ovulation, OH release normally extends after the critical period; whenever administered, PB blocks subsequent release of pituitary OH.

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Received Dec. 13, 1967. P.S.E.B.M., 1968, Vol. 128.

Pituitary Content of Somatotropin, Gonadotropin, and Thyrotropin in Rats with Stunted Linear Growth following Head X-Irradiation* (32933)

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X-irradiation limited to the head of the neonatal rat will result in stunted growth (1,2). We have reported that the degree of stunting is related to the size of dose and that the administration of bovine growth hormone and/or thyroxine failed to correct the stunting (2). Inasmuch as the pituitary and the central neuroendocrine system is in the path of the X-ray beam, we have carried out further studies of the pituitary hormonal system to clarify the role of endocrine systems in this form of stunted growth. In this report we give results of assays for pituitary content of somatotropin, gonadotropin, and thyrotropin in the irradiated stunted rats and consider the significance of the findings in the overall problem of the cause of stunting of the head-irradiated rat.

Materials and Methods. All of the irradiated rats and controls were produced in our colony of Long-Evans rats begun in July of 1964; additional rats of the same strain were added in September, 1965. The colony was isolated in a room with a sound-absorbing ceiling. The air was fresh, filtered and maintained at 45–55% relative humidity and 72–78°F. Lighting was set for a 14-hour day. Purina mouse breeder chow and tap water were given *ad libitum*. Lettuce was provided once weekly to nursing mothers.

A description of the caging, breeding technique, and physical data pertaining to the irradiation are given in our previous report (2). At 2 days of age the animals were restrained in teflon holders and placed beneath a ¼-inch thick lead shield. Those to be irradiated were placed with their heads exposed to the X-ray beam past the edge of a cut out portion in the center of the shield. Control rats were placed completely under the shield. At 23, 42, and 121 days of age 600R head X-irradiated and control rats were killed by ether fumes and the pituitaries immediately removed, weighed, and frozen in vials surrounded by dry ice. The pooled glands were stored at —70°C until assays

* Work supported in part by the Mental Health Fund of the State of Illinois, in part by a USPHS grant, and in part by the General Research Support Fund of the University of California, Irvine.

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TABLE I. Bioassay of X-Irradiated Rat Pituitaries.[a]

Animals			Tibial epiphyseal width (μ)			Ovarian (gonad) wt. (mg)		Thyroidal radioactivity (C/mole $\times 10^{-2}$)	
Age (days)	Sex		N	Mean ± SEM	p	Mean ± SEM	p	Mean ± SEM	p
23	Males	X	6	266.0 ± 10.72		12.2 ± 0.78		118 ± 13.5	
		C	9	235.4 ± 16.46		11.1 ± 0.71		121 ± 5.6	
	Females	X	9	245.2 ± 12.98		11.9 ± 0.90		182 ± 13.2	<.05
		C	9	265.3 ± 20.19		12.7 ± 0.80		144 ± 8.6	
	Saline		5	155.8 ± 7.97		6.2 ± 0.57		8 ± 0.8	
42	Males	X	9	249.1 ± 14.39	<.025	14.6 ± 0.41	<.02	198 ± 11.2	
		C	8	293.5 ± 9.48		20.3 ± 2.19		216 ± 18.4	
	Females	X	9	263.4 ± 12.95		9.3 ± 0.31		147 ± 12.9	
		C	8	248.7 ± 13.47		8.5 ± 0.30		191 ± 16.7	
	Saline		7	135.6 ± 3.52		6.3 ± 0.41		7 ± 0.7	
121	Males	X	9	282.6 ± 10.55		17.8 ± 1.36	<.02	129 ± 8.1	
		C	10	294.2 ± 14.09		13.7 ± 0.71		129 ± 9.5	
	Females	X	10	249.0 ± 13.35		16.5 ± 0.50	<.001	115 ± 12.1	
		C	9	248.9 ± 18.50		8.6 ± 0.67		101 ± 10.8	
	Saline		10	153.6 ± 5.28		6.0 ± 0.32		8 ± 0.3	

[a] Abbrev.: N = number of assay animals; SEM = standard error of the mean; p = level of significance with two-tailed t test; X = irradiated; and C = control.

were carried out. The glands were weighed on a Roller-Smith torsion balance after thawing and were homogenized in chilled isotonic saline at pH 9 (NaOH) with a glass and teflon homogenizer. The concentration of pituitary tissue in the homogenate was 1.25 mg/ml. The homogenate was transferred to sterile injection vials and kept at 3–4°C. Homogenate for an injection period was always prepared on the first day of injection or the day before.

The assay animals were female Sprague-Dawley rats hypophysectomized at 26 days of age. The assay animals were given Purina mouse breeder chow in pellet form and in a paste made of powdered chow and tap water *ad libitum*. The wet diet was prepared fresh each day. Fresh 5% glucose solution was continued throughout the experimental period. The hypophysectomized rats were weighed at 29 days and 38 days of age; those that gained more than 10 gm, lost more than 5 gm, or who appeared to be in bad condition were discarded. Assays were begun on the thirty-eighth day of age.

All assay animals received a total of 2.5 mg of pituitary from either irradiated or control animals. It was given intraperitoneally in four successive daily doses of 0.5 ml of the homogenate. After injecting the last dose of pituitary homogenate, the rats were injected subcutaneously with 1.0 μC of radio-^{131}iodine in 0.25 ml sterile isotonic saline. On the following day the rats were weighed and all groups were sacrificed by ether vapor at approximately the same time.

The right tibias were dissected free of soft tissue, split midsagittally and preserved in 10% formalin. Tibial epiphysial width measurements were later carried out according to the technique of Evans *et al.* (3). The sella turcica of each rat was inspected under the dissecting microscope for completeness of hypophysectomy. Ovaries were dissected by the same person and weighed combined on a torsion balance to the nearest 0.1 mg. The thyroid glands with the trachea were removed and placed in a counting vial. Radioactivity was measured with a 3-inch crystal well detector and radiation spectrometer. Count rates were corrected for decay and background.

TABLE II. Rat Pituitary Weights (mg), 600 R Dose.[a]

Age (days)	Item	Males		Females	
		X	C	X	C
23	N	30	28	33	36
	M	0.88	1.68	0.96	1.92
	SEM	0.033	0.065	0.329	0.048
42	N	14	12	14	22
	M	1.99	4.55	2.15	5.48
	SEM	0.103	0.267	0.147	0.203
121	N	9	13	11	11
	M	3.84	7.65	3.93	8.57
	SEM	1.084	0.261	0.271	0.517

[a] Abbrev.: N = number; M = mean; SEM = standard error of the mean; X = irradiated; and C = control.

Results. Table I shows the responses in the assay animals to the injections of pituitary homogenate and saline media without pituitary tissue. The responses to saline injection were uniform. The responses to pituitary injections were more varied. Table II shows pituitary weights of the groups of irradiated and control animals. At the different ages the mean weight of the irradiated pituitaries was approximately half that of the corresponding control weight. The assay results were as follows:

Somatotropin. Pituitaries from rats of the same sex sacrificed at 23, 42, and 121 days of age caused essentially the same tibial epiphyseal width whether or not the animals had been previously irradiated. There were no significant differences in the response at different ages between groups of the same sex.

Gonadotropin. Pituitaries from the irradiated or the control goups at 23 days of age caused essentially the same ovarian weight increase. However, differences were seen in the results from the injection of pituitaries of older animals. At 42 days of age the pituitaries of irradiated males stimulated less ovarian weight gain than did those of control males. This difference did not exist between the irradiated and control females of that age. At 121 days of age pituitaries of irradiated rats of either sex produced greater ovarian weight gain than did those of the controls. The greatest difference was caused by pituitaries of the females.

Thyrotropin. Pituitaries from males showed no difference between irradiated or control groups in the stimulation of thyroidal ^{131}I uptake. There was greater potency in pituitaries of 42 days of age than those of either 23 or 121 days of age in males. Pituitaries from irradiated females of 23 days of age produced a greater increase in ^{131}I uptake than did those of female controls of the same age, but differences at 42 days and 121 days of age were not statistically significant.

Comment. The hormonal concentration of the pituitaries of the irradiated rats was, in general, as great or greater than that of the nonirradiated control rats. The present data and our results reported previously (2) show that the mean pituitary weight of the head-irradiated rats of our colony given the same dose, 600 R, is about half that of the control rat at 121 days. Our assay data would then indicate that somatotropin, gonadotropin, and thyrotropin are about halved in amount *per pituitary*. An exception was seen in the assay of gonadotropin in irradiated female pituitaries of 121 days of age. Here, assuming linearity of response, the concentration was about double that of control pituitaries; hence, the pituitary content would be about equal that of the controls. Treatment of the assay animals with human chorionic gonadotropin to enhance responsiveness of pituitary gonadotropin was avoided lest this interfere with the tibial epiphyseal width assay.

Our principal aim was to compare somatotropin concentration in the pituitaries of the irradiated and nonirradiated rats; hence, we did not quantitate pituitary hormonal content against reference standards. We would note, however, that the observed values of tibial epiphyseal cartilage width fell within the linear range of log dose–response curves reported by Greenspan *et al.* (4); thus, one can reasonably assume that the concentrations of pituitary somatotropin in the irradiated and nonirradiated rats were similar.

We have previously reported that there is normal tibial epiphyseal width and skeletal

maturation in the irradiated stunted rats. We have also reported a lack of response in growth to administration of growth hormone and/or thyroxine. Gonads, thyroids, and adrenals are heavier in comparison to body weight in the stunted head-irradiated rat (2). The present experiments show that the anterior lobes store as much hormone per unit of tissue weight as the controls. Studies of the blood level of somatotropin or of serum sulfation factor would be of value in confirming that hormonal release mechanisms are intact; the bulk of the evidence, however, suggests that well-known endocrine mechanisms are not involved in the stunting in the head-irradiated rat.

Summary. The injection of homogenates of the pituitaries of rats stunted by 600 R of X-irradiation administered to the head and of nonirradiated controls into hypophysectomized immature female rats resulted in comparable responses in terms of tibial epiphyseal width, gonadal weight, and thyroidal ^{131}I uptake. An exception occurred in 121-day-old females in which the pituitary homogenate of the stunted animals produced about twice the gonadal weight in the assay animals as did those of controls. These findings combined with those of earlier experiments, suggest that the permanent stunting resulting from head X-irradiation is not the result of hormonal deficit.

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Received Dec. 14, 1967. P.S.E.B.M., 1968, Vol. 128.

Morphological Changes following Irradiation of a Segment of the Rat Thymus* (32934)

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Considerable evidence indicates that the thymus has a significant role in the development of peripheral lymphoid tissue and in the development of immunological responsiveness. Although the thymus has been studied for almost a century, many aspects of the population kinetics of the various types of thymic cells are controversial. One approach to the study of these problems has been to follow the pattern of regeneration of the thymus after experimental involution produced either by irradiation or by increased adrenal cortical activity. This approach, however, gives information only about the potentialities of the surviving cells which are primarily considered as epithelial derivatives of the thymus. The developmental potentialities of the mesodermal derivatives of the thymus and their role in the regeneration process cannot be adequately assessed since these cells are markedly reduced.

In this first paper in a series of studies of thymus cellular kinetics a method is described for producing by irradiation a narrow band of involution in the rat thymus bounded by essentially normal thymic tissue, and the pattern of regeneration of this involuted tissue is described.

Materials and Methods. Irradiations were performed with a Philips X-ray apparatus operated at 200 keV and 18 mA with 0.5-mm Cu and 1.0-mm Al filters. The animals were irradiated 30 cm from the source at a dose rate of 80 R/min. A 7-mm thick lead shield with a rectangular opening of 5 × 25 mm

* Supported in part by Cancer Research Funds and by the U. S. Atomic Energy Commission.

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