Dose Dependence and Sequential Changes in Mouse Small Intestinal Weight Induced by Ionizing Radiation.* (21196)

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The marked sensitivity of the gastrointestinal tract and particularly the small intestine of the rat to ionizing radiation has been recently demonstrated quantitatively by measuring weight changes in components of the gastrointestinal tract at various times after irradiation(1). Further work has shown that the mouse small intestine responds in a similar manner (though to a less extent) and that the degree of weight loss, when measured 2 days postirradiation, was dose dependent throughout the lethal range. This technic was designed and used successfully for biological measurement of rem dosage of neutron radiation in atomic bomb field tests. Studies of this dose dependent response of the small intestine of mice to 2000 KVP X-radiation and gamma radiation (Co^{60}) and the sequential weight changes with the latter radiation are presented in this paper.

Materials and methods. Male mice weighing between 18 and 28 g, of the CF (Carworth Farms) strain, were used in the study with 2000 KVP X-ray and male white Swiss strain were used in the gamma-ray study. All animals were randomly selected into control and experimental groups. *Groups of CF mice* were exposed, each mouse in a single cell, in a masonite-wooden constructed unit which was curved to conform to an arc of a circle at 2 meters distance from the radial beam of a 2000 KVP G. E. Industrial X-ray machine (see previous publication(2) for details of this exposure unit). Groups of 17 mice were exposed to doses of 400, 600, 800, 950, 1100, 1300, and 3000 r. Thirty-four control mice were placed in the exposure cages for comparable lengths of time but not irradiated. Radiation factors were: 2000 KVP, 1.5 ma; no added filter; intensity of the radial beam was 15.0 ($\pm$0.15) r per minute in air at 2 meters; h.v.l. 4.3 mm Pb. The mean effective energy of the beam was .58 Mev. The mice in this study were all sacrificed at 2 days postirradiation for gastrointestinal weights. Preliminary work showed that the 2-day time period gave the best index of dose dependence in gut weight. *Groups of white Swiss mice* were exposed to gamma-ray doses of 150, 350, 550, 750, and 950 r for each of the following sequential time studies: 12, 24, 36 hrs, and 2, 3, 4, 5, 7, 10, and 12 days postirradiation. Five experimental and 5 control animals were used for each group, except for the larger dose and time periods where extra experimental mice were used. This study was repeated about a month later in parallel using the same number of mice. The gamma-ray dose was delivered in a constant time of 10 minutes from an approximately 4 π source of cobalt^{60} (1.23 Mev) irradiator(3). The 30-day LD_{50} for these mice with this radiation was 690 r. Doses were measured in air with cages in place by a Victoreen roentgen rate meter using a 4 mm lucite cap. The cobalt was housed in aluminum capsules which filtered out the incident beta radiation. The radiation volume was uniform throughout an area 6 inches in length by 9 inches in diameter. The various prescribed doses were obtained by varying the number of cobalt capsules. In both studies the mice were sacrificed with chloroform and the small intestine was stripped free of mesentery after severing at the pyloric sphincter and ileocecal junctions respectively. The intestines were then opened, washed, and placed in small tared aluminum pans and placed in a 95°C oven and weighed after 18 hours. This time period proved adequate to achieve constant dry weights. The mean dry weights of each group were expressed as the percentage of the mean dry weight of the unirradiated controls. In the gamma-ray study, data from the 2 parallel studies were combined, since an analysis of variance showed that this was statistically valid. (Since this study acted as a

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TABLE I. Effect of Radiation on Mouse Small Intestinal Weight. Mean intestinal wt (mg) ± stand. error of mean.

Time after irrad.	No. mice /group*	2000 KVP 0 r	400 r	600 r	800 r	950 r	1100 r	1300 r	3000 r
Controls	34	313±6.9							
2 days	17		258±6.2	234±5.9	215±7.6	193±6.1	194±7.3	188±6.2	183±5.3

		Gamma radiation 0 r	150 r	350 r	550 r	750 r	950 r
Controls	99	263 ± 8.3					
12 hr	10		261 ± 7.7	252 ± 5.7	259 ±11.4	257 ± 9.0	231 ± 9.3
24	10		261 ±12.6	247 ± 8.9	238 ±11.5	227 ± 9.1	241 ± 8.8
36	10		257 ± 6.6	252 ±11.5	227 ± 7.5	212 ± 7.1	214 ± 8.7
2 days	10		261 ±10.6	238 ± 7.9	235 ± 6.5	200 ± 8.0	174 ± 8.6
3	10		247 ± 6.2	264 ±11.2	277 ±12.3	228 ±19.5	170 ± 9.6 (9 of 10)
4	10		264 ±16.3	256 ±13.3	290 ±10.9	296 ± 5.9	237 ±15.7 (7 of 10)
5	10		294 ±16.6	285 ± 9.6	249 ± 5.4	302 ±11.0	293 ±15.6 (8 of 10)
7	10		269 ± 9.2	266 ±11.9 (9 of 10)	272 ±11.5	267 ±23.4 (9 of 10)	266 ± 8.6 (17 of 18)
10	10		259 ± 9.9	272 ±12.1	266 ±11.5	234 ±10.3	198 ±16.4 (5 of 15)
12	10		265 ±13.2	293 ± 7.9	266 ±18.9	237 ±19.6 (9 of 10)	223 (1 of 20)

* Where numbers of mice in a group differ due to deaths or added mice, number of living followed by original number is presented in parenthesis after stand. error of mean.

control for another study, the mice received isotonic saline (1 ml/100 g wt) subcutaneously 25 minutes before irradiation).

Results and discussion. Table I shows the mean small intestinal weight and the standard error of the mean of groups of mice at various doses and times after 2000 KVP X-ray and gamma radiation. These data indicate a relation between the 2-day small intestinal weight and the amount of radiation that is described by the equation: $Y = a-bx$, where Y is intestinal weight and x equals dose in r. Using the variation in individual gut weights within groups as the error variance, this equation was found to adequately represent the relation between small intestinal weight and the dose of radiation. In Fig. 1, this relation has been converted into percentage values. Both studies showed dose dependence of intestinal weight loss through 950 r. Least square lines have been fitted to the data through 950 r.

The difference in response of the Swiss gamma-treated and the C. F. 2000 KVP X-ray treated mice may be due to strain differences. However, the difference may be partly due to difference in tissue dose from the two types of radiation.

In the 2000 KVP X-ray experiment, it can be seen (Fig. 1) that at 950 r a maximum weight loss of the intestine was attained on the second postirradiation day and a plateau effect developed with higher doses. At about this dose and above, one also sees early deaths (4 to 6 days postirradiation) in mice which is believed to be associated with severe gastrointestinal damage(4-6). Thus, a radiation dose of around 950 r and above probably represents a critical level of gastrointestinal damage which invokes the early mechanism of death. Williams'(7) studies on mitotic inhibition in the mouse small intestinal epithelium after X-irradiation indicate that doses of this magnitude also produce a maximum suppression of mitosis at about the same time (2 days). Comparing 2-day small intestinal weight loss in sexually mature Sprague-Dawley rats using 200 KVP X-ray with such changes in mature C. F. mice using 250 KVP X-ray, the rat intestine turns out to be about 1.7 times as sensitive as the mouse intestine based

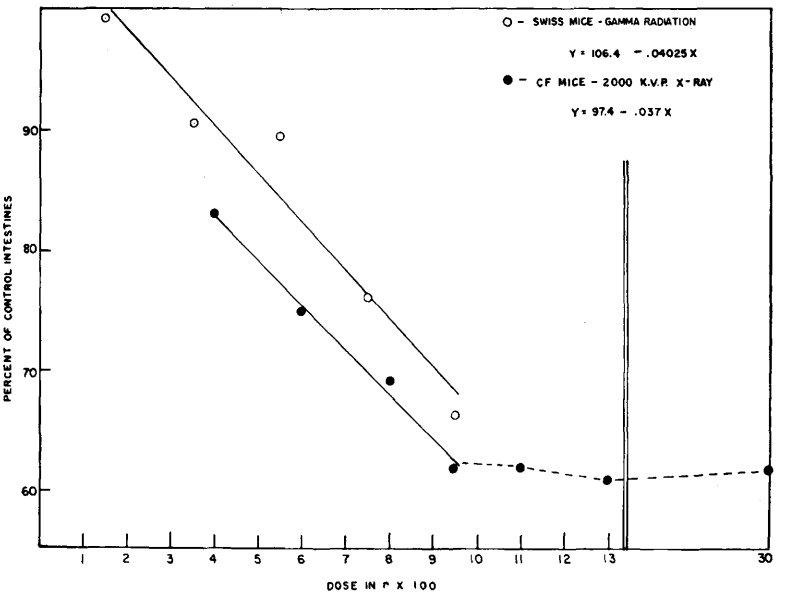

FIG. 1. Effect of irradiation on mouse small intestinal weights 2 days post-irradiation.

on this criterion. This conforms to the gross and histological observations that rats have more sensitive gastrointestinal tracts and exhibit early deaths at lower percentage mortality doses.

Radiation-induced weight loss in the thymus and spleen has also been shown by Carter, *et al.*(8) to be dose dependent when measured 5 days postirradiation and both have been used extensively as biological dosimeters. Body weight loss has also been shown to be an index of radiation dosage(9,10). This is probably a more complicated resultant of radiation damage, whereas weight loss in the thymus and spleen represents primarily lymphoid degeneration and in the gut is associated primarily with epithelial damage.

Sequential weight changes of groups of mice after exposure to various doses of gamma radiation are shown in Table I and Fig. 2. Since the 150 and 350 r doses showed the characteristic responses to a considerably less degree, only the curves for 550, 750, and 950 r were plotted. It can be seen that the gut lost weight rather rapidly to the second and third day, the maximum weight loss being somewhat later with increasing dose. This initial weight loss increased with dose to 950 r and as was shown above, the second day weight loss serves as a useful index of radiation dose. It has been pointed out(1) that this initial weight loss was believed to be largely due to destruction as well as failure of normal replacement of epithelium due to mitotic inhibition.

The initial weight loss was followed by recovery of weight with an overshoot above the control range, in all doses used. Recovery began somewhat later with increasing dose, the recovery peak tending to be delayed. The degree of overshoot appeared to increase with dose and initial weight loss up to 750 r. Thus, up to 750 r, it would appear that the amount of overshoot is in some way related to the amount of weight loss induced. However, even with 950 r (in the mice that did not succumb to early death) there was remarkable ability of the intestine to regenerate as shown by the 5-day recovery above the control range. Again, in regard to mitotic studies referred to above(7), it is interesting that there appeared to be an increase in mitosis above the normal rate in the gut epithelium which corresponded in general with the dose and time involved in these weight studies.

By the seventh day following the recovery peak, the intestine returned to the control weight range with all doses and remained with-

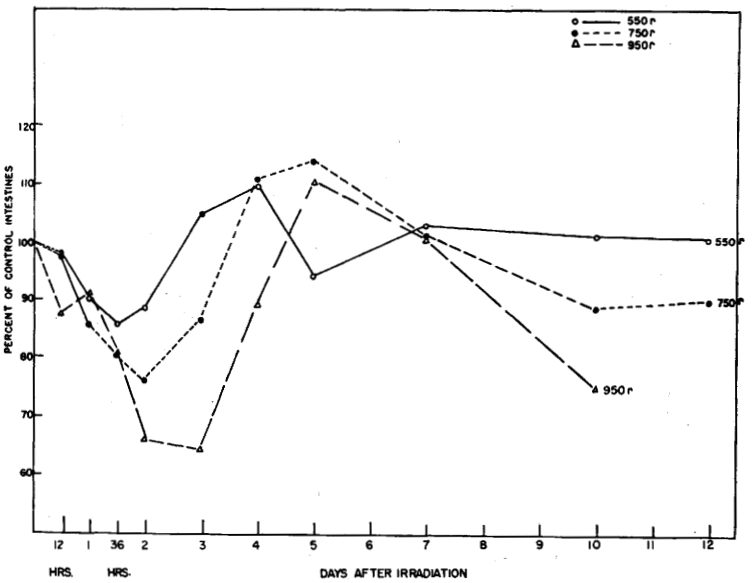

FIG. 2. Sequential weight changes of small intestine of mice exposed to various doses of gamma radiation.

in this range with doses through 550 r. However, with the 750 r and 950 r doses, a secondary depression of gut weight occurred, on the 10th and 12th days. However, (Table I) only survivors can be included with the higher doses (less than 10 animals) at later times of sacrifice, which would if anything lead to an underestimation of the weight loss at this time. This secondary weight loss may be related to general nutritional changes associated with the sequelae of pancytopenia which is believed to be responsible for mortality at this time.

The technic of measurement of radiation-induced weight change in the small intestine of animals, if used under properly controlled conditions, is a useful tool in the field of biological dosimetry. One may determine dosage or rem, relative biological effectiveness of various types of radiation, strain and species differences in response, effects of protective agents, and the relative sensitivity of the gastrointestinal tract to various qualities of radiation compared to other tissues.

Summary. The effect of 2000 KVP X-ray and gamma (Co^{60}) irradiation on the weights of the small intestine of C. F. and white Swiss mice was presented. Weight loss in the small intestine showed dose dependence when measured 2 days postirradiation with doses of 150-950 r. Doses above 950 r showed no further weight loss at 2 days resulting in a plateau effect. It was pointed out that under properly controlled conditions, this technic may be used as a biological dosimeter in determining dosage or rem, relative biological effectiveness of various types of radiation, strain and species differences in response, effects of protective agents, and the relative sensitivity of the gastrointestinal tract compared to other tissues. Sequential weight changes in the small intestine were studied after various times and doses of gamma radiation in white Swiss mice. Radiation was followed by rapid weight loss in the gut (dose dependent) reaching a maximum by the 2nd or 3rd day followed by recovery above the control range at 3 to 5 days. The magnitude of this peak recovery tended to increase with dose up to 750 r and to be somewhat delayed with increasing dose. These findings showed correlation with similar time and dose studies on mitotic inhibition of the mouse small bowel epithelium(7). By the 7th day the intestines returned to the control range and remained in the range with doses through 550 r. However with doses above 550 r a secondary depression in gut weight

occurred which was manifest on the 10th and 12th postirradiation days.

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Penetration and Local Effect of Vitamin A on the Skin of the Guinea Pig.*† (21197)

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Vitamin A penetrates the intact skin of the guinea pig largely by way of the pilosebaceous canals and the sebaceous glands, and the speed of its penetration is dependent upon the vehicle used. Topical applications of massive doses of vit. A over a period of 2 weeks cause an hypertrophy of the epidermis but no appreciable inhibition of keratinization. The growth of hair follicles is never sufficiently affected by the vitamin to be observable either in gross appearance or in histological sections.

Materials and methods. These experiments were carried out in duplicate: in one set crystalline vit. A alcohol was used; in the other, vit. A acetate. Since the results were identical, no attempt will be made to describe or discuss them separately.

In the study of penetration, 10 mg of the crystalline vitamin were dissolved in 2 ml of 95% alcohol, chloroform, oleic acid, linoleic acid or a paste made up of petrolatum, zinc oxide and talcum. After one topical application of vit. A dissolved in each of the above vehicles to the clipped skin of guinea pigs, biopsy punctures of skin were removed from each animal after 10 minutes, one hour, and 2 hours. In some cases, specimens were removed after 4 and 8 hours. The animals were maintained in a dark room to avoid inactivation of the vitamin by light. The skin samples were frozen without fixation. Sections were cut at 10 and 20 μ, mounted in glycerine, and studied under near-ultraviolet light filtered to transmit rays at 3600 Å. The penetration of vit. A can be traced by its brilliant fluorescence. Unlike the fluorescence of natural vit. A which fades quickly under ultraviolet light, that of the synthetic vitamin fades very slowly and is visible for about 20 minutes.

For the study of the effect of vit. A and of the vehicles alone on the skin, an aluminum ring ¼″ wide and ½″ deep was fastened to the clipped backs of guinea pigs with methyl methacrylate and adhesive tape (Fig. 1, 2). The well was left empty overnight to make

*Crystalline vitamins used were obtained from the National Biochemical Co. and oleic and linoleic acids from the Fisher Scientific Co., both through the courtesy of the Desitin Chemical Co., of Providence, R. I.

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