peak in female reproductive capacity as measured by number of females pregnant, with vulva open and the number lactating. It seems logical that these variations in reproductive function must be accompanied by metabolic and biochemical changes which if understood could provide insight into the regulation of circadian cyclic phenomena. Oxygen uptake, and lactic acid production indicate that during the period of degeneration, testes shift from a metabolism characterized by a high oxygen utilizing system to one in which glycolysis predominates. The fact that glucose uptake remains virtually unchanged suggests that one pathway is operating at the expense of the other. This trend is reversed during the period of redevelopment and it is interesting to note that endogenous oxygen uptake reaches a maximal point at this time. This observation is similar to that found in immature rat testes by Tepperman and Tepperman(16). Since testes increase approximately 4 times in size during the period of redevelopment, one would expect an increase in protein synthesis. This hypothesis is supported by the fact that RNA and protein content of testis and that the RNA/DNA ratio is greatly increased during this time. Deoxyribonucleic acid content also is increased and probably reflects an increase in cell number. Therefore these data indicate that cyclic breeding activity of the male cotton rat is correlated with profound physiological and biochemical changes in the testis of this seasonal breeding mammal.

Summary. A total of 91 cotton rats (*Sigmodon hispidus*) were collected from their natural habitat once a month for one year. Testes and accessory organs were excised and weighed. Teased tubule preparations of the testis were used to measure oxygen uptake, glucose uptake and lactic acid production. The remainder of testis was assayed for protein, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). These data indicate that characteristic metabolic and biochemical changes take place in testis during circadian breeding patterns.

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Response of Intraocular Ovarian Isografts to LH in Male Mice.* (30006)

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Ovariectomized mice, bearing bilateral intraocular ovarian isografts, show normal vaginal cycles. The grafts repeatedly develop mature follicles and corpora lutea in relation to proestrus-estrus and metestrus-diestrus, respectively(1). Identical grafts in *intact* males develop mature follicles which, while they

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may become atretic and be replaced by new ones, do not usually form corpora lutea(2). The present report concerns luteinization in such grafts in response to exogenous LH.†

Material and methods. Male mice, in groups of 6-8 or (usually) 12-15, received a graft of 1/8th of an isologous ovary in the anterior chamber of each eye(3,4). Recipients and donors were of the BALB/c strain, unless otherwise stated, and were 2¼-4¼ months of age. Two to 4 weeks later each animal received a single dose of LH, vehicle, or no treatment. Thereafter each graft was studied daily for 6 days using a dissecting microscope. The luteal response was calculated as the percentage of grafts in a group that formed corpora lutea during this period. Grafts that had corpora lutea, or had no mature follicles, at the time of LH administration were disregarded.

Dosage of LH ranged from 0.2 to 20 μg and the amount of vehicle was 0.1 cc. The route of administration was subcutaneous (SC), intraperitoneal (IP), or intravenous (IV), and the vehicle 16% gelatin‡ (G) or buffered sterile saline (S).

Results. After one week, grafts were well vascularized and had developed mature follicles. The mean number of such follicles per graft was 1.8 ± 0.26 at this time and increased significantly ($P<0.001$) to 4.2 ± 0.41 after 2 weeks or 3.9 ± 0.44 after 4 weeks and declined significantly ($P<0.05$) to 2.4 ± 0.35 after 8 weeks in 4 untreated groups of mice with 20-30 grafts per group.

Typically, grafts showed no change on the 1st day after LH administration. On the 2nd day, responding follicles had a cloudy appearance and, on the 3rd, had transformed into opaque white corpora lutea. These retained their integrity for one to 3 days and then lost definite outlines, usually being replaced by newly matured follicles. This timing did not appear to be affected by dose, route, or vehicle. Among 521 grafts, corpora formed on the 1st day in 0.3%, on the 2nd in 14%, on the 3rd in 62%, on the 4th in 21%, on the 5th in 2.6%, and on the 6th in 0.1%. When corpora were formed in one eye they did not necessarily develop in the other. Not all apparently mature follicles in a graft formed corpora and, regardless of the dose of LH, rarely did all grafts in a group show luteinization. Newly-formed corpora showed moderate hyperemia in only 29% of grafts and this hyperemia was never present on the next day.

TABLE I. No. of Transplants Forming Corpora Lutea Spontaneously in Pooled Groups Receiving Vehicle or No Treatment.

No. mice	No. transp	No. with spont lut on day: 1	2	3	4	5	% lut
18	30	–	–	–	–	–	0
24	42	–	–	–	2	1	7
34	60	–	3	–	2	1	10
36	64	–	1	2	1	2	9

Table I shows the number of grafts that developed corpora in pooled groups of animals that had received either vehicle alone or no treatment, over a 5-day period.

Dose response. Individual groups showed that a luteal response in approximately 90% of the grafts required about 20 μg of LH-SC-G, 10 μg of LH-SC-S, 2.5 μg of LH-IP-S, and 1 μg of LH-IV-S. In 4 different series of 3 simultaneously tested groups, 20, 10, and 5 μg of LH-SC-G gave a 91%, 79%, and 18% response; 10, 5, and 2.5 μg of LH-SC-S gave a 91%, 72%, and 7% response; 2.25, 1.5, and 0.75 μg of LH-IP-S gave a 75%, 45%, and 19% response; and 0.8, 0.4, and 0.2 μg of LH-IV-S gave a 83%, 33%, and 14% response. Table II gives the data from probit analysis of quantal response(5) for these series. The 50% effective dose decreased, or the potency increased, approximately 1.5-fold from SC-G to SC-S, 3-fold from SC-S to IP-S, and again by 3-fold from IP-S to IV-S administration. At the same time, the respective slopes of the regression lines did not differ significantly (Chi^2 test) between themselves from linearity or parallelism.

Mouse strain. Table III shows the luteal responses of C3H, Strong A and C57BL/BALB/c F1 hybrid mice to LH-IV-S and LH-IP-S; those of the 2 former were similar to that of BALB/c mice, but the hybrids

† NIH-LH-B1, bovine, generously supplied by the Endocrinology Study Section, Nat. Inst. Health.

‡ 16% gelatin vehicle, Armour, generously supplied by Dr. P. L. Brule, Armour Pharmaceutical Co.

TABLE II. Effective Dose for 50% Luteal Response, Slope of Line, and Relative Potency of LH Administered by Different Routes with Statistical Limits by Log-Probit Analysis.

Route	No. of transp	ED^{50} with 95% limit	Slope with S.E.	Rel pot with 95% limit*
IV-S	21-23	.46	3.43	3.23
		.41	± *.78*	*2.73*
		.52		*3.84*
IP-S	20-21	1.45	3.16	3.04
		1.29	± *.90*	*2.55*
		1.64		*3.64*
SC-S	11-13	4.47	4.66	1.65
		3.92	±*1.23*	*1.41*
		5.08		*1.95*
SC-G	22-26	7.37	4.44	
		6.68	±*1.07*	
		8.13		

* Comparing IP-S/IV-S; SC-S/IP-S; and SC-G/SC-S.

TABLE III. Luteal Response to LH of C3H, Strong A and C57BL/BALB/c F1 Hybrids.

Strain	No. of transp	Dose of LH, μg IV-S 0.2	IV-S 0.4	IP-S 1.0	IP-S 2.0
		(%)	(%)	(%)	(%)
C3H	13-14	36	46		
	27-28			59	75
Str A	12-14	25	43		
C57BL/ BALB/c	15-17	0	0		
F1	19-27			28	50

gave no response to the IV doses and a somewhat lesser response than the other strains to the IP doses.

Discussion. The pituitary in the male mouse apparently releases LH only sporadically at a level which permits luteinization of ovarian isografts. LTH release also appears to be at much lower levels than in females for, after administration of LH, functional luteal hyperemia(6) occurred in only 29% of grafts and had a maximum duration of one day. In contrast, cyclic females(1) showed luteal hyperemia in 92.5% of grafts and it persisted for 2 or more days in 40% of grafts.

The mechanism of action of LH evidently differs from that of LTH for the former was most effective when given by a method promoting rapid absorption and the latter by one giving delayed absorption(7). The results from injection of LH suggest a threshold response since the sequence of resulting morphological events occurred in the same time period regardless of dosage or mode of administration. This is in accord with the concept of a pre-ovulatory surge of LH release described in the cyclic female rat(8). Finally, the dose-response relationship for IV-S administration suggests that the male mouse, with ovarian isografts, can be used for bio-assay of LH with a sensitivity comparable to the most specific methods currently employed(9).

Summary. Intraocular ovarian isografts in intact male mice develop mature follicles that show only sporadic luteinization. A single dose of LH-NIH-B1 produced a peak of formation of corpora lutea on the 3rd day after administration. The 50% effective dose was 0.48 μg (IV in saline), 1.55 μg (IP in saline), 4.27 μg (SC in saline), or 7.34 μg (SC in gelatin).

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