

lysis treatments) which have evaded attention simply because of their lack of activity.

From the above data we see that one color unit of pure androsterone is represented by 90 gamma of the crystalline material, while one color unit of urine extract is equivalent in biological activity to only 10 gamma of androsterone. This is an expected finding, but one that must be remembered when using the colorimetric method as a guide to quantitative studies.

10097

Effect of Estrogen Injections on Lactogen Content of Female Rat Pituitary.

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Earlier work¹ has demonstrated that pituitary glands from sexually mature ovariectomized rats injected with estrogen contained more lactogen than glands from sexually mature ovariectomized non-treated rats. Ovariectomy itself, however, decreased the lactogen content of the rat pituitary gland.¹ We were interested, therefore, in ascertaining the influence of estrogen injections on the lactogen content of pituitary glands from animals with their ovaries intact.

Three series of rats, with 20 rats in each series, were employed in this study. The animals designated as experimental animals in the third series, however, are the same animals as those designated experimental animals in the second series. Rats were paired on the basis of body weight and they were all fed the same stock diet. Daily vaginal smears were made from injected animals, and from the control animals during the last 4 days of the experimental period. All animals were sacrificed the day following the last day of injection, their pituitaries and other endocrine organs removed and weighed. The lactogen content of the pituitary glands was determined by the assay method employed by Reece and Turner.²

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¹ Reece, R. P., and Turner, C. W., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 283.

² Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1937, 266.

Series I. Ten rats that had been running normal estrous cycles were injected subcutaneously daily for 15 days with 200 i.u. of estrogen (Progynon-B†). The control animals were normally cyclic rats. Vaginal smears of the injected rats revealed a complete cornification of the vaginae on the 2nd day of injection. During the remainder of the injection period the vaginal smears consisted mainly of cornified cells with leucocytes appearing at irregular intervals. The estrogen injections elicited an increase in the weight of the pituitary gland, a decrease in ovarian weight, and no significant change in thyroid or adrenal weight. The pituitary glands from the injected rats contained 40% more lactogen than did glands from non-injected animals. This increase in lactogen content was due entirely to increased pituitary size because the lactogen concentration was decreased.

Series II. Ten normally cyclic rats were injected subcutaneously daily for 15 days with 1000 i.u. of Progynon-B. The control rats were normally cyclic animals. The vaginae of the injected rats were completely cornified on the 2nd or 3rd day of injection and cornified cells were present in the vaginal smears throughout the injection period. Beginning on the 6th day and continuing throughout the injection period nucleated epithelial cells and/or leucocytes were present in the majority of the vaginal smears. In this connection it should be mentioned that Wolfe³ has reported that the vaginal smears of rats injected with 1000 i.u. of Progynon-B daily for 8 to 15 days revealed a complete cornification of the vaginae for the first 3 to 5 days of the experiment. This condition did not persist throughout the experiment. On autopsy, as has been reported by other investigators,^{3, 4} the ovaries of the injected rats contained corpora lutea which were similar in size and appearance to the corpora lutea of rats sacrificed in late pregnancy.

Estrogen injections induced an increase in pituitary size but not as great as that observed in Series I. The ovaries of 7 out of 10 experimental animals were heavier than those of the control animals. There was no significant difference in the weights of the thyroid and adrenal glands of the control and experimental animals. The pituitary glands from the injected animals contained 28% more lactogen than did those from non-injected animals, and, as was observed in Series I, this increased hormone content was due entirely to increased pituitary size, for there was a slight decrease in hormone concentration.

† The Progynon-B used in this study was kindly supplied by Dr. E. Schwenk of the Schering Corporation.

³ Wolfe, J. M., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 757.

⁴ Hohlweg, W., *Klin. Wchnschr.*, 1934, **13**, 92.

TABLE I.
Effect of Estrogen Injections on Lactogen Content of Female Rat Pituitary.

No. of Animals	Physiological condition	Avg body wt beginning of exp. g	Avg body wt when sacrificed g	Avg pituitary wt, mg	Avg thyroid wt, mg	Avg adrenal wt, mg	Avg ovarian wt, mg	Bird units per pituitary gl	Bird units per mg pituitary tissue	Bird units per 100 g body wt
10*	Normally cyclic rat	149	160	8.9	15.2	46.7	59.1	7.93	0.89	4.96
10†	200 i.u. Progynon-B/day for 15 days	152	161	15.7	17.4	45.7	47.5	11.10	0.71	6.89
10*	Normally cyclic rat	160	167	7.9	16.3	42.1	58.9	12.6	1.59	7.54
10†	1000 i.u. Progynon-B/day for 15 days	161	159	13.3	19.3	41.5	64.4	16.1	1.21	10.13
10*	Ovariectomized + 1000 i.u. Progynon-B/day for 15 days	162	160	21.4	14.1	42.9		14.3	0.67	8.94
10†	1000 i.u. Progynon-B/day for 15 days	161	159	13.3	19.3	41.5	64.4	14.1	1.06	8.87

* Control Animals.

† Experimental Animals.

Series III. For control animals 10 normal cyclic rats were ovariectomized and injected subcutaneously daily for 15 days with 1000 i.u. of Progynon-B. The experimental animals in this series were the same as those in Series II.

The vaginal smears of the control animals indicated cornification of the vaginae on the 2nd day of the injection period. The smears, during the remainder of the injection period, consisted largely of cornified cells. An occasional smear, however, contained large nucleated epithelial cells and/or leucocytes.

Of most interest in this series is the observation that each one of the pituitary glands from the control animals was heavier than the gland from its experimental mate. On the average, the pituitaries from the control animals weighed 21.4 mg and those from the experimental animals weighed 13.3 mg. This observation is somewhat surprising inasmuch as Brooksby⁵ reported that 500 gamma of progesterone daily enhanced the action of estrogen in causing macroscopic enlargement of the pituitary.

Each one of the thyroid glands from the experimental animals was heavier than the thyroid gland from its control mate. There was no difference in adrenal weight between the 2 groups of animals.

The lactogen content of the pituitary glands from the control and experimental animals was nearly identical when average figures are considered. When the pairs are considered separately, 6 of the glands from the control animals contained more lactogen than the glands from the experimental animals, one was equal, and 3 contained less lactogen.

The results are summarized in Table I.

Conclusions. The injection of Progynon-B into rats with their ovaries intact augmented the lactogen content of their pituitary glands. The pituitary glands from ovariectomized rats injected with 1000 i.u. of Progynon-B daily for 15 days were significantly larger than those from rats with their ovaries intact and similarly treated, but the lactogen content per pituitary was similar.

⁵ Brooksby, J. B., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 832.