Mouse Thymocyte Beta-Glucuronidase Activity After Whole-Body X-Irradiation.* (30982)

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Studies on thymocytes from neonatal rats (1) and from mice during radiation induced thymic atrophy and regeneration(2) revealed changes in alkaline phosphatase and amidase activities which were significantly correlated with changes in the medium and large thymocyte population. Changes in β-glucuronidase activity have been observed during embryonic development in the chicken(3), regeneration of colonic mucosa in dog(4) and subsequent to whole body X-irradiation in rat thymus and spleen(5) and rat testes(6). These findings have prompted us to investigate the effect of whole body sublethal X-irradiation on β-glucuronidase activity and cell size distribution of mouse thymocytes.

Materials and methods. Two-month-old female LAF_1 mice (NRDL Colony), fed Purina Fox Chow and water *ad libitum* were used. A Westinghouse X-ray therapy unit operated at 250 kvp and 15 ma was used. One mm of Al and 0.5 mm Cu were added as filters; hvl was 1.28 mm Cu; target distance was 40 in. and exposure rate was 29 rad/min. The mice received 600 R of whole body X-irradiation. Cell suspensions were prepared from the thymuses as previously described (1) and volume distribution analysis determined with a Coulter Counter Model B. The distribution curves were divided into 3 compartments designated A, B and C representing the small, medium and large thymocyte populations respectively(2). Beta-glucuronidase activity was assayed fluorimetrically(7) and enzyme activity expressed as moles of fluorogen $\times 10^{-11}$/cell/hr.

Results. It can be seen from Fig. 1 that β-glucuronidase activity increases immediately after radiation reaching a first peak by day 2, a maximum activity by day 7 and returns to normal levels by day 11. Corresponding cell volume distribution analyses were made on all samples and representative curves for control, days 5 and 7 postirradiation are shown in Fig. 2. Integrals of the respective areas under all the curves were determined and expressed as percent of total. It can be seen in Table I that the small thymocyte population (A) decreases after radiation to a minimum value of 48% by day 5 and then returns to normal by day 11. Populations B and C increase immediately after radiation reaching maxima of 33 and 19% respectively by day 5 and then return to control values by day 11.

Discussion. The changes in β-glucuronidase activity in the present study follow a pattern similar to that observed for alkaline phosphatase, leucyl amidase and alanine amidase activities subsequent to whole body X-irradiation(2). In previous work(1,2), we suggested that enzyme activity was related to changes in the medium and large thymocyte population. Multiple linear regression analysis of the data(2) did indeed reveal a significant correlation of enzyme activity with changes in the B and C cell populations. Other workers have observed increases in β-glucuronidase activity during the develop-

TABLE I. Cell Volume Distribution Analysis.

Days after 600 rad X-irradiation	Vol (μ^3) (A) (157–270)	(B) (270–405)	(C) (405–700)
	%		
Control (0)	67	24	9
1	60	30	10
2	59	31	10
3	57	32	11
4	51	33	16
5	48	33	19
7	63	26	11
11	66	25	9

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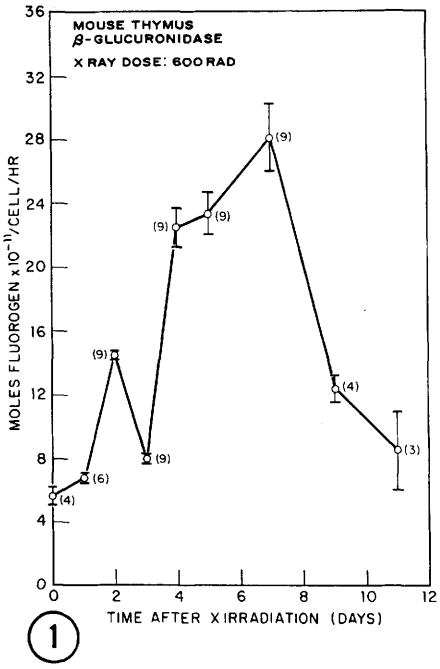

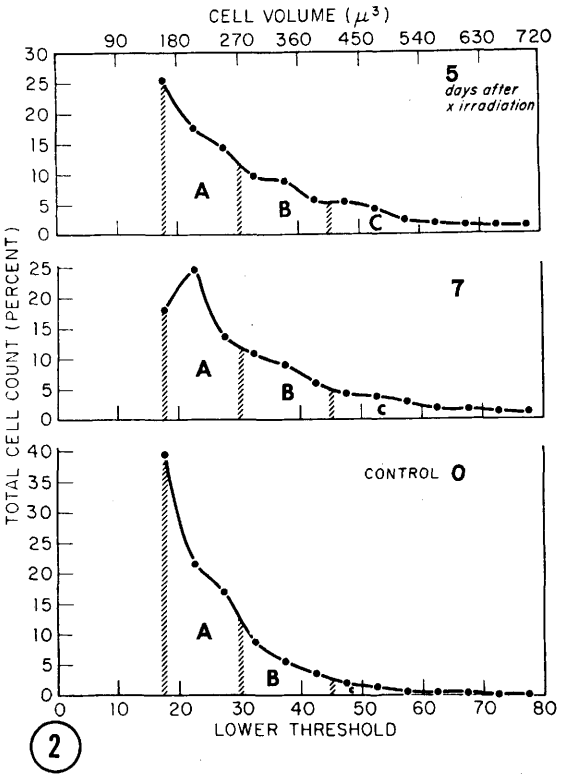

FIG.1. Change in β-glucuronidase activity in mouse thymus after whole body X-irradiation. Cross bars represent ± one standard error. Numbers in parenthesis refer to number of animals used for each point.

FIG. 2. Volume distribution analysis of mouse thymocyte suspensions. Areas designated A, B, C refer to volume classes indicated in Table I.

ment of the Mullerian ducts in chick embryo (3), with rat kidney hypertrophy(8), and during the regeneration of colonic mucosa in dog(4) subsequent to artificial lesions. Rahman(5) observed increases in β-glucuronidase specific activity in rat thymus subsequent to whole body X-irradiation. After 200 rad enzyme specific activity reached a maximum by 24 hours and then decreased by 48 hours postirradiation. When the dose was 1000 rad, however, enzyme specific activity was maximal after 48 hours postirradiation. Increases in the activity of this enzyme have also been seen in rat testes(6) after a dose of X-rays which produces temporary sterility, and also in rat duodenal mucosa after doses of 400 r and 1200 r of local X-irradiation(9).

While the physiological significance of the thymocyte β-glucuronidase pattern in the present study remains obscure, the changes in enzyme activity are obviously associated with thymus cell proliferation and differentiation following radiation injury. We are curcently extending these studies to the single cell level, as well as developing additional fluorogenic substrates as markers of cell proliferation and differentiation.

Summary. Beta-glucuronidase activity of mouse thymocytes, at various time intervals after a single whole body sublethal dose of X-rays (600 rad), was studied. Activity per cell showed an initial peak at day 2, a maximum by day 7 and returned to normal levels by day 11. Concomitant cell volume distribution analyses were carried out; the data were interpreted to indicate a positive correlation of changes in β-glucuronidase activity with changes in the medium and large thymocyte populations.

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Thorotrast Inhibition of Amylase Synthesis by the Isolated, Perfused Rat Liver.* (30983)

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The removal of intravenously injected crystalline hog amylase from the serum of dogs was previously investigated in this laboratory. It was found that at the end of 6 hours, the decrease of amylase in the serum was accompanied by an increase in the amylase activity of the liver equivalent to one-half to two-thirds of the enzyme injected. It was also found that the rise in amylase activity of the liver was almost completely inhibited in dogs with Thorotrast blockade of the reticulo-endothelial system (RES)—which includes the Kupfer cells. It seems possible that in dogs the RES is involved in the mechanism which regulates the amylase level of the serum and the amylase activity of the liver(1,2).

Several investigators have shown that the isolated, perfused rat liver synthesizes amylase and releases it into the perfusion medium(3,4). Many of the cells of the rat liver are RE cells, Kupfer cells. As in the dog, these may be involved in the mechanism that regulates the serum and liver amylase. The present study investigates the release of amylase into the perfusion medium by isolated livers from rats with Thorotrast blockade of the RES.

Methods. Rats of the Slonaker-Addis strain, weighing between 250 and 400 g were used in these experiments. The plasma amylase activity was determined by the method of Van Loon *et al*(5) and the plasma volume by the Evans blue method(6). The total circulating amylase activity was then calculated. The RES in the experimental group of rats was blocked by intravenous injection of 3 ml per kilo of Thorotrast (25% ThO_2) 4 to 6 hours before their use as liver donors(7). The control group was not treated. The liver was removed by the method of Brauer *et al* (8). It was weighed and a small biopsy taken for the determination of amylase activity by the method of McGeachin *et al*(9). The liver was then perfused on the apparatus developed in this laboratory by Rosenfeld *et al*(10), using 150 ml of perfusion fluid. This consisted of either 100 or 120 ml of heparinized rat blood diluted to 150 ml in Ringer's solution. A specimen of perfusion fluid was removed for hematocrit and amylase determination before the perfusion was begun and thereafter at half-hourly intervals for 3 hours. At the completion of the perfusion period, the liver was again weighed and another biopsy taken for determination of the amylase activity. The plasma volume of the perfusion fluid was calculated from the hematocrit.

Results. In 5 normal rats, the total circulating amylase activity (plasma volume $\times$ plasma amylase concentration) averaged 187 units (Table I).

The isolated livers of 6 normal rats were perfused. The total amylase in the perfusing medium increased by an average of 329 units after 2 hours, and by 376 units after 3 hours

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