A UTERINE LUTEOLYTIC FACTOR IN THE SYRIAN HAMSTER

RONALD STEVEN MAZER
University of New Hampshire, Durham

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IN THE SYRIAN HAMSTER-

BY
RONALD STEVEN MAZER
A.B., Bowdoin College, 1964
M.S., University of New Hampshire, 1966

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In all of the following figures (1-19) symbols have been used as follows:

C = length of control (normal, intact) pseudopregnancy.

E1 = length of pseudopregnancy after hysterectomy.

E2 = length of experimental pseudopregnancy upon daily intraperitoneal injection, on the 4th through 7th days of the cycle, of 1 cc of extract or control vehicle as indicated.

E3 = length of first pseudopregnancy following experimental pseudopregnancy.

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<td>Effects of heat on extract of uteri removed on 7th day of pseudopregnancy</td>
<td>52</td>
</tr>
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<td>18</td>
<td>Effects of dialysis on extract of uteri removed on 7th day of pseudopregnancy</td>
<td>54</td>
</tr>
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<td>19</td>
<td>Effects of dialysis on extract of uteri removed on 7th day of pseudopregnancy</td>
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INTRODUCTION

Although requirements for establishing functional corpora lutea in several mammalian species are reasonably well worked out and accepted, the mechanism for concluding the functional life of corpora lutea, often called luteolysis, remains a perplexing problem for the reproductive physiologist. Evidence has accumulated which indicates that the uterus plays a role in the life span of the corpus luteum by producing a specific, blood-borne luteolytic factor. Recent reviews (Anderson et al., 1963; Bland and Donovan, 1966) cover pertinent literature in this and related facets of the problem, and indicate that species differences may be enormous. In addition, Ginther (1967) cites some of the growing support for the occurrence of local (i.e. non-blood-vascular) utero-ovarian relationships for many species.

Attempts to produce cell-free extracts which have demonstrable luteolytic activity, on the other hand, have not given uniform results. Cooper and Hess (1965) obtained in vitro inhibition of progesterone synthesis when guinea pig uterine extracts were introduced into a medium of cultured luteal cells. No inhibition of progesterone synthesis was observed when rat uterine extracts (stage of cycle not specified) were added to cultured rat luteal cells (Cooper and Hess, 1965). However, pseudopregnant cycles of normal length were observed in hysterectomized rats injected with endometrial suspensions of estrous uterine horns (Bradbury et al., 1950). Neither aqueous nor ether extracts of bovine endometrium significantly affected the life
span of corpora lutea in hysterectomized pseudopregnant rats (Malven and Hansel, 1965) nor did ether-soluble extracts or lyophilized homogenates of estrous rat uteri shorten pseudopregnancy in hysterectomized rats (Kiracofe and Spies, 1966a). Kiracofe et al. (1966b) found that no luteolytic effect could be attributed to ovine extracts injected into ewes.

The work of Duncan et al. (1960) supported the possibility of a uterine luteolytic hormone in that filtrates of endometrial tissues taken from gilts on the 16th and 18th days of the estrous cycle decreased in vitro synthesis of progesterone. Flushings from sow uteri added to cultured pig luteal cells produced a luteolytic effect (Schomberg, 1967).

Using acetone-dried preparations of bovine uterus, Williams et al. (1967) found, in 64% of rabbits treated, "regression of corpora lutea, development of follicles, and a depression of acetate incorporation into progesterone during the in vitro incubation of the ovaries." These results contradict earlier work of Mishell and Motyloff (1941) in which bovine endometrium extracted with organic solvents increased, rather than decreased, longevity of corpora lutea.

Earlier work in our laboratory has shown that a highly significant reversal of the effects of hysterectomy on pseudopregnancy in the hamster could be accomplished by homologous uterine transplantation to the cheek pouch (Caldwell et al., 1967). The present research is a continuation of the above report in the hope of further elucidating the relationship between the uterus and ovary.
MATERIALS AND METHODS

Female virgin hamsters (7-15 weeks of age) from a local breeder were individually housed in 12½'' by 15½'' opaque plastic cages covered with 1'' wire screening. Only hamsters weighing between 80-120 g and showing satisfactory weight increments during a preliminary observation period were used. Food consisted of Purina laboratory chow (micro and macro mixed) ad lib., supplemented twice weekly with Geisler hamster food. Water was continuously available.

A time switch automatically provided 12 hr of light alternating with 12 hr of continuous darkness in the light-tight, air-conditioned animal room. Lights were off from 3:30 AM to 3:30 PM in order to observe estrual behavior between the hours of 8 and 10 AM, corresponding to late evening for the hamster. In addition, the temperature and humidity were regulated near the optima (70 ± 5°F and 50 mm Hg respectively). All animals were allowed 7 days to adjust to these conditions. Control pseudopregnant and pregnant animals were maintained at regular intervals to check against any seasonal variation, but none was observed during the time limits of these experiments.

Two normal cycles were observed before a female was subjected to any experiments. Years of prior experience has revealed that the most convenient and reliable method of checking for estrus in the hamster was the lordosis reflex by females in the presence of males. Pseudopregnancy was induced in normal females by mating with vasectomized males; intact males were used with hysterectomized females. Pregnancy was induced by allowing normal males to remain
with the female for $\frac{1}{2}$ hr.

Nembutal at a dosage of 8 mg/100 g body weight was administered intraperitoneally and induced a 20-minute anaesthesia in about 5 min. Booster shots, if needed, were given at a dosage of 1.5 mg. Hysterectomies and ovariectomies were performed by making a single median incision starting about one inch from the anus and proceeding craniad for approximately 1 inch. Care was taken not to disturb any ovarian blood vessels in cases where normal ovarian vascularity was important. The entire operative procedures usually required no more than 15 minutes. All operated animals were allowed a 10-day recuperative period before being subjected to experimental procedures.

For making extracts, freshly-excised uterine horns were placed in a mortar at 0 C, cut into small pieces and then ground with a pestle in Hank's balanced saline solution at pH 7.4. This material was further ground in a Virtis "23" micromonogenizer for 30 min at 0 C, and filtered. Pregnant uterine horns were removed, held vertically with the cervix up, and flushed to remove blastocysts with 0.5-1 cc of Hank's balanced solution before being subjected to the same procedure. All extracts were stored in a freezer at 0 C until injected, but for periods no longer than 10 days. All injections were made over a four-day span, beginning on day four of the cycle.

In crude fractionation of uterine extracts, either 0.25 M aqueous sucrose or Hank's balanced solution was used, an equivalent of one uterine horn per ml of solution being maintained. After homogenization, the suspension was spun at 0 C in a Sorval RC 2-B refrigerated centrifuge at 755 g for 15 min. The supernatant was removed, the sediment resuspended and the procedure repeated. The sediment was
resuspended and constituted fraction I. The supernatant was then centrifuged at 8700 g for 30 min. The sediment was resuspended and centrifuged again for 30 min at 8500 g. The new sediment was resuspended and labeled fraction II. The remaining supernatant was designated fraction III.

For histological study, tissues were fixed in Bouin's solution, embedded in paraffin, sectioned serially at 8-10 micra and stained in hematoxylin and eosin. Sections of uterine horns from various days of the pseudopregnant cycle were stained simultaneously to avoid differences due to slight variation in staining technique.
RESULTS

For sake of uniformity, results in Figures (1-19) are reported as a mean figure based on observation of four cycles. Appearance of estrus in all cases is considered to mark day 0. A normal pseudopregnancy (C) was observed for each animal in order to check for normal cycling behavior. \( E_1 \) represents the length of the first pseudopregnancy following hysterectomy obtained only after a post operative rest period and is used as a comparison with results obtained in the various experimental procedures (\( E_2 \)). \( E_3 \) designates the length of pseudopregnancy following the experimental cycle. Complete data needed for comparisons among groups are found in Tables 1 and 2.

Results show clearly that the hamster uterus does produce a luteolytic factor (ULF) for a very limited time during pseudopregnancy. Injections made using seven-day pseudopregnant horns (Group E) shortened pseudopregnancy by more than 6 days. Histological examination also verified regression of the corpora lutea in this group. Apparent were shrunken elliptical nuclei and an increase in epithelial connective tissue, quite unlike the healthy tissue observed when saline alone was injected (Group A). Extracts of uteri from day 6 (Group D) produced a significant shortening but only of 2.75 days, indicating that the uterus probably releases this factor late on day 6, continuing through day 7. Extracts of uteri obtained on days 4, 5, and 8 (Groups B, C, and F) did not shorten pseudopregnancy. Hematoxylin and eosin staining revealed no discernible difference in uterine secretory activity on any day of pseudopregnancy, but more
Table 1. Effects of uterine extracts on lengths of hamster pseudopregnancy

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Type of extract</th>
<th>Dosage</th>
<th>Control pseudopreg. (Horn/day)</th>
<th>Control hysterec. pseudopreg. (Days)</th>
<th>Experimental hysterec. pseudopreg. (Days)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>Saline</td>
<td>0</td>
<td>9.67 ± .07a</td>
<td>17.33 ± .08a</td>
<td>17.08 ± .13a</td>
<td>≥ .05</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>4th day pseudopreg.</td>
<td>1</td>
<td>9.90 ± .08</td>
<td>17.17 ± .64</td>
<td>17.83 ± .69</td>
<td>≥ .05</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>5th day</td>
<td>1</td>
<td>9.50 ± .50</td>
<td>16.58 ± .57</td>
<td>17.33 ± .74</td>
<td>≥ .05</td>
</tr>
<tr>
<td>D</td>
<td>12</td>
<td>6th day</td>
<td>1</td>
<td>9.75 ± .08</td>
<td>16.50 ± .38</td>
<td>13.75 ± .94</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>E</td>
<td>12</td>
<td>7th day</td>
<td>1</td>
<td>9.42 ± .67</td>
<td>17.50 ± .59</td>
<td>10.75 ± .93</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>F</td>
<td>12</td>
<td>8th day</td>
<td>1</td>
<td>9.67 ± .08</td>
<td>18.33 ± .59</td>
<td>19.58 ± .50</td>
<td>≥ .05</td>
</tr>
<tr>
<td>G</td>
<td>12</td>
<td>7th day</td>
<td>1</td>
<td>9.58 ± .15</td>
<td>17.17 ± .44</td>
<td>10.92 ± .34</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>H</td>
<td>12</td>
<td>7th day</td>
<td>(\frac{1}{2})</td>
<td>9.70 ± .08</td>
<td>18.42 ± .55</td>
<td>11.33 ± .46</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>I</td>
<td>12</td>
<td>7th day</td>
<td>(\frac{1}{4})</td>
<td>9.58 ± .15</td>
<td>17.83 ± .49</td>
<td>15.08 ± .52</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>J</td>
<td>12</td>
<td>Immature horns</td>
<td>1</td>
<td>9.50 ± .19</td>
<td>16.58 ± .42</td>
<td>16.50 ± .51</td>
<td>≥ .05</td>
</tr>
<tr>
<td>K</td>
<td>12</td>
<td>Pregnant horns</td>
<td>1</td>
<td>9.42 ± .19</td>
<td>17.17 ± .41</td>
<td>15.92 ± .45</td>
<td>&lt; .05</td>
</tr>
</tbody>
</table>

\(\text{a Mean ± standard error of the mean.}\)

Intergroup P values:
- A:B \(P > .05\)
- A:C \(P > .05\)
- A:D \(P < .01\)
- A:E \(P < .01\)
- A:F \(P > .05\)
- A:G \(P < .01\)
- A:H \(P < .01\)
- A:I \(P < .05\)
- A:J \(P > .05\)
- A:K \(P > .05\)
<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Test</th>
<th>Dosage of horn (Given/day)</th>
<th>Control hysterectomy pseudopreg. (Days)</th>
<th>Experimental hysterectomy pseudopreg. (Days)</th>
<th>P values (Col 5:Col 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>12</td>
<td>Fractionation Saline 755 g (nuclear)</td>
<td>1</td>
<td>18.17 ± .59a</td>
<td>17.25 ± .67a</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>M</td>
<td>12</td>
<td>Fractionation Saline 8700 g (mitochondrial)</td>
<td>1</td>
<td>18.50 ± .47</td>
<td>12.41 ± .29</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>Saline supernatant</td>
<td>1</td>
<td>18.25 ± .65</td>
<td>16.83 ± .39</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>O</td>
<td>6</td>
<td>Fractionation .25 M sucrose 755 g (nuclear)</td>
<td>1</td>
<td>19.17 ± .95</td>
<td>18.67 ± .95</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>P</td>
<td>6</td>
<td>Fractionation .25 M sucrose 8700 g (mitochondrial)</td>
<td>1</td>
<td>17.83 ± .71</td>
<td>17.33 ± .80</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Q</td>
<td>6</td>
<td>.25 M sucrose supernatant</td>
<td>1</td>
<td>16.83 ± .41</td>
<td>10.17 ± .09</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>R</td>
<td>6</td>
<td>Heat 70 C 15 min.</td>
<td>1</td>
<td>17.17 ± .75</td>
<td>16.50 ± .80</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>S</td>
<td>6</td>
<td>Dialysate</td>
<td>1/2</td>
<td>17.50 ± .60</td>
<td>16.17 ± 1.1</td>
<td>&gt; .05</td>
</tr>
</tbody>
</table>

a Mean ± standard error of the mean.

Intergroup P values:  
L:M P < .01  
N:M P < .01  
L:N P > .05  
0:P P > .05  
0:Q P < .01  
P:Q P < .01
precise indicators of endocrine activity will be used in further study.

From preliminary observation, threshold and maximal dosages of ULF must be very close, for $\frac{1}{4}$ horn (Group I) did not significantly shorten pseudopregnancy but the equivalent of $\frac{1}{2}$ (Group H) and 1 (Groups E, G) horn per day for four days produced maximal effects.

Extracts made from pregnant hamster horns (Group K) indicate that pregnancy somehow inactivates ULF either by preventing its release or synthesis, for no shortening of pseudopregnancy was observed. Since no significant differences were observed using pregnant horns from days 5, 8, and 9, results were combined to form Group K. The reverse procedure of injecting the active extract (i.e. from day 7 of pseudopregnancy) into hamsters beginning on day 5 of pregnancy showed that the extract was unable to overcome the established pregnancy, for 11 out of 12 animals went to full term.

Immature uterine horns ($3\frac{1}{2}-4\frac{1}{2}$ wk old) were also ineffective in shortening hysterectomized pseudopregnant cycles (Group J).

Data reported in Table 2 (Groups O, P), indicate that nuclear or mitochondrial fractions (Fractions I and II) of uterine extract (in 0.25 M sucrose) gave negative results. However, the supernatant (Fraction III, Group Q) remaining after mitochondrial fractionation, in dosages of the equivalent of one horn per day for four days, shortened pseudopregnancy by more than 6 days. With saline (Hank's balanced) solution, however, the mitochondrial fraction (Fraction II, Group N) curtailed hysterectomized pseudopregnancy whereas the other two fractions, I and III, (Groups L, N) caused no shortening. This discrepancy can be accounted for by the difference in density and sedimentation rates of the two solutions. Clearly, Fraction III,
using 0.25 M sucrose, contained ULF as did Fraction II using saline solution.

Further analysis revealed that ULF was thermolabile when heated to 70°C for 15 minutes (Group R). The material was also found to be nondialyzable (7-day saline extract against distilled water, 4°C for 16 hours) since the dialysate (in dosage equivalent to ½ uterine horn) had no significant effects on pseudopregnancy (Group S), and the original saline retained its activity.

Experiments reported in Table 3 indicate that the hamster, like several other species, displays local as well as systemic ovarian-uterine relationships. Unilateral hysterectomy with removal of the opposite ovary prolonged the life span of corpora lutea to 15.17 days whereas removal, ipsilaterally, of ovary and adjacent uterine horn, resulted in pseudopregnancy of 11.83 days. When both ovaries remained, with removal of one uterine horn, the length of pseudopregnancy was 13.17 days. The possibility exists that the lymphatic system as well as the blood vascular system may play a role in the transport of ULF.
Table 3. Positional control of hamster pseudopregnancy

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of control animals</th>
<th>Control hysterect. pseudopreg.</th>
<th>Number of experimental animals</th>
<th>Type of experiment</th>
<th>Length of experimental pseudopreg.</th>
<th>P Values (Col 3:Col 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>6</td>
<td>$17.83 \pm .73^a$</td>
<td>6</td>
<td>ipsilateral uterus-ovary removed</td>
<td>$11.83 \pm .60^a$</td>
<td>$&lt; .01$</td>
</tr>
<tr>
<td>U</td>
<td>6</td>
<td>$17.67 \pm .57$</td>
<td>6</td>
<td>contralateral uterus-ovary removed</td>
<td>$15.17 \pm .41$</td>
<td>$&lt; .05$</td>
</tr>
<tr>
<td>V</td>
<td>6</td>
<td>$17.50 \pm .63$</td>
<td>6</td>
<td>unilateral hysterectomy only</td>
<td>$13.17 \pm .48$</td>
<td>$&lt; .01$</td>
</tr>
</tbody>
</table>

$^a$ Mean ± standard error of the mean.

Intergroup P values:  
T:U $P < .01$  
T:V $P > .05$  
U:V $P < .05$
DISCUSSION

In consideration of the data presented there can no longer be doubt that the hamster uterus produces a luteolytic factor and that there is a specific time of release and duration of secretion of this hormonal principle. Contradictory and negative results of earlier workers (Malven and Hansel, 1965; Kiracofe and Spies, 1966; Kiracofe et al., 1966; Howe, 1965) undoubtedly resulted from differences in times of removal of the uterus. Hechter et al. (1940) found a profound elongation (to 22 days from normal of 14 days) of the rat pseudopregnant cycle when the uterine horns were exised between the 4th and 7th day of pseudopregnancy. No such effect was observed by Velardo et al. (1953) when the horns were removed on the ninth day of pseudopregnancy. Kiracofe and Spies (1966) obtained negative results using extracts of estrous rat uteri. Schomberg (1967) reported that only sow uterine extracts made from days 12 and 14 through 16 of the estrous cycle showed a luteolytic effect. Similar results, indicating a specific time during which the uterus has an effect on the ovary have been reported for pig (Anderson et al., 1963), heifers (Wiltbank and Casida, 1956; Anderson et al., 1965), and guinea pigs (Rowlands, 1961; Butcher et al., 1962). The time of appearance of a uterine factor seems to be specific for each individual species of mammal.

Pregnancy, on the other hand, manages to overcome the deleterious effect of ULF on the life span of corpora lutea. In the current investigation over 90% of the animals were able to go to full term, though given intraperitoneal injections of active uterine
extract. These results are in agreement with the earlier work of Moor and Rowson (1966a) who found in ewes that implantation up to the 12th day of pseudopregnancy of early embryos flushed from other donors caused corpora lutea to persist for a time equivalent to normal pregnancy and embryos were carried to term. In addition, intra-uterine infusions of 14 or 15-day sheep embryo homogenate showed an extension of cycle length of 22.4 days from the normal of 16.6 days (Moor and Rowson, 1966b). Since implantation in the hamster takes place on day 3, the embryos could well have prevented the luteolytic effect of pseudopregnant uterine extracts by producing a ULF-inhibiting or inactivating substance. Furthermore, the inability of flushed pregnant horns (i.e. free of embryonic tissue) to induce any effect on the length of pseudopregnancy in hysterectomized hamsters indicates that embryos or embryonic membranes prevent either synthesis or release of ULF by the uterus.

In previous work, no significant differences were noted in the length of pseudopregnancy in hamsters when (1) ovary and uterus were transplanted into the same cheek pouch (12.70 days) or (2) ovary and uterus placed into opposite pouches (13.80 days) (Caldwell et al., 1967). The present results, however, show some local uterine-ovarian relationship since cycle length was almost four days shorter when ovary and uterus were on the same side (ipsilaterally) than that seen when they were opposite (contralaterally). The difference is not easily explained. It may well be that multiple mechanisms operate to influence how long corpora lutea function. Perhaps, the primary relationship between uterus and ovary is local (via lymphatics) with a systemic control (i.e., blood-vascular) being secondarily present. As noted
above, evidence for local uterine ovarian relationships in various species is impressive (Ginther, 1967). If transport of ULF is primarily via lymphatics, transplantation of either or both ovary and uterus to the hamster cheek pouch would disrupt the normal in situ relationships between ovary and uterus, while allowing new blood-vascular pathways to be established. Any uterine secretion would then reach the ovary only via the blood vascular system. This problem remains unresolved.

Current research is underway to obtain a better understanding of the chemical characteristics of ULF. Chemical isolation of this factor would allow control over the cyclic behavior of virtually any mammal.
SUMMARY

1. The Syrian hamster uterus produces a uterine luteolytic factor (ULF) on the 6th and 7th day of pseudopregnancy.

2. Dosages of the equivalent of $\frac{1}{2}$ and 1 horn per day were maximally effective in shortening pseudopregnancy in the hysterectomized animal, but $\frac{1}{4}$ horn was ineffective.

3. Extracts made from pregnant hamster horns indicate that pregnancy abolishes the synthesis of the uterine luteolytic factor.

4. Active extracts of ULF did not interrupt established pregnancies.

5. Immature uterine horn extracts were ineffective in shortening hysterectomized pseudopregnant cycles.

6. The microsomal fraction of homogenized tissue appears to be the origin of the uterine luteolytic factor.

7. The luteolytic factor is nondialyzable and thermolabile.

8. The hamster displays a local as well as a systemic ovarian-uterine relationship.
BIBLIOGRAPHY


Figure 1. Group A. Saline extract given at dosage of 1 horn per day.
Figure 2. Group B. Extract of uteri removed on 4th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
Figure 3. Group C. Extract of uteri removed on 5th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
GROUP C

CYCLE LENGTH

C  E1  E2  E3  E4

CYCLE NO.
Figure 4. Group D. Extract of uteri removed on 6th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
CYCLE LENGTH

8 9 10 11 12 13 14 15 16 17 18 19 20 21

GROUP D

C E1 E2 E3 E4

CYCLE NO.
Figure 5. Group E. Extract of uteri removed on 7th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
Figure 6. Group F. Extract of uteri removed on 8th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
GROUP F

CYCLE NO. C E1 E2 E3 E4

CYCLE LENGTH

21- 20- 19- 18- 17- 16- 15- 14- 13- 12- 11- 10- 9- 8-
Figure 7. Group G. Extract of uteri removed on 7th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
Figure 8. Group H. Extract of uteri removed on 7th day of pseudopregnancy.

Dosage: equivalent of $\frac{1}{2}$ horn per day.
Figure 9. Group I. Extract of uteri removed on 7th day of pseudopregnancy.

Dosage: equivalent of $\frac{1}{4}$ horn per day.
Figure 10. Group J. Extract of immature uteri removed at $3\frac{1}{2}-4\frac{1}{2}$ weeks of age.

Dosage: equivalent of 1 horn per day:
Figure 11. Group K. Extract of pregnant uteri removed on the 5th, 8th and 9th days of pregnancy.

Dosage: equivalent of 1 horn per day.
Figure 12. Group L. Nuclear fraction of saline extract of uteri removed on 7th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
Figure 13. Group M. Mitochondrial fraction of saline extract of uteri removed on 7th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
GROUP M

CYCLE LENGTH

C  E1  E2  E3  E4

CYCLE NO.
Figure 14. Group N. Microsomal fraction of saline extract of uteri removed on 7th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
Figure 15. Group 0. Nuclear fraction of .25 M sucrose extract of uteri removed on 7th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
Figure 16. Group P. Mitochondrial fraction of .25 M sucrose extract of uteri removed on 7th day of pseudopregnancy. Dosage: equivalent of 1 horn per day.
GROUP P

CYCLE LENGTH

C  E1  E2  E3  E4

CYCLE NO.
Figure 17. Group Q. Microsomal fraction of .25 M sucrose extract of uteri removed on 7th day of pseudopregnancy.
Dosage: equivalent of 1 horn per day.
Figure 18. Group R. Thermolability of extract of uteri removed on 7th day of pseudopregnancy.

Dosage: equivalent of 1 horn per day.
Figure 19. Group S. Dialysis of extract of uteri removed on 7th day of pseudopregnancy.

Dosage: equivalent of \( \frac{1}{2} \) horn per day.