Serum Intestinal Alkaline Phosphatase in Rats After 800 R Whole-Body or Regional X-Irradiation (34654)

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(Introduced by Paul D. Altland)

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In earlier studies it was found that X-irradiation of the whole-body (1, 2), or abdomen alone (3), produced a marked fall in plasma alkaline phosphatase in rats. The values reached a low at 3–6 days and returned to near normal levels at 9–12 days after whole-body exposure to 800 R (2). It has also been shown that administration of certain fats such as olive oil to fasting normal rats increases plasma or serum alkaline phosphatase (SAP) values which reach a peak in about 24 hr (4). This rise has been attributed to an increased contribution to the serum of intestinal alkaline phosphatase (IAP) due to increased production of alkaline phosphatase by the intestinal epithelium in association with the transport and absorption of fat (5, 6). This activity, as demonstrated histochemically, reaches a maximum about 24 hr after a fat meal (6). The purpose of this study was to determine, in X-irradiated rats, the effect of olive oil on SAP values to indicate possible functional impairment of the irradiated intestinal mucosa.

Materials and Methods.[1] Several series of Sprague-Dawley male rats, 2 to 5 months old and weighing about 350 g, were housed, 4 to 6 per cage, in a room maintained at about 23° and given Purina rat chow and water *ad libitum*. They received 800 R, as described previously (2, 3), using an X-ray unit operated at 300 kvp and 20 mA with added filtration of 2 mm Cu. The TSD was 57 cm, and the average dose rate was about 105 R/min.

[1] The "Principles of Laboratory Animal Care" as promulgated by the National Society for Medical Research were observed during this study.

In each of 23 experiments, 7 to 14 rats were used, including 2 nonirradiated controls and 1 or more groups receiving either whole-body or regional irradiation achieved by appropriate shielding. In one experiment, for example, 2 rats were not irradiated and 12 were irradiated. In 6 of the 12, the abdomen was shielded during irradiation while in the other 6, irradiation was limited, by shielding, to the abdomen. As many as 8 unshielded rats were irradiated simultaneously in a wooden cage partitioned into 8 compartments. The regionally shielded rats were irradiated individually under sodium barbital anesthesia in a Lucite frame, as described previously (3). To shield the abdomen during irradiation, lead plates were placed on the Lucite frame overlying the area between the xiphoid and symphysis pubis. A horizontal line at the lower costal margin was used to separate the upper and lower abdomen. The midabdomen consisted of adjacent halves of the upper and lower abdomen. A number of control rats were given a barium mixture by stomach tube, and radiographs were taken about 30 min later with the partially shielded rats placed in the Lucite frame to determine what portions of the intestinal tract were exposed to the X-ray beam in similarly shielded X-irradiated rats. Such radiographs revealed that shielding the upper half of the upper abdomen shielded virtually the entire liver and stomach, while shielding the lower half shielded chiefly the duodenum and only a small portion of the jejunum.

Tail blood samples were obtained from each rat immediately before the administration by stomach tube of 1 ml/100 g of olive oil, usually at 3:30 p.m. They were bled

TABLE I. Mean Values and Rise in Serum Total (SAP) and L-Phenylalanine-Inhibited Alkaline Phosphatase (SPIAP) Induced in 16–24 hr by 1 ml/100 g of Olive Oil Given by Stomach Tube to Starved Rats 3 Days after Whole-Body Exposure to 800 R.

Days after 800 R[a]	No. of rats	SAP (Sigma units)			SPIAP (Sigma units)		
		Before oil	After oil	Rise	Before oil	After oil	Rise
Not irrad.	25	2.4 ± 0.2[b]	5.7 ± 0.3	3.3 ± 0.4	1.2 ± 0.1	3.1 ± 0.2	1.9 ± 0.3
1	11	1.9 ± 0.1	5.8 ± 0.4	3.9 ± 0.4	0.9 ± 0.1	2.8 ± 0.2	1.9 ± 0.2
2	9	2.7 ± 0.3	4.0 ± 0.5	1.3 ± 0.6	1.4 ± 0.2	2.2 ± 0.4	0.8 ± 0.4
3	7	2.8 ± 0.1	3.9 ± 0.3	1.1 ± 0.3	1.4 ± 0.1	2.0 ± 0.1	0.6 ± 0.1
4	17	0.9 ± 0.1	0.9 ± 0.1	0.0 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
7	16	0.6 ± 0.1	1.8 ± 0.1	1.2 ± 0.1	0.2 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
9	12	1.4 ± 0.1	4.1 ± 0.2	2.7 ± 0.3	0.6 ± 0.1	2.2 ± 0.2	1.6 ± 0.2

[a] Values denote number of days after 800 R when last tail blood sample was taken.
[b] Mean ± SEM.

again the next morning about 17 hr later. The rats were fasted, but given water *ad libitum*, beginning 24 hr before the administration of olive oil.

SAP values in Sigma units were determined by the Sigma method, using *p*-nitrophenyl phosphate as a substrate and a buffer adjusted to pH 9.2 (7). One Sigma unit of phosphatase will liberate 1 μmole of *p*-nitrophenol/hr under the specified conditions. The determinations were repeated after adding L-phenylalanine (8, 9), 41 mg/50 ml of the combined substrate and buffer, resulting in a 0.005 M concentration. This compound selectively inhibits a major portion of the activity of intestinal alkaline phosphatase in the serum (SIAP). Human intestinal alkaline phosphatase (IAP) was inhibited 78% by L-phenylalanine (8) and rat intestinal alkaline phosphatase up to 66% (9). The degree of IAP inhibition by L-phenylalanine varies with the pH, substrate, and other conditions (9) and was not determined in our study. The difference between the SAP values obtained with and without the use of L-phenylalanine, therefore, represents the amount of SIAP inhibited by L-phenylalanine (SPIAP). The value of this L-phenylalanine inhibited alkaline phosphatase fraction (SP IAP), although less than that of SIAP, can serve as an index of the relative concentration of SIAP in the different experimental groups and of the relative amount of alkaline phosphatase produced and contributed to the serum by the intestinal mucosa before and after stimulation by olive oil. A diminished rise in SPIAP after olive oil would suggest functional impairment and diminished ability of the intestinal mucosa to produce alkaline phosphatase.

TABLE II. Rise in Serum Total (SAP) and L-Phenylalanine-Inhibited Alkaline Phosphatase (SPIAP) Induced in 17 hr by 1 ml/100 g of Olive Oil Given by Stomach Tube to Regionally Shielded or Irradiated Rats 3 Days After Exposure to 800 R.

Region shielded	No. of rats	Sigma units		Region irradiated	No. of rats	Sigma units	
		SAP	SPIAP			SAP	SPIAP
None	17	0.1 ± 0.1[a]	0.0 ± 0.1	None	20	3.5 ± 0.3	2.0 ± 0.2
Abdomen	10	2.8 ± 0.3	1.5 ± 0.2	Abdomen	9	0.1 ± 0.1	0.1 ± 0.1
Upper abdomen	12	3.3 ± 0.3	1.9 ± 0.2	Upper abdomen	9	1.5 ± 0.2	0.7 ± 0.1
Midabdomen	10	3.7 ± 0.4	2.0 ± 0.3	Midabdomen	9	0.5 ± 0.1	0.1 ± 0.1
Lower abdomen	16	1.6 ± 0.2	1.0 ± 0.2	Lower abdomen	13	2.3 ± 0.4	1.1 ± 0.2
Duodenum	5	1.8 ± 0.6	0.9 ± 0.4	Duodenum	6	2.2 ± 0.5	1.1 ± 0.4
				Liver	8	4.2 ± 0.9	2.3 ± 0.4

[a] Mean ± SEM.

The duodenum of a number of rats was removed and rapidly frozen in isopentane, and cryostat sections, about 6-μ thick, were stained for IAP by an azo-dye coupling technique using naphthol AS-MX as substrate and fast red violet LB salt (10); incubation time was 5 min at 7°. The duodenum was selected because its relatively fixed position facilitated selection of comparable sections from different animals, and because in the rat it contains the highest concentration of IAP (9).

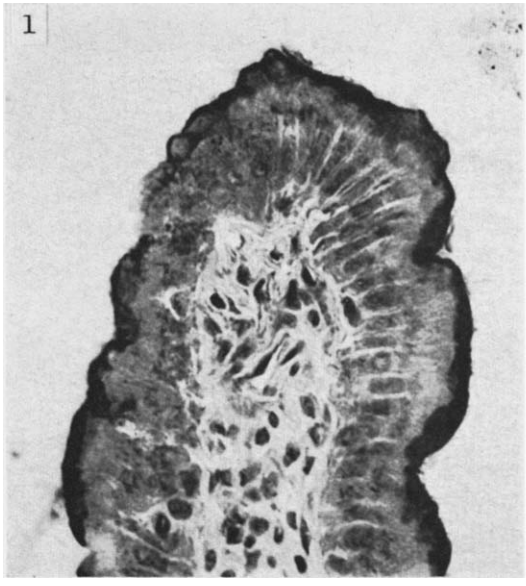
Results. As shown in Table I, the rise in SAP and SPIAP in response to olive oil was not altered significantly the first day but then dropped sharply and virtually disappeared on the fourth day after 800 R. There was a slight rise in SAP and SPIAP after the olive oil at 7 days and a moderate rise at 9 days suggesting some functional recovery of the intestinal mucosa.

As shown in Table II, shielding the abdomen or its upper or middle portion during irradiation gave fairly complete protection and permitted a normal rise in SPIAP after administration of olive oil, while shielding the lower abdomen or the duodenum gave only a slight to moderate protection. On the other hand, irradiating the entire abdomen or the midportion of the abdomen alone resulted in virtual absence of a rise in SPIAP in response to olive oil suggesting that injury to the gut in the midabdomen was responsible for the failure to respond to olive oil. Irradiating the upper or lower abdomen or duodenum caused a moderate suppression of the rise in SPIAP in response to the olive oil, while irradiating the liver had no suppressive effect.

In a supplementary study, using cardiac blood from nonfasted rats, SAP values in 2 nonirradiated rats were determined to be 6.94 and 4.98 Sigma units, respectively. The mean SAP value $\pm$ SEM in 7 irradiated rats, 4 receiving whole-body and 3 abdominal exposure to 800 R 4 days previously, was 0.48 $\pm$ 0.07 units. SAP determinations were made also on the serum of each of the 2 nonirradiated rats mixed with an equal volume of serum from each of the 7 irradiated rats. The mean SAP value of the 7 mixtures with the serum of the first nonirradiated rat was 3.84 $\pm$ 0.05 units, approximating the calculated mean value of 3.71 $\pm$ 0.02 units. The mean SAP value of the mixtures with the serum of the second nonirradiated rat was 2.70 $\pm$ 0.04 units, compared with a calculated value of 2.73 $\pm$ 0.03 units. The calculated value of a given mixture is the average SAP value, determined before mixing, of the 2 sera in each mixture. In no case was the difference between the calculated and determined value of a given mixture greater than 0.3 Sigma units. Since the serum from the irradiated animals caused no apparent fall in the SAP values of the nonirradiated rats, these results indicate that the low SAP values in the irradiated animals was not due to an inhibitor of alkaline phosphatase in their circulation.

Examination of frozen sections of unfixed duodenum stained for alkaline phosphatase revealed abundant alkaline phosphatase in the epithelium of the villi in abdominally shielded irradiated rats (Fig. 1) and absence or marked diminution of alkaline phosphatase in the duodenal epithelium of unshielded rats both before and after administration of olive oil (Fig. 2). Some irradiated villi showed no epithelial alkaline phosphatase, while others showed some alkaline phosphatase in a few epithelial cells, chiefly near the tip, probably representing unshed irradiated lining cells. The irradiated duodenal villi were irregular and shortened, and the epithelial lining cells were greatly reduced in number and often misshapen (Fig. 2). Mitotic activity in the irradiated crypts varied widely.

Discussion. The findings in this study confirm our previous report that injury to the intestinal mucosa is chiefly responsible for the fall in circulating alkaline phosphatase following irradiation (3) and that this fall is due largely to a reduction in IAP. Our findings also show that the SPIAP response to olive oil was lowest at about 4 days after irradiation corresponding to the greatest fall in SAP. The suggested impairment in IAP synthesis is confirmed by our histochemical studies showing at 4 days after irradiation absence or marked diminution of alkaline phosphatase in the duodenal epithelium even



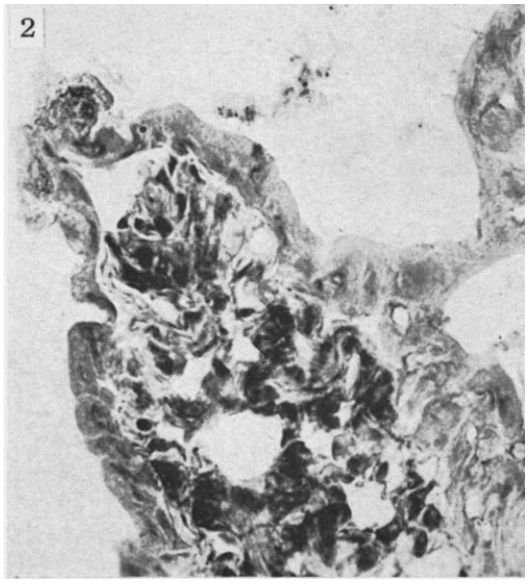


FIG. 1. Duodenum of abdominally shielded rat 4 days after exposure to 800 R showing epithelium of villi containing abundant alkaline phosphatase appearing darkly stained by a modified Burstone method (500 ×).

FIG. 2. Duodenum of rat 4 days after exposure of abdomen to 800 R and 17 hr after administration of olive oil showing no epithelial alkaline phosphatase demonstrable by a modified Burstone method (500×).

after administration of olive oil. Kosmider *et al.* (11) demonstrated histochemically some reduction in alkaline phosphatase in the jejunal mucosa in guinea pigs at 1 day and a more marked reduction at 4 days after exposure to 400 R, but they did not study the effect of administration of a fatty meal. The deficiency in the SPIAP response to olive oil in the irradiated animals suggests an associated impairment in fat absorption and transport (due to impaired synthesis of IAP) which could play a role in the development of the acute gastrointestinal syndrome after irradiation (12).

Our findings indicate that when only a small portion of the intestinal tract such as the duodenum is irradiated, there is only a moderate deleterious effect on the SPIAP response, perhaps due to compensatory increased functional activity and IAP synthesis in shielded portions of the intestinal tract such as the jejunum. When a larger portion of the tract is injured, such as occurs when the midabdomen is irradiated, there is virtual absence of a response to olive oil, probably because the nonirradiated portions of the intestinal tract cannot compensate adequately for the injured mucosa, and their contribution of intestinal alkaline phosphatase is insufficient to cause a marked rise in SAP. In the rat (10) the greatest activity in the production of IAP is in the duodenum with activity decreasing distally. The midabdomen includes the duodenum and jejunum, the areas of greatest activity, whereas the lower abdomen includes chiefly the ileum and only a portion of the jejunum. It is, therefore, not surprising that shielding or irradiating the duodenum has about the same effect as shielding or irradiating the lower abdomen (Table II), which contains a much greater length of intestine (ileum). Irradiating the liver had no significant effect indicating that possible liver injury by radiation is not the cause of the fall in SAP. This confirms our previous observation (3). More than one-third of IAP in rats is not inhibited by L-phenylalanine (9). This supports the view (3) that most of the SAP in rats is IAP and may explain why the response of SAP to olive oil is similar to that of SPIAP.

In the duodenum of the rat, AkP produc-

tion is limited largely to nondividing differentiated columnar cells lining the villi (10). These are derived from proliferating stem cells in the crypts of Lieberkühn. The new cells migrate up the villi to replace the short-lived older cells being shed constantly at the tips of the villi. The turnover time required by the proliferaing crypt cells to replace completely a population of differentiated cells lining the duodenal villi is about 1.6 days in the normal rat (12) and somewhat longer in the irradiated gut (13), the time varying with the dose.

It is well known that within several hours after a single moderate irradiation exposure, mitosis is inhibited and degenerative changes and necrosis appear in the crypts of Lieberkühn, thereby stopping temporarily the supply of new epithelial cells to replace the cells being shed. Although mitotic activity is usually resumed within a few days, and was pronounced in some duodenal crypts of our irradiated rats at 4 days, the number of cells lining the villi is greatly reduced. Epithelial denudation of the villi is delayed or prevented because of contraction and shortening of the villi and because the reduced number of existing cells tend to flatten out so as to cover the mucosal surface (Fig. 2). The first cells to migrate up the villi are often abnormally large and irregular in shape (13). These cells, as well as any unshed residual lining cells, except for a few near the tips of some villi, evidently show little or no alkaline phosphatase activity at 4 days after exposure to 800 R (Fig. 2).

SPIAP levels in our rats were markedly reduced (Table I) at 4 days after 800 R and were not fully restored to normal at 9 days. These findings, and the histochemical findings in our rats and those of Kosmider *et al.* (11) in irradiated guinea pigs, indicate that following irradiation IAP production by the differentiated cells lining the villi is diminished or lost. The virtual absence of demonstrable IAP at the base of the villi suggests that the early generations of cells ascending from the crypts up the villi, though protecting the surface of the villi, exhibit no significant IAP activity. These findings indicate that some time is required for the stem cells in the crypts to recover sufficiently from the effects of irradiation to repopulate the villi with new generations of differentiated epithelial cells sufficient in number and functional capacity to restore normal intestinal alkaline phosphatase activity.

Summary. To determine if the fall in serum alkaline phosphatase (SAP) in rats after whole-body or abdominal X-irradiation was due largely to decreased ability to produce intestinal alkaline phosphatase (IAP), young adult male Sprague-Dawley rats were exposed to 800 R whole-body or regional irradiation. Determinations were made of SAP with or without the addition of L-phenylalanine. The reduction in value of SAP by this inhibitor was used to indicate the relative amount of IAP contributed to the serum. Administration of olive oil by stomach tube, which stimulates production of IAP, caused a sharp rise in the L-phenylalanine-inhibited fraction of SAP in normal starved rats, but virtually no rise 4 days after whole-body or abdominal irradiation. Shielding the abdomen or its upper or middle portions during irradiation gave fairly complete protection and permitted a normal response to olive oil, while shielding the lower abdomen or duodenum gave only slight to moderate protection. Irradiating the entire abdomen or the midportion gave the same effect as whole-body irradiation, while irradiating the upper or the lower abdomen or duodenum caused a moderate suppression of the normal response to olive oil. These findings indicate that injury to the duodenum and jeiunal mucosa is mainly responsible for the fall in IAP in the serum following irradiation and that the injury is sufficient to diminish or prevent synthesis of intestinal aklaline phosphatase even after stimulation by olive oil. This appears to be due primarily to a reduced population of differentiated cells lining the villi.

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