larly in RIF-free chick embryo tissue culture, is of importance in the development of safe attenuated rubella virus vaccines for parenteral administration. Nine virulent or attenuated rubella virus strains were propagated for at least 3 passages in RIF-free chick embryo tissue culture or duck embryo tissue culture. Several strains showed growth in duck and chick tissue through the 5th to 7th passages. Highest titers were usually obtained when passages were made at intervals of 7 or more days. Virus titers varied from 1.2 to 4.5 $TCIND_{50}$ log $_{10}$/ml in duck tissue culture at the 5th to 7th passages, and 1.0 to 1.5 $TCIND_{50}$ log_{10}/ml in chick tissue culture at the 5th passage level. Virus growth was demonstrated in early passages in embryonated duck eggs but no detectable growth was obtained in chick eggs. The propagation of rubella virus in RIF-free chick embryo tissue culture warrants further efforts at the development of an attenuated rubella vaccine in this tissue culture system.

1. Parkman, P. D., Meyer, H. M., Kirschstein, R. L., Hopps, H. E., New Eng. J. Med., 1966, v275, 569.

2. Meyer, H. M., Parkman, P. D., Panos, T. C., ibid., 575.

3. Hilleman, M. R., Clin. Pharmacol. Therap., 1966, v7, 752.

4. Stokes, J., Weibel, R. E., Buynak, E. B., Hilleman, M. R., Pan American Health Organization 1st International Conference on Vaccines Against Viral and Rickettsial Diseases of Man, 1967, v147, 402.

5. Sever, J. L., Schiff, G. M., Traub, R. G., J.A. M.A., 1962, v182, 663.

6. Sever, J. L., Monif, G. R. G., Cusumano, C. L., Schiff, G. M., Huebner, R. J., Am. J. Epidemiol., 1966, v84, 163.

7. Schiff, G. M., Sever, J. L., Huebner, R. J., Science, 1963, v142, 58.

8. Reed, J. L., Muench, H., Am. J. Hyg., 1938, v27, 493.

Received May 1, 1967. P.S.E.B.M., 1967, v125.

Changes in Pituitary Prolactin Release and Hypothalamic PIF Content During the Estrous Cycle of Rats.*† (32265)

M. SAR AND JOSEPH MEITES

Department of Physiology, Michigan State University, East Lansing, Mich.

In randomly cycling rats, considerable variability has been observed in pituitary prolactin content, pituitary prolactin release *in vitro,* and in hypothalamic prolactin inhibiting factor (PIF) content(1-3). These variations may be due to differences in secretion of the gonadal hormones during each stage of the estrous cycle. Changes during each phase of the estrous cycle of rats have been observed in pituitary LH concentration (4), plasma LH content(5) and hypothalamic content of luteinizing hormone releasing factor (LRF)(6). It was of interest therefore, to determine whether differences could be detected during each stage of the estrous cycle of rats in pituitary prolactin content, pituitary prolactin release *in vitro* and in hypothalamic PIF content.

Materials and methods. Mature female Sprague-Dawley rats (Spartan Research Labs., Inc., Haslett, Mich.) were housed in a temperature (75 ± 1°F) and light (14 hr/day) controlled room. Vaginal smears were taken each morning between 9-10 A.M. beginning one week after arrival of the rats. Only rats which had exhibited at least 2 regular cycles of 4 or 5 day length were used in this study. At each stage of the estrous cycle (except metestrus), the rats were killed by guillotine, and the anterior pituitaries and hypothalami were quickly removed. The posterior lobe was discarded and the anterior pituitary was weighed, frozen and stored at −20°C until assayed. The hypothalamus and median eminence were collected in chilled .1N HCl (10 hypothalami/2 ml)

* This study was supported in part by NIH grant AM 04784-06.

† Published with approval of Director of Mich. Agr. Exp. Station as journal article No. 4075.

TABLE I. Prolactin Content of Rat Anterior Pituitaries During the Estrous Cycle.

Exp No.	No. of rats	Cycle phase	Body wt (g)	No. of pigeons	Prolactin (RTU/AP)*	% Difference
I	6	Proestrus	222.3 ± 1.5	7	4.5 ± .5	64**
		vs				
	6	Diestrus	234.5 ± 5.5	7	2.7 ± .5	
II	12	Estrus	214.1 ± 3.1	6	4.8 ± .4	71***
		vs				
	12	Diestrus	225.4 ± 2.6	6	2.8 ± .2	
III	6	Proestrus	222.3 ± 1.5	7	4.5 ± .5	7†
		vs				
	12	Estrus	214.1 ± 3.1	6	4.8 ± .4	

* RTU/AP = Reece Turner units/anterior pituitary.
** $P < .05$.
*** $P < .01$.
† Not significant.

and frozen at −20°C until assayed a few days later.

Preparation of hypothalamic extracts. On the day of incubation the hypothalami were thawed and homogenized with a glass homogenizer, and centrifuged at 12,000 *g* for 40 minutes at 4°C. The supernatants were placed in protein free medium 199 (Difco Labs, Detroit, Mich.) and the pH was adjusted to 7.4 by adding a drop at a time of 1N NaOH and testing with glass electrodes.

Incubation. Adult male rats of the same strain were killed by guillotine and the anterior pituitaries were removed and each anterior pituitary was hemisected. One half was placed into separate 25 ml Erlenmeyer flasks containing 2 ml of medium 199. The equivalent of 2 or 3 anterior pituitaries (4 or 6 halves) was placed into each flask. The neutralized hypothalamic extract (equivalent of 2 hypothalami per incubated pituitary) was added rapidly to each flask. Incubation was carried out in a Dubnoff metabolic shaker (60 cycles/min) under constant gassing with 95% O_2-5CO_2 at 37° ± 0.5°C for 4 hours to measure PIF activity in the hypothalamus. A 2 hour incubation was used to measure prolactin release by the anterior pituitaries of rats in different stages of the cycle. At termination of each incubation, the pituitary halves were weighed and the media were frozen at −20°C and stored until assayed.

Prolactin assay. Prolactin was assayed in 6-8 week old White King squabs by the intradermal pigeon-crop technique of Lyons(7) as modified by Reece and Turner(8). A direct comparison was made between samples by injecting one sample over one side of the crop sac and the other sample over the other side of the crop of the same bird. Significance of differences in pituitary prolactin content and hypothalamic PIF content during the different stages of the estrous cycle was analyzed by the "t" test for paired observations.

Results. Pituitary prolactin content during the different phases of the estrous cycle is shown in Table I. Prolactin is expressed in Reece-Turner units (RTU) per pituitary gland in all experiments. In Exp. I and II, pituitary prolactin content of proestrous and estrous rats was compared with pituitary prolactin content in diestrous rats. Prolactin in the pituitaries of the proestrous (Exp. 1) and estrous rats (Exp. 2) was significantly greater, by 64 and 71% respectively, than in the pituitaries of the diestrous rats. No significant difference in pituitary prolactin content was observed between the proestrous and estrous rats (Exp. III).

Prolactin release *in vitro* during a 2 hour incubation by the pituitaries of rats in different stages of the cycle is shown in Table II. Prolactin release from the pituitaries of proestrous and estrous rats was significantly greater, by 55 and 54% respectively, than from pituitaries of diestrous rats.

The hypothalamic PIF contents in proestrous, estrous and diestrous rats are seen in Table III. Anterior pituitary halves in-

TABLE II. Effect of Estrous Cycle on Pituitary Prolactin Release *in vitro*.

Exp No.	No. of rats	Cycle phase	No. of pigeons	Prolactin released (RTU/AP)*	% Difference
I	3	Proestrus	6	2.6 ± .4	55***
		vs			
	3	Diestrus	6	1.7 ± .3	
II	3	Estrus	6	2.2 ± .4	54**
		vs			
	3	Diestrus	6	1.4 ± .4	

* RTU/AP = Reece Turner units/anterior pituitary.
** P <.05.
*** P <.01.

TABLE III. PIF Content of Hypothalamus During the Estrous Cycle.

Exp No.	No. of rats	Cycle phase	No. of flask pairs	No. of pigeons	Prolactin released into medium (RTU/AP)*	% Difference
I	14	Proestrus	3	11	1.3 ± .3	84***
		vs				
	14	Diestrus		11	.7 ± .2	
II	12	Estrus	3	6	1.7 ± .3	42**
		vs				
	12	Diestrus		6	1.2 ± .2	
III	4	Proestrus	1	6	2.4 ± .5	0
		vs				
	4	Estrus		6	2.4 ± .8	

* RTU/AP = Reece Turner units/anterior pituitary.
** P <.02.
*** P <.001.

cubated with hypothalamic extract from proestrous rats released an average of 84% more prolactin than the corresponding anterior pituitary halves incubated with hypothalamic extract from diestrous rats (Exp. 1). Anterior pituitary halves incubated with hypothalamic extract from estrous rats (Exp. II) released an average of 42% more prolactin in the medium than by the corresponding pituitary halves incubated with hypothalamic extract from the diestrous rats. No differences in prolactin release were observed when anterior pituitary halves were incubated with hypothalamic extracts from proestrous and estrous rats (Exp. 3).

Discussion. These results show that anterior pituitaries of proestrous and estrous rats contain significantly more prolactin than anterior pituitaries of diestrous rats, that the former release significantly more prolactin when incubated *in vitro* than the latter, and that proestrous and estrous rats have significantly less PIF in the hypothalamus than diestrous rats. The findings on pituitary prolactin content are in agreement with a previous study showing that the pituitaries of proestrous and estrous rats and guinea pigs contain more prolactin than the pituitaries of diestrous rats and guinea pigs(8,9). The stimulus from ovarian estrogen during proestrous and estrous is believed to decrease hypothalamic PIF content and to increase prolactin release from the incubated anterior pituitary. Estrogen has been observed to depress PIF content in the rat hypothalamus (3) and directly to stimulate prolactin release by the rat anterior pituitary *in vitro* (10).

Many reports indicate that estrogen increases pituitary prolactin synthesis and release. Thus estrogen administration has been observed to increase pituitary prolactin content and initiate lactation in rats(10), and to increase activity by the acidophil cells of the rat anterior pituitary(11). Implantation of estrogen into the anterior pituitary of rats

(12) or rabbits(13) increased prolactin release *in vivo,* and injections of estrogen into rats increased prolactin release by the pituitaries when subsequently incubated *in vitro* (14).

The present results are believed to help explain the considerable variations previously observed in randomly cycling rats in prolactin release by anterior pituitary tissue incubated *in vitro,* and also the fluctuation in hypothalamic PIF content. Recently it was shown that the use of pituitaries from mature male rats show much more uniformity in prolactin release *in vitro* than pituitaries from randomly cycling female rats(15). We are currently using male rat pituitaries for assaying PIF *in vitro,* but are incubating them for 4 instead of the 2 hours used previously for pituitaries from female rats. This is because male rat pituitaries release considerably less prolactin than female rat pituitaries. Use of hypothalami from male rats or from female rats during a particular stage of the cycle should also give more uniform results in measuring PIF content than hypothalami from randomly cycling rats.

Summary. Comparisons were made of pituitary prolactin content, pituitary prolactin release *in vitro* and hypothalamic PIF content at different stages of the estrous cycle of the rat. The anterior pituitaries of proestrous and estrous rats contained significantly more prolactin than anterior pituitaries of diestrous rats; the former released significantly more prolactin when incubated *in vitro* than the latter, and proestrous and estrous rats had significantly less PIF in the hypothalamus than diestrous rats. Ovarian estrogen secreted during proestrus and estrus is believed to increase prolactin release by depressing hypothalamic PIF production and by directly stimulating the pituitary.

1. Nicoll, C. S., Meites, J., Endocrinology, 1962, v70, 927.
2. Talwalker, P. K., Ratner, A., Meites, J., Am. J. Physiol., 1963, v205, 213.
3. Ratner, A., Meites, J., Endocrinology, 1964, v75, 377.
4. Schwartz, N. B., Bartoski, D., ibid., 1962, v71, 756.
5. Ramirez, V. D., McCann, S. M., ibid., 1964, v74, 814.
6. Ramirez, V. D., Sawyer, C. H., ibid., 1965, v76, 282.
7. Lyons, W. R., Cold Spring Harbor Symposium on Quantitative Biology, 1937, v5, 198.
8. Reece, R. P., Turner, C. W., Missouri Univ. Agri. Exp. Sta. Research Bull., No. 266, 1937.
9. ———, Proc. Soc. Exp. Biol. & Med., 1939, v42, 54.
10. Meites, J., Nicoll, C. S., Proc. First International Congress on Hormonal Steroids, Academic Press, New York, 1965, v2, 307.
11. Wolfe, J. M., Chadwick, C. S., Endocrinology, 1936, v20, 503.
12. Ramirez, V. D., McCann, S. M., ibid., 1964, v75, 206.
13. Kanematsu, S., Sawyer, C. H., ibid., 1963, v72, 243.
14. Ratner, A., Talwalker, P. K., Meites, J., Proc. Soc. Exp. Biol. & Med., 1963, v112, 12.
15. Kragt, C. L., Meites, J., Endocrinology, 1967, v80, 1170.

Received May 8, 1967. P.S.E.B.M., 1967, v125.

Endogenous Creatinine Clearance by Rats.* (32266)

RALPH GOLDMAN (With the technical assistance of Pedro Fiorello)
Department of Medicine, University of California School of Medicine, Los Angeles

The present study examines 3 aspects of the clearance of endogenous creatinine in rats: (1) Twenty-four hour, undisturbed creatinine clearances, (2) urinary non-creatinine chromogen content, and (3) correlation of clearance with body weight, kidney weight and surface area. Previous endogenous clearances have been performed during short term studies, usually one hour or less. In general, these studies have involved

*This research supported by USPHS Grant HE 07852. Computational assistance was obtained from the U.C.L.A. Health Sciences Computing Facility supported by NIH Grant FR-3.