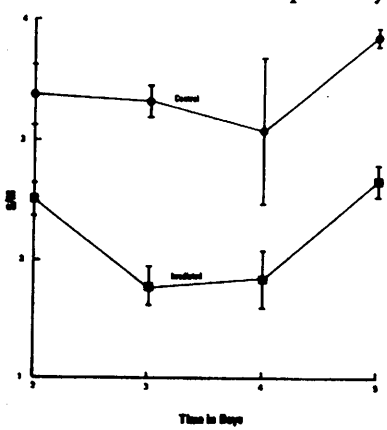

FIGURE 1. Influence of irradiation on duodenal calcium transport as measured by an everted gut-sac technique *in vitro*. The irradiated group received 900 rads on day 0. Each point represents the mean S/M of 5 rats ± S.E.M.

In the adaptation-irradiation experiments, both duodenal and jejunal calcium transport were examined at 10-day intervals and at 3 days after irradiation (day 33). The LCD produced a marked increase in duodenal calcium transport at 20 to 30 days after initiation of the experimental diets (Fig. 2). Duodenal transport in the LCD group

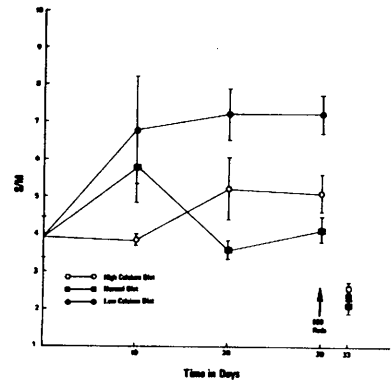

FIGURE 2. Influence of dietary calcium and irradiation on duodenal calcium transport as measured by an everted gut-sac technique *in vitro*. Each point represent the mean S/M of 5 rats ± S.E.M.

was significantly greater than in the HCD group at 10 days ($P < 0.005$), significantly greater than in the ND group at day 20 ($P < 0.001$), and at 30 days greater than in either the HCD group ($P < 0.025$) or the ND group ($P < 0.001$). Three days after irradiation with 900 rads (day 33), there was a marked decrease in the transport of all three dietary groups with no significant differences between them.

The calcium-transporting capacity of the jejunal segments was slightly enhanced by the LCD (Fig. 3). At days 10 and 20 jejunal transport in the LCD group was significantly greater than in either the ND or HCD group ($P < 0.05$), and at day 30 it was greater than in the ND group ($P < 0.05$). Irradiation produced a small decrease in jejunal transport in the LCD and HCD

groups at day 33, but did not alter transport in the ND group.

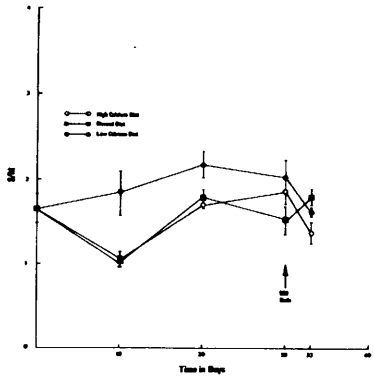

FIGURE 3. Influence of dietary calcium and irradiation on jejunal calcium transport as measured by an everted gut-sac technique *in vitro*. Each point represents the mean S/M of 5 rats ± S.E.M.

DISCUSSION

Calcium transport, which is most active in the proximal end of the rat intestine, decreases progressively in more posterior segments and becomes passive in the mid-intestine (9). For this reason, duodenal segments were used in the initial transport experiments to define clearly the influence of whole-body irradiation. Duodenal calcium transport was markedly depressed at days 2 through 5 post-irradiation (Fig. 1), a finding which is consistent with previously observed effects of irradiation on other active transport systems in the intestine (4, 5, 6, 8). The time-course of irradiation effects on intestinal transport capacity probably reflects the fact that the mucosal cells which occupy the absorptive surface of intestine at days 2 to 5 were immature, mitotically active, and, therefore, extremely sensitive to radiation damage at day 0 when irradiation occurred.

During periods of calcium deprivation, the intestine adapts to the dietary deficiency by transporting calcium more actively (14). Parathyroid hormone is believed to be responsible for acute adaptation (15). In the present study, it was reasoned that low calcium adaptation might protect the calcium-transporting mechanism against radiation. However, it appears that adaptation offered little or no radioresistance in duodenal segments (Fig. 2). The jejunum, which transported calcium less actively than the duodenum, was not significantly influenced by radiation (Fig. 3).

Lengemann (16) and Lengemann and Comar (17), who measured skeletal retention of orally administered radiocalcium, reported an increased calcium absorption at 24 to 48 hr post-irradiation. This observation appears to be in conflict with the present results. However, the fact that the duodenum represents only a very small fraction of small intestine, the known influence of radiation on peristalsis (3), and the presently observed lack of radiation influence on the jejunum indicate that the previously reported increased calcium absorption is due to the greater residency time of ingesta in the small intestine (17). Therefore, it appears that the net intestinal absorption of calcium may be enhanced by radiation while duodenal transport is inhibited.

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