Influence of Hypophysectomy on Porphyrin Synthesizing Enzyme Activity of Rat Harderian Glands and Livers.* (25413)

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It was previously shown that extracts of Harderian gland and liver homogenates of normal rats exhibit a high porphyrin-producing enzyme activity(1), and that activity of the Harderian gland extract was considerably higher than that of liver in the same animal. The porphyrin-producing enzyme activity of the mouse Harderian gland extracts(2) varied with age of animal and the strain, but age of animal was the most important variable. The amount of porphyrin-producing enzyme appeared to increase rapidly just before mice became sexually mature, when the hypophysis increases its output of gonadotropic and perhaps other hormones. It was, therefore, of interest to examine the quantitative aspects of porphyrin-producing enzymes in Harderian glands of hypophysectomized rats to see if the gonadotropic hormones or secondarily produced sex hormones might have some influence on increasing rate of production of Harderian gland porphyrins. Enzyme activities of homogenates of liver tissues were also determined to serve as control tissue. Hypophysectomy in the male albino rat brought increased porphyrin-producing enzyme activity of the liver but not of the Harderian gland. Neither of these results had been anticipated.

Materials and methods. Harderian glands and liver tissues were obtained from adult normal and hypophysectomized male albino rats under ether anesthesia. Animals were housed in metal cages and given Purina Laboratory Chow and water *ad lib*. The method of Davidheiser and Figge(3) was used to determine quantity of porphyrin produced by extracts of Harderian gland and liver tissues from normal (control) and hypophysectomized rats. In brief, tissues assayed for enzyme activity were carefully weighed and ground with sand in buffered saline (pH 7.2). The sand and solid glandular homogenates were centrifuged to obtain aqueous extract of the homogenate. The clear aqueous extract was added to Klett tubes in varying amounts (0.1-1.6 cc). Buffered saline was added to bring volume of each tube to 1.6 cc. The substrate added to each tube was 1.4 cc of 0.01 M δ-aminolevulinic acid. After incubation at 37°C for 24 hours, concentrated 37% HCl was added to give a 25% HCl concentration. This stopped the reaction and cleared the solution so that the amount of porphyrin formed could be determined by comparison with standard porphyrin solutions in a Klett colorimeter. Protein content of each tissue was determined by Clark's method(4).

Results. The quantity of porphyrin produced by enzyme extracts of livers and Harderian glands from normal and hypophysectomized rats is shown in Table I. The amount of porphyrin produced by extracts of liver from the hypophysectomized rat is approximately twice that of liver extracts of normal animals. In the normal animal, the porphyrin-producing enzyme concentration of liver was less than that of the Harderian gland. However, in the hypophysectomized rat the reverse was noted. Protein concentration of each of these tissues was also determined. The protein content of livers of hypophysectomized animals was about 3% higher than in the control normal animal, probably because of a higher water content of livers of normal non-hypophysectomized animals. This dif-

TABLE I. Porphyrin Production by Aqueous Extracts of Homogenates of Harderian Gland and Liver Tissue from Normal and Hypophysectomized Rats.

Tissue	Type of rat	Enzyme extract added 0.4 cc	1.6 cc
		mg of porphyrin/24 hr	
Liver	Normal (control)	28.0	70.4
	Hypophysectomized	55.6	149.6
Harderian gland	Normal (control)	29.8	100.4
	Hypophysectomized	30.0	100.0

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ference would not be large enough to account for the 100% increase in enzyme activity. Harderian gland extracts of normal and hypophysectomized animals exhibited no significant difference in amount of porphyrin produced.

Discussion. Since such a marked change in amount of porphyrin-producing enzyme activity of the Harderian gland at puberty had been observed, it was anticipated that there would be some corresponding influence of hypophysectomy on porphyrin production of extracts of Harderian glands, but this was not observed.

In addition to great increase in porphyrin production by the Harderian gland at puberty, other observations also indicated that there might be some relationship between the hypophysis and the function of other porphyrin-producing glands such as sebaceous glands. It has been mentioned that there appears to be a cytological and functional similarity between Harderian gland and sebaceous glands(5,6). Sebaceous glands in the adult are also porphyrin-producing glands. It is a striking fact, that the sebum of the pre-pubertal child contains little or no porphyrin as judged by fluorescence of the sebum. At puberty, the sebum in certain areas of the skin becomes red fluorescent because there is a gradual increase in amount of porphyrin which continues until the age of 25. Red fluorescence of the sebum then appears to level off and a constant intensity is observed until age 55 or 60 when there is a decrease in amount of porphyrin in sebaceous material. That the hypophysis influences sebaceous glands was shown by Lasher, Lorincz, and Rothman(7) who observed that there was atrophy of cutaneous sebaceous glands in ovariectomized and hypophysectomized white rats. It was also observed by Lorincz and Lancaster(8) that hypophysectomized castrated male rats were affected by injections of sebatropic preparations. It is well established that the hypophysis affects growth of the Harderian gland(9,10). It has been suggested that the growth hormone and thyrotropic hormone are responsible for such growth stimulation. It was therefore surprising to find that hypophysectomy appeared to have no influence on porphyrin-producing potentialities of the Harderian gland as determined by testing the extracts of homogenized tissues.

It was equally surprising to find that the amount of porphyrin-producing enzyme in liver extracts of hypophysectomized rats was double that of controls. The fact that liver tissues have such a great capacity to manufacture porphyrin indicates that rate of turnover of these substances may be relatively rapid. Why hypophysectomy should cause increased rate of porphyrin manufacture in the liver, however, is not understood.

Summary and conclusions. 1. A quantitative study was made of porphyrin-producing enzyme activities of Harderian glands and livers of normal and hypophysectomized albino rats. 2. Buffer-saline extracts were made from weighed quantities of each homogenized tissue. Measured amounts of extracts were mixed with 0.01 M solution of δ-aminolevulinic acid and incubated at 37° for 24 hours. 3. Extract of Harderian glands of normal and hypophysectomized rats showed approximately equal amounts of porphyrin-producing enzyme activity. The hypophysis appears to have little or no influence on this phase of activity of the Harderian gland. 4. Extracts of livers of hypophysectomized animals exhibited approximately twice as much porphyrin-producing enzyme activity as extracts of liver of normal animals. Extracts of liver from hypophysectomized rats also showed greater activity than extracts of Harderian glands. 5. It was concluded that the hypophysis exerts a relatively potent influence on porphyrin production by the liver.

1. Davidheiser, R. H., Figge, F. H. J., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 775.

2. Figge, F. H. J., Davidheiser, R. H., *ibid.,* 1957, v96, 437.

3. Davidheiser, R. H., Figge, F. H. J., *ibid.,* 1955, v90, 461.

4. Clark, E. P., *Semicro Quantitative Organic Analysis,* Academic Press, N. Y., 1943, 37.

5. Figge, F. H. J., *Bull. Sch. Med. Univ. Md.,* 1942, v26, 165.

6. ———, A.A.A.S. Research Conf. on Cancer, 1944, 117-128.

7. Lasher, H., Lorincz, A. L., Rothman, S. J., *Invest. Dermatol.*, 1955, v24, 499.

8. Lorincz, A. L., Lancaster, G., *Science*, 1957, v126, 124.

9. Boas, N. F., Bates, R. W., *Endocrin.*, 1954, v55, 601.

10. Smelser, G. K., *Anat. Rec.*, 1943, v86, 41.

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Effect of Ovariectomy and Replacement Therapy Upon Thyroxine Secretion Rate of Rat.* (25414)

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The role of ovarian hormones in influencing thyroid function has been the subject of many investigations. It has been reported that ovariectomy depresses(1,2) or has no effect upon thyroid function(3,4). Moreover, estrogen administration may increase(5,6,7), decrease(8,9), or exert little effect(3) upon thyroid activity depending upon dose and length of treatment. Progesterone, on the other hand, increased thyroid function(5,10) but when administered concomitantly with estrogen, a depressing effect has been reported (11). In the above studies various indices of thyroid activity have been used, such as release of thyroidal-I^{131}, I^{131} uptake, thyroid histology and cytology and hyperplasia. Since there is little agreement concerning the problem, it seemed desirable to determine quantitatively the influence of ovariectomy upon thyroxine secretion rate (TSR) at various periods postcastration and effect of ovarian hormone replacement therapy.

Materials and methods. Young mature female rats of Sprague-Dawley-Rolfsmeyer strain with initial weight of 190-230 g were housed at uniform temperature of 78 ± 1°F in a room artificially illuminated during normal daylight hours. They were given Purina Lab Chow and fresh water daily. Each rat was injected i.p. with 2 μc carrier-free I^{131}. Forty-eight hours were allowed for I^{131} fixation by the thyroid. External neck counts were taken at this time and every 48 hours thereafter by first anesthetizing each animal with ether, then placing it on a lead plate with neck resting on a scintillation probe containing a 2″ NaI crystal. Care was taken in placement of animal to insure same geometrical relationship at each successive counting period. Thyroidal radioactivity was measured with scintillation counter, Nuclear-Chicago (N.C.) Model DS5, connected to rate meter, N.C. Model 1620 A. Conventional corrections were made for decay of isotope and background. *Determination of thyroxine secretion rate (TSR).* Each rat was injected subc. with .5 μg/100 g l-thyroxine for 2 consecutive days beginning 4 days after I^{131} injection, in such manner that ovariectomized rats received initial thyroxine injection either on day of ovariectomy or at days 6, 20, 70, and 84 postcastration. Thyroxine dose was increased at .5 μg/100 g increments, each dose injected for 2 consecutive days, with neck counts made on day of each increase. TSR was determined by plotting the percent previous count with thyroxine dose. The dose which prevented further thyroidal-I^{131} output in each rat (95-100% previous count) was estimated as its TSR. Some ovariectomized rats received daily subc. injections of 1 μg estradiol benzoate (EB) and/or 3 mg progesterone (P), commencing day of initial thyroxine injection and continuing until their TSR was established. Intact rats of same age and body weight range served as controls for each series of TSR determinations.

Results. Effect of ovariectomy and replacement with EB and/or P upon average daily thyroxine secretion rate (TSR) is pre-

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