TABLE I. Effect of Serum from Plateaued Rats on Growth Response to GH-Containing Extract.

No. rats	Daily dose	10-day wt gain (g)
5	25 mg* + 1.75 ml treated serum	31.2 ± 3.3†
5	25 " + 1.75 " control "	34.4 ± 3.5
10	25 " + 1.75 " saline	30.0 ± 1.3
8	25 " undiluted	35.7 ± 2.5
10	10 " "	13.7 ± 2.2
17	None	1.9 ± .8

* Dosage is expressed as wet wt of anterior lobe tissue represented in the daily dose; 25 mg of extract is equivalent to 0.8 mg pure GH by 10-day normal rat wt gain assay.

† Stand. error of mean.

average of 113.5 g ± std. error of 4.0 g over their controls, compared to 4.0 ± 4.5 g over controls the last 40 days. These rats were exsanguinated under ether 12 hr after last injection and their serum incubated with aliquots of above pituitary extract in ratio of 1.75 ml serum (8.5% calculated serum of average donor) to 25 mg of extract (10% of daily dose per donor rat during last 40 days of treatment). After 30 min incubation at 10°C, pH 9.0 (no turbidity, possibly due to pH), the mixture was divided into injection vials and stored at –30°C until 30 min prior to injection. Mixtures of same volume of serum from control rats and extract, and of normal saline and extract were prepared and handled identically. These 3 mixtures and 2 dose levels of undiluted extract were assayed for growth potency by daily s.c. injections for 10 days in 5 groups of normal 7-mo-old Long-Evans female rats.

Results. Growth response to the GH extract was not altered significantly by "treated serum" (Table I). The much smaller response of the 10 mg group compared to any of the 25 mg groups indicates that appreciable attenuation would have been detectable. Arthus type reaction with edema, inflammation and some necrosis at site of injection occurred in all 5 rats receiving both extract and treated serum and in no rat in other groups, indicating antibodies to some of the proteins of the extract.

Discussion. The procedures employed do not detect GH-inactivating antibodies in serum of rats plateaued on high dosage of a GH-containing extract. It is conceivable that donor rats were producing GH-inactivating antibodies but at a rate such that the daily GH dosage cleared the antibody from serum for 12 or more hours. It is also possible that plateauing may be due to fixed GH-inactivating antibodies.

Summary. Serum from female rats plateaued while on treatment with a GH-containing extract (after reaching 2.4 times normal adult female size) was ineffective in attenuating GH response of previously uninjected assay rats.

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Spleen Adenosine Deaminase and Guanase Activities after Whole-Body X-Irradiation of Rats. (26024)

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It has been shown(1) that the activities per mg of nitrogen (specific activity) of 2 purine-metabolizing enzymes of liver homogenate, adenosine deaminase and inosine phosphorylase, were unchanged from 16 hours to 10 days after rats were given 600 r of whole-

body X-irradiation. At the same time, it was pointed out that specific activity of inosine phosphorylase of spleen homogenate was increased about 50% although total organ activity of the enzyme was significantly lowered. Other spleen enzymes recently studied in this laboratory have been ribonuclease(2) and DPNH cytochrome *c* reductase(3) both of which exhibited increases in homogenate specific activity, but to varying degrees, 13 to 16 hours after irradiation. As an extension of the investigation of enzymes involved in metabolism of ribonucleic acid and purines, this paper reports the effect of 600 r of whole-body radiation on rat spleen adenosine deaminase and guanase and discusses the interpretation of these results as well as previous data obtained with spleen enzymes.

Methods. A. Irradiation procedure. Three experiments were carried out with male rats of the Wistar strain obtained either from a commercial breeder (Exp. I and II) or directly from Wistar Inst. (Exp. III). A total of 40 animals (190-200 g) were studied for enzyme activity in Exp. I, 24 (120-160 g) in Exp. II, and 24 (185-210 g) in Exp. III. In the first 2 experiments, the rats were given a single dose of 600 r of 240 KV X-rays filtered through 1.0 mm Cu and 1.0 mm Al at an average rate of 66.8 r/minute. In the third experiment, 600 r of 220 KV X-rays were delivered through 0.25 mm Cu and 1.0 mm Al at an average rate of 92.6 r/minute. Target distance was 50 cm in all experiments. The animals were irradiated 2 at a time in a large crystallizing dish covered with a sheet of light cardboard. Food was withheld from all animals for 24 hours after irradiation, then controls were pair-fed against X-rayed rats with a stock commercial diet. Water was supplied *ad libitum*.

B. Tissue preparation and enzyme assays. Spleens were removed from the rats under ether anesthesia after exsanguination by heart puncture, washed free of adhering blood, and homogenized 1:10 with cold glass-distilled water in a Ten Broeck homogenizer. For the adenosine deaminase tests, the homogenates were further diluted to 1:250 in water. Deaminase activity was assayed spectrophotometrically by following the decline of the 265-mμ peak of adenosine with time (adenosine$\rightarrow$inosine + NH_3). Components of the reaction mixture, in their order of addition to cuvettes, were 0.1 or 0.2 ml of homogenate, 0.5 ml of 0.1 M KH_2PO_4-K_2HPO_4 buffer, pH 7.4, and 0.3 micromole of adenosine. Readings were taken at 1-minute intervals for 6 minutes.

Before studying effect of irradiation on guanase activity, a number of preliminary experiments were carried out, for although the presence of this enzyme in rat spleen has long been known, no data were available concerning application of Kalckar's spectrophotometric technic(4) to normal spleen guanase assays. Guanase activity of rat spleen homogenates (1:50 in water) was followed by the rate of decline of the 250-mμ peak of guanine (guanine$\rightarrow$xanthine + NH_3). As a check on the reaction under study, xanthine formation was determined by fortifying the reaction mixtures with purified milk xanthine oxidase which converted xanthine stoichiometrically to uric acid. The latter was measured by the appearance of its 292-mu band. Since absorbancy changes at 250 mμ for consecutive time intervals were not always regular, due probably to settling of particles occasioned by use of more concentrated homogenates than in the adenosine deaminase test, it was decided to study guanase activity in the soluble fractions prepared from spleens of normal and X-rayed rats. This was possible, for in aqueous homogenates centrifuged at 56,550 $\times$ g for 45 minutes, about 90% of total guanase activity was recovered in the supernatant fluid to give a 2-fold concentration of activity with respect to nitrogen. The enzyme was found to be stable in either homogenates or soluble fractions for at least 3 to 5 hours at 2°. After removing 0.6 ml of each spleen homogenate for adenosine deaminase assays and nitrogen analyses, remainder of each homogenate was centrifuged at 56,550 $\times$ g for 30 minutes. All supernatant samples were carefully decanted into chilled tubes and volumes recorded. For guanase assays, the cuvettes contained 0.025 or 0.05 ml of the soluble fractions, 1 ml of 0.1 M phosphate buffer, pH 7.4, water to 3 ml, and 0.42 μmole of guanine. Readings were taken at 2-minute

TABLE I. Effect of Whole-Body X-Irradiation on Specific Activity of Adenosine Deaminase of Rat Spleen Homogenates.

Exp.	Group	Avg specific activities* with ranges at varying days after irradiation 1	2	3	4	5	8
I	C†	.109‡ (.091-.120)	.096 (.084-.110)	.107 (.104-.108)		.115 (.107-.126)	.094 (.082-.100)
	X	.132 (.120-.143)	.138 (.112-.172)	.141 (.125-.169)		.112 (.105-.124)	.115 (.104-.133)
II	C	.114 (.093-.139)	.122 (.088-.153)	.114 (.102-.122)			
	X	.144 (.120-.178)	.116 (.082-.138)	.118 (.067-.172)			
III	C	.088 (.076-.105)	.110 (.101-.116)		.100 (.091-.109)		
	X	.103 (.093-.110)	.129 (.104-.142)		.118 (.100-.137)		

* μmoles adenosine deaminated/min./mg N. † C = control, X = irradiated.
‡ Each value represents avg of 4 animals.

intervals for 8 minutes. All assays for guanase and adenosine deaminase were carried out as soon as possible at 2 levels of tissue at a temperature of 25° with a thermostated Beckman model DU instrument. Activities were directly proportional to concentrations of tissue used, and reaction rates were linear during periods of observation. Specific activity of each enzyme was calculated by use of the molar extinction coefficients reported by Kalckar(5). Total activity was obtained by multiplying specific activity by total nitrogen. Nitrogen in homogenates and soluble fractions was determined by a micro-Kjeldahl procedure.

Results. For the 3 experiments, specific activities of adenosine deaminase are presented in Table I. Intervals studied after irradiation are listed in the table, for convenience, as days 1, 2, 3, 4, 5, and 8. Actually, these figures represent postirradiation periods of: 15, 39, 63, 87, 111, and 183 hours. In Exp. I, activities of irradiated rats were increased above control level by 21, 44, and 32% on the first, second, and third days, respectively, after irradiation. By day 5, activities had returned to normal level. In Exp. II, spleen adenosine deaminase activity was increased again on first day after exposure (+26%) but returned to control level on second and third days. In Exp. III, adenosine deaminase activity was uniformly 17% higher than that of controls on first, second, and fourth days after irradiation. While the results of each experiment were not identical, the trend towards a small increase in activity was consistent. In considering the results of Exp. II where no change in adenosine deaminase activity was found on days 2 and 3, the fact that the rats were much younger than those used in Exp. I and III should be taken into account. In Exp. I, employing combined control and irradiated spleen homogenates, no evidence was obtained for formation (or release) of a deaminase activator or destruction of a deaminase inhibitor. Table II summarizes results obtained for specific activities of guanase in spleens of normal and irradiated rats. On the first, second, and fourth days after irradiation, activity was increased by 34, 20, and 24% respectively.

Despite the general increase in specific activity of both enzymes, total organ adenosine deaminase and guanase activities decreased (Table III) due to considerable loss in spleen nitrogen (−35 to −50%) following irradiation. In general, spleens of X-rayed animals showed

TABLE II. Effect of Whole-Body X-Irradiation on Specific Activity of Guanase of Rat Spleen Supernatant Fractions.

Group	Avg specific activities* with ranges at varying days after irradiation 1	2	3
Control	.030† (.028-.032)	.037 (.031-.042)	.038 (.034-.040)
X-irradiated	.041 (.036-.045)	.044 (.042-.048)	.047 (.043-.050)

* μmoles guanine deaminated/min./mg N.
† Each value represents avg of 4 animals.

TABLE III. Effect of Whole-Body X-Irradiation on Total Activities of Adenosine Deaminase and Guanase of Rat Spleen.

Enzyme	Exp.	Group	Avg total activities* at varying days after irrad. 1	2	3	4	5
Adenosine deaminase	I	C†	4.33	4.04	4.98		2.92
		X	3.58	3.99	2.94		2.87
	II	C	1.63	1.62	1.62		
		X	1.13	1.14	.87		
	III	C	1.33	1.43		1.28	
		X	1.08	.96		.80	
Guanase	III	C	.187‡	.194		.183	
		X	.155	.109		.092	

* Values represent avg spleen total enzyme activity (μmoles adenosine or guanine deaminated /min./spleen). Each value represents avg of 4 animals.

† C = control, X = irradiated.

‡ Values represent activity of whole soluble fraction.

a 17 to 45% decrease in total adenosine deaminase activity between 1 and 4 days after irradiation. Total guanase activity present in the soluble fractions obtained from homogenates of X-irradiated rats averaged 17, 44, and 50% less than that found in corresponding fractions of control spleens during the 3 days studied. Total adenosine deaminase activity of control spleens in Exp. I was 2.5 to 3 times greater than that of control spleens in Exp. III. This discrepancy in total activity is due to the much larger spleens found in the rats used in the first experiment, despite the fact that the animals weighed about the same in Exp. I and III. The reason for the wide variation in organ weights is not known, but it is of interest that the specific activity of adenosine deaminase in control spleens (Table I) is independent of organ size. Hence, it would appear that the hypertrophied spleens studied in Exp. I did not affect activity of the enzyme.

Discussion. As in the case of another purine-metabolizing enzyme of spleen studied earlier, namely inosine phosphorylase(1), both adenosine deaminase and guanase have been shown in the present paper to undergo small increases in specific activity following 600 r of whole-body X-irradiation. On the other hand, total organ activity of each of the 3 enzymes was significantly decreased as compared to that of control animals. In agreement with Feinstein's(6) interpretation of increased specific activity of inosine phosphorylase, we recently(7) ascribed this and many other modest increases in spleen enzyme activity to an increased concentration of the enzymes with respect to nitrogen due to the marked nitrogen loss seen in spleens of irradiated animals. Based on the consideration of all combinations of changes in spleen enzyme activities that may be encountered, we presented a guide to interpretation of enzyme changes in spleen and other organs which undergo atrophy with a corresponding nitrogen loss after irradiation(7). According to this guide, spleen enzymes may be classified as follows: if specific activity is unchanged but total activity is decreased, the enzyme falls into case 1; if both specific and total activities decrease, the enzyme falls into case 2; if specific activity increases up to 100% but total activity decreases or remains the same, the enzyme falls into case 3a; and finally, if, along with a nitrogen loss of 50%, specific activity increases by more than 100% and total activity increases, the enzyme falls into case 3b. Under the experimental conditions employed, both adenosine deaminase and guanase may be classified in case 3a (Tables I to III). This means that increases in specific activity of these enzymes cannot be interpreted as activation of enzymes or as being due to enzyme synthesis but rather reflect an enrichment of enzymes with respect to nitrogen. It has been pointed out(7), based on a survey of results reported in the literature, that moderate increases in specific activities of spleen enzymes after whole-body irradiation may be more the rule than the exception. Or, it would appear more likely, for any spleen enzyme selected at random, that its

specific activity will be somewhat increased rather than decreased or unaffected. Such moderate increases in specific activity, unaccompanied by increases in total activity but usually associated with a loss of enzyme units, indicate that some enzyme has in fact been destroyed but rate of destruction has been exceeded by rate at which other nitrogen has been lost. These factors should be borne in mind in any comparison of response to irradiation of the same enzyme in a radiosensitive tissue and in a relatively radioresistant one.

Finally, it may be of interest that according to Smith and Low-Beer(8), 2 spleen enzymes involved in pyrimidine metabolism, uridine phosphorylase and cytidylic acid phosphatase, exhibited a slight increase in specific activity, and no increase in total activity (*i.e.*, case 3a) in rats exposed to 650 r, while a third enzyme, uridylic acid phosphatase, showed decreases in both specific and total activities (*i.e.*, case 2).

Summary. Adenosine deaminase and guanase activities were studied in homogenates and supernatant fluid fractions, respectively, prepared from spleens of control rats and animals given 600 r of whole-body X-irradiation. Specific activities of both enzymes were increased slightly in irradiated animals but total organ activities of both enzymes were decreased as compared to levels obtained in control rats. Interpretation of these and similar findings is discussed, and it is concluded that they do not represent direct or indirect enzyme activation or enzyme synthesis but rather reflect loss of nitrogen content and weight which are concomitants of splenic involution.

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Maintenance of Gonads of *Xenopus laevis* in Organ Cultures.* (26025)

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Some *in vivo* and *in vitro* effects of testosterone on gonads of larvae of *Rana catesbeiana* and *Xenopus laevis* have recently been reported(1). The present studies were made to determine whether gonads of *Xenopus,* placed in organ cultures at varying stages of development, would differentiate and respond to treatment with estrogen and chorionic gonadotropin in the same manner as *in vivo.*

Materials and methods. Larvae were made bacteriologically sterile(2) and cultures were set up as previously described(3). One part of glass distilled water in the medium was replaced by the hormones, estradiol (Progynon, Schering Corp.) or chorionic gonadotropin (Antuitrin-S, Parke, Davis and Co.). Developmental stages of larvae are numbered according to Nieuwkoop and Faber(4).

Results. Controls. Undifferentiated gonads from larvae of stages 49-50, attached to mesonephroi, showed no development in culture. In sectioned material no gonads nor germ cells could be positively identified. However, in gonads from larvae of developmental stage 52 and above, ovaries and testes could be distinguished. Some of these showed pro-

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