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We are indebted to Maria T. Torres for competent assistance.

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Received July 23, 1962. P.S.E.B.M., 1962, v111.

Action of Purified Gonadotropins on Corpora Lutea in the Cyclic Mouse.* (27892)

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Isologous intraocular ovarian transplants restore normal estrous cycles in castrated female mice(1). Corpora lutea of each cycle initially show a distinct hyperemia that persists for only one or 2 days. This hyperemic phase is extended to approximately one week in pseudopregnancy, in the presence of an anterior pituitary transplant(2), and in lactation(3). Deciduomata can be evoked only when such corpora are present; the hyperemia appears to indicate function under the influence of pituitary luteotropin. The present report concerns the influence of exogenous FSH, LH and LTH on hyperemic corpora.

Material and methods. Female CE/BALB /c F1 hybrid mice, 2 to 3 months of age, in groups of 5 to 14 animals, were castrated.

* Supported by research grants from Nat. Cancer Inst., N.I.H., U.S.P.H.S.

Each received bilateral intraocular ovarian isotransplants. After 2 weeks (for establishment of transplants), vaginal and ovarian cycles were followed daily throughout the experimental period. One group of 10 mice received no treatment (controls). In the other groups, after at least 3 normal cycles, gonadotropin administration was commenced on the 1st day of appearance of hyperemic corpora (usually at metestrus) in the next cycle of individual animals. It was continued over from part of one to all of 3 subsequent cycles. Animals in groups I, II, III, IV, VII and VIII (Table I) received a constant dosage of 0.1 mg FSH, 0.1 mg LH, 0.2 mg LTH, 0.1 mg LTH, or 0.1 mg of both LH and LTH, respectively, administered subcutaneously in a 16% gelatin vehicle (Armour) every 12 hours.† Some animals in group V received a constant dosage of 0.025 mg LTH over 3 cycles (Va) while others had the dosage increased to 0.1 mg in the 3rd cycle (Vb). This group was duplicated in BALB/c mice (VI) but here all animals received the increased dosage in the 3rd cycle. A control group of the same strain received no treatment.

In some animals receiving LH (II), LTH at the 0.1 mg and 0.025 mg levels (IV, Va), and both LH and LTH (VII), the uterus was traumatized (by 3 silk thread loops passing through the anti-mesometrial wall) in the first experimental cycle when hyperemic corpora had been present for 2 days. Examination for deciduomata was made 4 days later. The 4th right mammary gland of animals in groups III, IV and Va (receiving LTH at 3 different dose levels), and of controls and intact virgin hybrids of the same age, was biopsied at the end of the period of gonadotropin administration.

Results. Representative individual protocols of hybrids receiving LTH or LTH with LH, and of controls, are shown in Fig. 1. Table I shows mean duration of hyperemia of corpora and length of vaginal cycles (proestrus to proestrus), with standard errors, for

† NIH-FSH-S1,NIH-LH-S1, and Prolactin, ovine NIH-P-S3 (LTH) generously supplied by the Endocrinology Study Section, N.I.H.

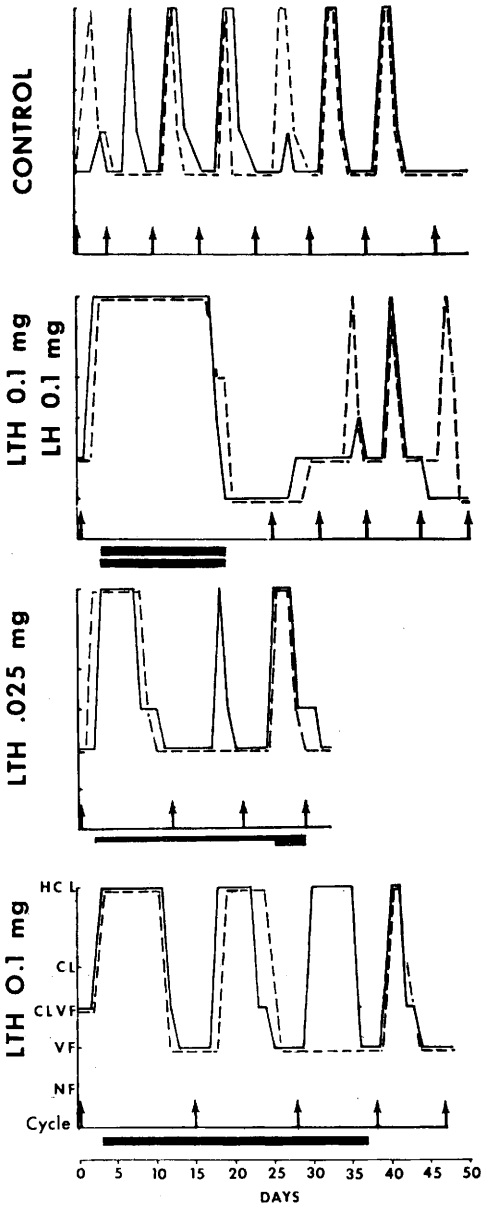

FIG. 1. Representative individual protocols of ovarian and vaginal cycles in castrated female CE/BALB/c mice with bilateral intraocular ovarian isotransplants for untreated (control) animals and for those receiving either 0.1 mg of LH and LTH, 0.1 mg of LTH, or 0.025 mg of LTH every 12 hr. (Arrow, proestrus; solid bar, period of gonadotropin administration; level of HCL, hyperemic corpora lutea present in ovarian transplant; of CL, only non-hyperemic corpora present; of CLVF, non-hyperemic corpora and vesicular follicles present; of VF, only vesicular follicles present; of NF, no follicular structures present; solid line and broken line, transplants in right and left eyes respectively.)

TABLE I. Mean Duration of Hyperemia of Corpora Lutea and Length of Corresponding Vaginal Cycle, with Standard Errors, in Mice Receiving Purified Pituitary Gonadotropins Every 12 Hr.

Group	Mean length hormone admin.	No. trs.	Duration of hyperemia of corpora and cycle length during and after gonadotropin admin. 1st	2nd	3rd	4th	5th
I	FSH .1 mg	10	—	—	—	—	—
	22 d.		—	—	20.6 ± 2.18	—	—
II	LH .1 mg	22	—	—	—	—	
	14 d.		23.6 ± 2.11	†9.4 ± 1.03	—	—	
III	LTH .2 mg	10	6.6 ± .62	6.0 ± .98	—		
	20 d.		12.0 ± 1.07	12.5 ± .64	—		
IV	LTH .1 mg	28	8.2 ± .40	6.5 ± .32	*4.5 ± .86	—	—
	38 d.		13.3 ± 1.17	*15.6 ± 2.62	11.8 ± 1.11	*11.6 ± 1.33	—
Va	LTH .025 mg	26	5.7 ± .29	—	—	—	
	29 d.		11.5 ± .98	†10.3 ± 1.26	10.5 ± .45	10.5 ± .45	
Vb					†3.7 ± .71		
VI	LTH .025 mg	16	4.9 ± .33	†2.6 ± .41	*4.0 ± .61		
	33 d.		—	—	†10.3 ± 1.21		
VII	LH, LTH .1 mg	10	10.0 ± .33	—			
	10 d.		18.4 ± .40				
VIII	LH, LTH .1 mg	10	16.6 ± 1.82	—	—	—	
	19 d.		29.8 ± 1.85	—	—	—	

* Values differed at less than 1% level; other values differed at less than 0.1% level.
† Values differed at less than 5% level.
(—) Values not significantly different from controls.

all treated groups *if* these values differed from those of controls at less than the 5% level of probability. *Controls.* In hybrid mice, hyperemia had a mean duration of 1.6 (S.E. ± 0.33) days and the vaginal cycle of 6.3 (S.E. ± 0.71) days for 67 cycles observed. Hyperemic corpora did not appear in either transplant in the same animal in 7 cycles, appeared on one transplant only in 23 cycles, and in both transplants in 37 cycles. Hyperemia persisted for only one day on 54 occasions, for 2 days on 29, for 3 days on 10, for 4 days on 2, and for 5 days on 2. The vaginal cycle was usually prolonged when hyperemia persisted for over 3 days and such cycles were, presumably, those of spontaneous pseudopregnancy.

In BALB/c mice, hyperemia had a mean duration of 1.5 (± 0.12) days and the vaginal cycle of 7.5 (± 0.29) days; the detailed pattern did not differ from that in hybrid mice. *FSH; (Table I, I).* Five animals received FSH until the day after disappearance of hyperemia of corpora in the 3rd experimental cycle. Duration of hyperemia was not affected in these cycles, nor in 2 afterwards (mean, 1.3 ± 0.21 days). Cycle length was increased in the 3rd cycle (mean, 20.6 days; $p < 0.1\%$) relative to the other cycles or to those in controls but was not significantly different in any other cycle (mean, 8.0 ± 1.10 days). Except for this, the pattern for ovarian and vaginal cycling was similar to that in controls (Fig. 1). *LH; (Table I, II).* Five animals received LH over part of the 1st cycle. Hyperemia was not significantly prolonged (mean, 2.19 ± 0.29 days) but cycle length was greatly increased (mean, 23.6; $p < 0.1\%$). Except for this, the pattern of ovarian and vaginal cycling was similar to that in controls (Fig. 1). Hyperemia was unaffected in 3 cycles after cessation of administration (mean, 1.5 ± 0.38 days); the 2nd cycle was slightly prolonged (mean, 9.4 days; $p < 5\%$) but the 3rd and 4th were unaffected (mean, 8.1 ± 1.38 days).

Six other animals received LH for 5 or 6 days after hyperemia first appeared in the 1st cycle; its duration was also unaffected. Deciduomata did not occur in these animals after uterine traumatization. *LTH; 0.2 mg (Table I, III).* Five animals received this dosage of LTH through the 1st cycle and until 2 days after hyperemia had disappeared in the 2nd cycle. Both the duration of hy-

peremia (mean, 6.6 and 6.0 days) and of the cycle (mean, 12.0 and 12.5 days) were longer, to a highly significant degree ($p<$ 0.1%) than in controls. These values did not differ significantly within the group nor from those in animals on the 0.1 mg dosage of LTH (IV). In the 3rd cycle, duration of hyperemia (mean, 1.7 $\pm$ 0.47 days) and of the cycle (mean, 7.5 $\pm$ 1.18 days) did not differ from that in controls. Except for this cycle, the pattern of ovarian and vaginal cycling was similar to that in animals on the 0.1 mg dosage of LTH (Fig. 1). *LTH; 0.1 mg (Table I, IV; Fig. 1).* Fourteen animals received this dosage of LTH through the 1st cycle; 5 continued to receive it until 2 days after disappearance of hyperemia of corpora in the 3rd cycle. Hyperemia persisted for 8.2, 6.5, and 4.5 days respectively, in the 3 cycles of administration, a highly significant increase ($p<$ 1% or 0.1%) relative to controls. The decrease in duration between the 1st and 2nd cycles was significant ($p<1\%$), as was that between the 2nd and 3rd cycles ($p<5\%$). Hyperemia in the 2 cycles after cessation of administration (mean, 1.25 $\pm$ 0.14 days) did not last longer than in controls.

Cycles during administration, and the 4th cycle, were approximately double the length of those in controls ($p<1\%$ or 0.1%) but the 5th cycle (mean, 9.0 $\pm$ 1.07 days) did not differ from them.

Four of the 5 animals that were subjected to uterine trauma in the 1st cycle exhibited deciduomata. *LTH; 0025 mg (Table I, Va and b, VI; Fig. 1).* Thirteen hybrids received this dosage of LTH through the 1st cycle; 4 received uterine trauma and did not exhibit deciduomata at autopsy. Five of the remaining 9 continued on the same dosage until 2 days after loss of hyperemia of corpora in the 3rd cycle but the other 4 received an increased dosage of 0.1 mg from the time of appearance of this hyperemia; this administration also ceased 2 days after loss of hyperemia.

Hyperemia persisted significantly longer (mean, 5.7 days; $p<0.1\%$) in the 1st cycle but not in the 2nd (mean, 1.9 $\pm$ 0.26 days) nor in the 3rd at the 0.025 mg dosage (mean, 1.3 $\pm$ 0.33 days) and the cycle after cessation of administration (mean, 1.5 $\pm$ 0.5 days). Cycle length, however, was significantly extended in all 4 cycles. On the other hand, in animals receiving 0.1 mg LTH in the 3rd cycle, hyperemia persisted significantly longer (mean, 3.7 days; $p<5\%$) than in either the controls or animals still on the 0.025 mg dosage.

Hyperemia had a significantly shorter duration ($p<0.1\%$) in the first 2 cycles than in animals on the 0.1 mg dosage (IV, Table I); it did not differ significantly in the 3rd cycle for animals (Vb) on the increased dosage.

Eight BALB/c mice (Table I, VI) also received the 0.025 mg dosage through the first 2 experimental cycles, when 4 were sacrificed. The dosage given the remaining 4 was raised to 0.1 mg as soon as hyperemic corpora appeared in the 3rd cycle. It was continued at this level until 2 days after hyperemia was lost. The duration of hyperemia was significantly greater than in control BALB/c mice in the 1st (mean, 4.9 days; $p<0.1\%$), 2nd (mean, 2.6 days; $p<5\%$), and 3rd cycles (mean, 4.0 days; $p<1\%$). The duration in the 2nd cycle was significantly less than in the 1st ($p<0.1\%$); however, duration in the 3rd cycle did not differ significantly from either that in the 1st or 2nd cycles. Cycle lengths differed significantly from those in controls only in the 3rd cycle (mean, 10.3 days; $p<5\%$). *LH and LTH; (Table I, VII, VIII; Fig. 1).* Five animals received both gonadotropins, each at the 0.1 mg level, for 10 days in the 1st cycle (VII). Three transplants had lost hyperemic corpora one or 2 days before administration ceased. The mean duration of hyperemia was significantly longer (mean, 10.0 days; $p<0.1\%$) than in controls; so also was the cycle length (mean, 18.4 days; $p<0.1\%$). Hyperemia in the subsequent cycle did not differ from that in controls (mean, 1.2 $\pm$ 0.17 days). Deciduomata occurred in 4 of the 5 animals after uterine trauma in the 1st cycle.

A second group of 5 animals (VIII) received the same dosage of LH and LTH during the 1st cycle until 2 days after loss of hyperemic corpora. The mean duration of hyperemia was 16.6 days and cycle length

29.8 days; both values were significantly greater ($p<0.1\%$) than corresponding ones in controls and all other groups (except for 1st cycle length on LH, group II). Hyperemia and cycle length in 3 subsequent cycles did not differ from the values in controls (mean, 1.2 $\pm$ 0.20 days for hyperemia; 7.3 $\pm$ 1.30 days for cycle length). *Mammary glands.* In intact virgin females, the duct system was moderately branched with occasional to frequent alveoli and no end buds; in controls (*i.e.*, with ovarian tissue intraocularly rather than *in situ*) there was also moderate duct branching (with fewer fine terminals) but end buds were present and alveoli rare. At the 0.025 mg LTH dosage (mean duration, 29 days), development varied from that seen in controls to that in intact virgins. At the 0.1 mg LTH dosage (mean duration, 38 days), branching was maximal, end buds absent, and alveoli numerous but only rarely in lobules. At the 0.2 mg LTH dosage (mean duration, 20 days), branching was also maximal, end buds absent, and the numerous alveoli arranged in lobules but not dilated by secretion; this development resembled that found in mid-pregnancy.

Discussion. In intraocular ovarian transplants, hyperemic corpora lutea had a mean life of 5.5 days in pseudopregnancy(1), of 6.4 days in the presence of an anterior pituitary transplant(2), and of 6.0 days during lactation(3); during the first cycle of administration of LTH, the period was 8.2, or 6.6 days at the 0.1 mg or 0.2 mg dosages, respectively. This remarkable constancy suggests an intrinsic life span of such corpora. However, when LH was given in addition to LTH, their persistence was extended to 16.6 days, although LH had no effect alone. The limited duration of hyperemia under other conditions may have been due to failure of LH secretion by the host pituitary rather than the loss of responsiveness by the corpora. In other words, functional corpora require both gonadotropins.

Hyperemic corpora had a shorter life (mean, 5.7 days) on the lowest dosage of LTH (0.025 mg) than on the higher ones (0.1 mg, 0.2 mg) and no deciduomata were obtained after uterine trauma. However, as trauma occurred on the 2nd day of hyperemia and uterine examination 4 days later, hyperemia was either no longer present or was about to disappear at this examination. Deciduomata were not found on the day after hyperemia disappeared in animals with ovarian-anterior pituitary transplants(2).

A continuing dosage of 0.025 mg of LTH failed to give extended hyperemia after the 1st cycle, unlike the 0.1 or 0.2 mg dosages. This failure may have been due to inactivation of ovine LTH by host antibodies, this reaction being overwhelmed by higher dosages. Alternatively, animals may have developed tolerance to exogenous LTH at the low dosage. Nonetheless, the mouse ovary can give an undiminished and cyclic response to LTH secreted by an anterior pituitary transplant over several months(2).

Summary. The life of hyperemic (functional) corpora lutea in intraocular ovarian transplants in castrated female mice was between one and two days. It was not affected by administration of 0.1 mg of FSH or of LH every 12 hours. It was extended to approximately 5, 8 and 7 days, respectively, by 0.025, 0.1, or 0.2 mg of LTH and to 17 days by 0.1 mg of LH with 0.1 mg of LTH. Deciduomata occurred in response to uterine trauma in animals receiving 0.1 mg LTH, or 0.1 mg LH and LTH, but not in those receiving 0.025 mg LTH or 0.1 mg LH.

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Received July 23, 1962. P.S.E.B.M., 1962, v111.