# Luteotropic and Luteolytic Effects of Rat Chorionic Mammotropin[1] (35303)

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In summarizing his own and others' work on the luteotropic and luteolytic activities of hypophyseal mammotropin (MH, prolactin, lactogen), Malven, (1, 2), referred to the suggestion of Alloiteau (3) that rat chorionic mammotropin (RCM), a mammogenic and lactogenic hormone which is also a potent luteotropin (4, 5), may be luteolytic as well. The results of the following experiments are interpreted as demonstrating this additional activity of the hormone [see also Matthies and Lyons (6)].

*Materials and Methods.* The procedures were essentially the same as those used in demonstrating the luteotropic activity of RCM in fetal placentas or maternal blood on day 12 of pregnancy (5). Sperm detection in the vaginal smear is considered as day 1 of pregnancy.

Long-Evans rats, 26 days old, were injected with 5 IU of pregnant mare serum gonadotropin (PMSG) 4 days prior to hypophysectomy. On the day of pituitary ablation one group of animals was injected subcutaneously with serum taken from rats 12 days pregnant. In other groups, intervals of 24, 48, and 72 hr, respectively, were permitted to elapse before the same treatment protocol was begun. Some groups were injected daily for 4 days, while other groups were injected for 7 days. Necropsies were performed 24 hr after the last injection.

In the 4-day tests, evidence for the luteotropic or luteolytic response was sought in all animals in histological sections of uteri and ovaries (as described below) fixed in Bouin's solution and stained by the periodic acid–Schiff and hematoxylin method. In the 7-day test, the left uterine horn was threaded on the third day of the injection regimen and at necropsy was inspected for deciduoma formation. If the degree of swelling of the traumatized region of the uterus was equivocal, histological sections were utilized for the determination of the presence or absence of the deciduoma reaction. Confirmation of the presence or absence of progestogen secretion was available by histological examination of the epithelium of the right, untraumatized, uterine horn, [Vokaer reaction (7, 8)], and of the corpora lutea for luteal cell hypertrophy.

The pituitary fossa of all test animals was carefully examined for tissue remants before the animal was included in the experimental data reported below.

*Results.* In Table I, 7 groups of rats are listed according to the treatment regimen. The 7-day deciduoma test for progestogen secretion was used in all but group 4. None of the 3 rats in each of groups 1 (uninjected) and 2 (injected with day 17 pregnancy serum commencing 3 days posthypophysectomy) showed any evidence of either luteotropic or luteolytic response. The day 17 serum is not capable of inducing a tropic response even if administered at high dosages commencing on the day of hypophysectomy (5). It was tested here for the purpose of detecting, if possible, a lytic substance distinct from RCM.

Group 5 was composed of 4 subgroups of 3 to 6 rats injected daily with 0.25 ml of Day 12 pregnancy rat serum commencing at 0, 1, 2, and 3 days posthypophysectomy, respectively. Using all three criteria, *i.e.*, luteal cell hypertrophy, deciduoma formation, and the Vokaer reaction, it was possible to conclude that the luteal cells of some of the rats were able to respond to the tropic effects of the hormone 48 hr after hypophysectomy but other rats on the same regimen responded

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TABLE I. Test for Luteotropic and Luteolytic Effects of Serum and Fetal Placenta from Rats 12 Days Pregnant.

| Group | No. Rats | No. Days | Daily inject[a] | Inject begun post-H (days) | Corpus luteum Stim. | Corpus luteum Degen. | Ut. epith. Pos. | Ut. epith. Neg. | Deciduoma Pos. | Deciduoma Neg. |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 7 | — | — | 0 | 3[b] | 0 | 3 | — | — |
| 2 | 3 | 7 | 0.25[c] | 3 | 0 | 3[b] | 0 | 3 | 0 | 3 |
| | 3 | 7 | 0.1 | 0 | 3 | 0 | 3 | 0 | 3 | 0 |
| 3 | 6 | 7 | 0.1 | 1 | 6 | 0 | 6 | 0 | 6 | 0 |
| | 6 | 7 | 0.1 | 2 | 4 | 2 | 2 | 4 | 2 | 4 |
| | 4 | 7 | 0.1 | 3 | 0 | 4 | 0 | 4 | 0 | 4 |
| 4 | 3 | 4 | 0.25 | 0 | 3 | 0 | 3 | 0 | — | — |
| | 3 | 4 | 0.25 | 1 | 3 | 0 | 3 | 0 | — | — |
| | 3 | 4 | 0.25 | 2 | 1 | 2 | 1 | 2 | — | — |
| | 3 | 4 | 0.25 | 3 | 0 | 3 | 0 | 3 | — | — |
| 5 | 3 | 7 | 0.25 | 0 | 3 | 0 | 3 | 0 | 3 | 0 |
| | 3 | 7 | 0.25 | 1 | 3 | 0 | 3 | 0 | 3 | 0 |
| | 9 | 7 | 0.25 | 2 | 3 | 6 | 3 | 6 | 3 | 6 |
| | 3 | 7 | 0.25 | 3 | 0 | 3 | 0 | 3 | 0 | 3 |
| 6 | 6 | 7 | 0.5 | 2 | — | — | — | — | 4 | 2 |
| | 3 | 7 | 0.5 | 3 | — | — | — | — | 0 | 3 |
| 7 | 3 | 7 | 2.5 | 2 | — | — | — | — | 2 | 1 |
| | 3 | 7 | 2.5 | 3 | — | — | — | — | 0 | 3 |

[a] Serum (ml) in groups 2–6; placental equivalents in group 7.
[b] Minimal signs of regression referred to as "maintained but not stimulated."
[c] Day 17 of pregnancy; all others day 12.

with luteolysis. No animals manifested the luteotropic response if the RCM injections were delayed for 3 days after hypophysectomy; whereas all rats which were injected, commencing with the day of operation or 1 day later, showed positive evidence of progestogen secretion.

The infranuclear "lucid zone" (Vokaer reaction), seen as a luteotropic response of the uterine epithelium (Fig. 1), was absent in uninjected control animals (Fig. 2), animals injected with day 17 pregnancy serum, and animals injected with solutions containing RCM but which responded with the luteolytic reaction rather than the luteotropic.

Hypotrophic luteal cells of group 1 control animals demonstrated the characteristic "nuclear crowding" of the maintained but unstimulated corpus luteum (Fig. 3), as was the case with group 2 controls. Luteal cell hypertrophy, characteristic of the progestogen secreting response to a luteotropic stimulus (Fig. 4), was seen in sections of ovaries from some rats treated with RCM commencing 48 hr after hypophysectomy. In contrast, other animals deprived for the same time of the pituitary and then treated with exogenous RCM possessed degenerating corpora lutea (luteolysis) Fig. (5). A more advanced degree of luteolysis (Fig. 6) was seen in ovaries of animals treated with RCM commencing 72 hr after hypophysectomy. In routine histological preparations luteolysis is identifiable by the presence of pyknotic nuclei, vacuolated luteal cell cytoplasm, and an infiltration of the corpus by stromal cells.

The results in group 5 were duplicated in groups 3 (injected with 0.1 ml of day 12 serum for 7 days) and 4 (0.25 ml serum was injected daily for 4 days). The results in the 4-day test were as convincing as those in the 7-day deciduoma test.

To test further the critical posthypophysectomy interval of 2 to 3 days and to

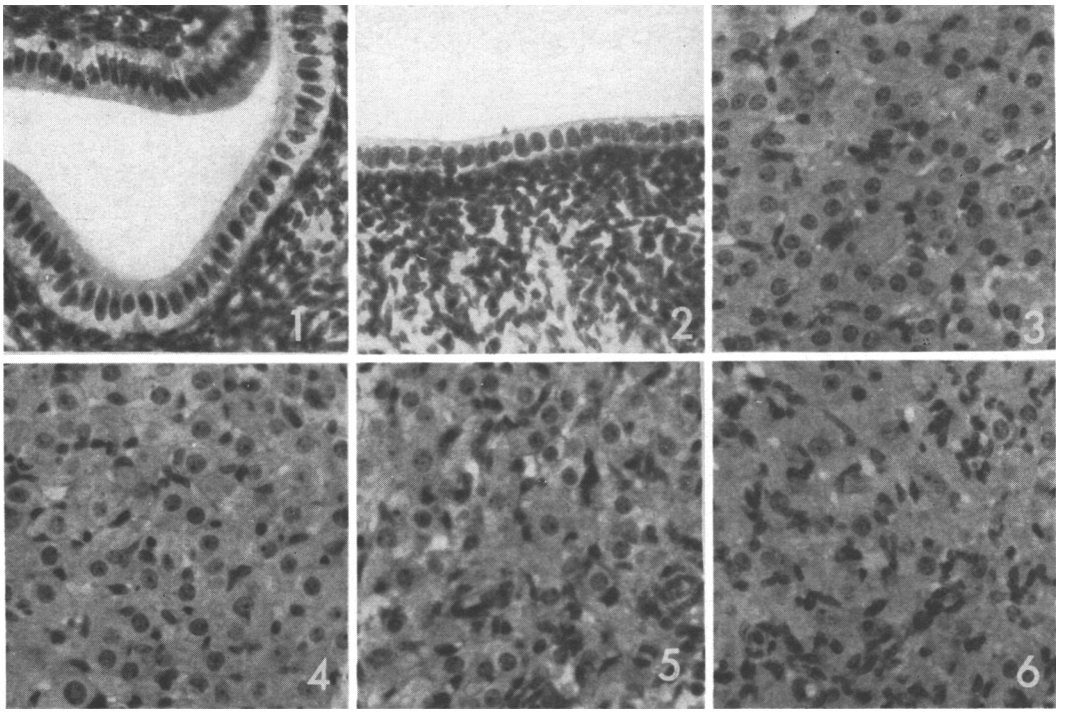


FIG. 1. Stimulated uerine epithelium showing infranuclear "lucid zone" (Vokaer reaction) and considered to be a response to progestogen secretion, in a hypophysectomized rat receiving 0.25 ml/day of serum from day 12 pregnant rats for 7 days commencing on day of operation.

FIG. 2. Unstimulated low cuboidal epithelium from uterus of control, uninjected hypophysectomized rat.

FIG. 3. "Maintained" corpus luteum from same control animal as in Fig. 2 showing hypotrophic luteal cells.

FIG. 4. Hypertrophied luteal cells of a stimulated corpus luteum from test animal deprived of luteotropin for 48 hr after hypophysectomy and then injected daily with 0.25 ml of day 12 pregnancy serum for 7 days.

FIG. 5. Degenerating corpus luteum showing luteolysis, from a different animal treated by the same protocol as the animal in Fig. 4.

FIG. 6. A more advanced degree of luteolysis from a test animal deprived of luteotropin for 72 hr after hypophysectomy and then injected daily with 0.25 ml of day 12 pregnancy serum for 7 days.

determine whether or not the luteotropic vs luteolytic effects were dose dependent, higher levels of 12 day pregnancy serum and a dosage of 12 day placental extract estimated to be 2.5 times the MED for luteotropic effect (5), were used in groups 6 and 7, respectively. As in the previous groups some of the rats showed the tropic influence and others the lytic effect after the 2-day interval but all showed luteolysis if treatment was delayed for 3 days.

*Discussion.* In the past we have used the expression "maintained but not stimulated" for the type of corpus luteum that Smith (9) found persisting in the ovaries of rats hypophysectomized 9 months previously. The implication was that, although such corpora were maintained with only slight evidence of some cell degeneration, they were nevertheless nonfunctional. The absence of corpus luteum function following hypophysectomy has been readily explained ever since the isolation of a homogeneous hypophyseal peptide with a corpus luteum-activating (luteotropic) property (10).

The immunity of these "maintained" corpora lutea to lysis was recently discussed by Malven and Sawyer (11). They reported that

approximately 48 hr after hypophysectomy "prolactin administration causes structural luteolysis." Others had reported that the time interval between hypophysectomy and treatment with the hypophyseal luteotropic factor was critical in terms of functional luteal responsiveness (10, 12). Desclin (12) narrowed the interval to 24 hr; and although he did not prove his suspicion that LH was the luteolytic contaminant, neither did he eliminate prolactin as the luteolytic factor. MacDonald and Greep (13) have used a 2-day posthypophysectomy period to demonstrate luteolysis following hypophyseal prolactin injections and also showed that the addition of LH did not alter the degree of corpus luteum regression. Contrary to a conjecture by Malven (1), Evans *et al.* (10) did not consider their mammotropin luteolytic because of LH contamination since some of their preparations, prepared by one of us (WRL), were homogeneous; and all were free of LH.

Acceptance of the proof of two distinctly opposite functions of ovine hypophyseal MH (*i.e.*, luteotropic and luteolytic) led to the test of the other potent rat luteotropin, RCM. Alloiteau (3) had already proposed that "rat prolactine placentaire" (RCM) was, under some circumstances, capable of active destruction of the corpus luteum. His rats, hypophysectomized on day 3 of pregnancy and maintained by progesterone until day 15, showed luteolysis; whereas pseudopregnant rats, similarly treated, did not. Our support of Alloiteau's suggestion should also serve to explain the findings of Long and Evans (14) that the destruction of the 3 and 4 crops of residual estrous cyclic corpora lutea was greatly accelerated if a rat became pregnant.

The finding that the placental luteotropic factor resembles that from the pituitary in being luteolytic after the luteal cells have been deprived of stimulus for approximately 2 days by hypophysectomy does not explain, but only adds support to, what had appeared to be paradoxical. It would seem that the luteal cell is capable of surviving independently of the pituitary; but that it may generate enzymes cytolytic to itself, depending on the level of steroid enzymes influenced by pituitary or placental luteotropin.

*Summary.* The results of the experiments reported herein support the findings of Malven and Sawyer (11) that a luteotropic hormone, capable of stimulating luteal cell synthesis and secretion of progestogen, can also be luteolytic. Rat chorionic mammotropin, a potent luteotropic hormone detectable during midpregnancy may under some circumstances have an opposite effect on luteal cells and become luteolytic. The dose of 0.1 ml of serum from rats 12 days pregnant promoted hypertrophy in newly formed luteal cells and continuing progestogen secretion if injected commencing on the day of hypophysectomy or 1 day later. This luteotropic potency is equal to about 1.5 IU of ovine pituitary LTH as tested in 1-month-old rats. Both luteotropic hormones induce luteolysis at the same dose level (or higher levels) if the luteal cells are deprived of trophic hormone for 48 hr or more. There has been no evidence favoring a rat placental ICSH (LH) as the luteolytic factor in these experiments but this possibility, under different circumstances, is not excluded.

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