THE FAILURE OF PROGESTERONE TO AFFECT MYOMETRIAL ACTIVITY IN THE GUINEA-PIG

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SUMMARY

A study was made of the effects of both systemic and intrauterine progesterone and medroxyprogesterone acetate (MPA) administration on the duration of pregnancy in the guinea-pig. In no case was pregnancy prolonged beyond normal term. When progesterone and MPA were administered to non-pregnant guinea-pigs (in doses up to 50 times that required to block myometrial activity in the rabbit) there was no effect on the amplitude and frequency of intrauterine pressure cycles recorded in vivo by means of intrauterine balloons. The response of the uterus to a standard dose (20 m-u.) of oxytocin was unchanged by progesterone. The administration of progesterone by an intrauterine, instead of a systemic, route did not alter the result. It is concluded that progesterone is not a myometrial blocking agent in the guinea-pig.

INTRODUCTION

The necessity of progesterone for the maintenance of pregnancy in the rabbit has been clearly demonstrated (Allen & Corner, 1929; Allen & Heckel, 1939) and Csapo & Takeda (1965) have shown that it depends on the ability of the hormone to block myometrial activity. It has been suggested further, that the effect of progesterone on the uterine smooth muscle is a fundamental one which occurs generally in mammals (Csapo, 1963). However, the failure of progesterone to prevent abortion in women (Fuchs & Stakemann, 1960) and to delay labour in women (Pose & Fielitz, 1961; Csapo, Sousa-Filho, De Souza & De Souza, 1966) and guinea-pigs (Zarrow, Anderson & Callantine, 1963; Schofield, 1964) raises the question whether the progesterone block theory is generally applicable.

The species in which progesterone appears to have little effect on the mechanical activity of the parturient uterus are those in which the ovaries may be removed without terminating pregnancy, and in which it is known that the placenta secretes progesterone (Zander & von Münstermann, 1954; Lurie, Reid & Villee, 1966; Heap & Deanesly, 1966). In an attempt to meet the objections to the progesterone block theory in these species, Csapo (1956, 1969) has suggested that placental progesterone reaches the uterine muscle directly through the endometrium. According to this hypothesis, concentrations of progesterone occur in the myometrium in vivo which
it is not possible to reproduce by systemic injection because of the poor solubility of the steroid in blood. This hypothesis would explain the failure of exogenous progesterone to prolong pregnancy in women and guinea-pigs, and is supported by experimental evidence (Goto & Csapo, 1959; Macedo-Costa & Csapo, 1959; Daniel, 1960; Kuriyama & Csapo, 1961; Wiest, 1967; Porter, 1968).

Nevertheless, in a pilot study reported earlier (Porter, 1969) in which progesterone was injected into the amniotic sacs of pregnant guinea-pigs, in an attempt to simulate a local effect of progesterone and thus to prolong pregnancy where systemic injections failed, large doses of the hormone had no effect on the length of gestation. These experiments have been extended and, in addition, studies were made of the effect on uterine activity in non-pregnant guinea-pigs, of progesterone and a synthetic steroid with potent progestational activity (6α-methyl 17α-hydroxyprogesterone, Provera, Upjohn; MPA).

**MATERIALS AND METHODS**

**Pregnant guinea-pigs**

Pregnant guinea-pigs of known mating date were obtained by placing oestrous females with a buck and checking for plugs (day of plug = day 1) the following morning. A total of 28 guinea-pigs (55–65 days pregnant) were used as follows:

**Group I (systemic administration) (four animals)**

Each animal received two s.c. implants of progesterone pellets (approx. 100 mg. total) at 57–61 days of pregnancy. The pellets were weighed before insertion and were recovered after delivery, dried and re-weighed to determine the rate of absorption.

**Group II (intrauterine administration) (24 animals)**

The animals were treated as follows: (a) (3 animals). 0·1, 0·4 and 0·5 g. respectively of progesterone crystals suspended in arachis oil were injected into one amniotic sac in each horn. (b) (4 animals). A total of 0·1, 0·2, 0·25 and 0·25 g. respectively of MPA in aqueous suspension was distributed equally among the amniotic sacs. (c) (10 animals). Implants of 0·05–0·2 g. (total weight) of solid progesterone were made between the foetal membranes and the endometrium near to one placenta in each horn (4 animals) or near to each placenta in both horns (6 animals). (d) (7 animals). Pellets of progesterone, weighed before insertion, were placed near to each placenta in both horns. The pellets were recovered at autopsy before term or at delivery and re-weighed.

The subcutaneous pellets of progesterone were implanted antiseptically under local anaesthesia, and the intrauterine treatments by laparotomy performed aseptically under ether anaesthesia. After all treatments the animals were inspected daily for delivery.

**Non-pregnant guinea-pigs**

A small (2·5 ml. capacity without stretch) latex balloon connected to polyethylene tubing (i.d. 0·38 mm., e.d. 1·09 mm.) was placed in one uterine horn of each of
14 non-pregnant guinea-pigs. The method used was similar to that described for rabbits by Csapo, Takeda & Wood (1963), and was carried out with aseptic precautions through a flank incision under ether anaesthesia. The animals were in various stages of the oestrous cycle and ovariectomy was not performed. In addition, two of the animals were equipped with a perforated indwelling catheter which was attached to the polyethylene tube of the recording balloon, to permit injections directly into the uterine lumen. The tubing was led out of the abdomen and passed subcutaneously to a point at the back of the neck, where it was brought to the exterior and anchored. The animals were allowed 48 hr. to recover, after which the balloons were filled with a small volume of water varying from 0·25 to 2·00 ml. depending on the resting pressure produced in the uterus. Intrauterine pressures were recorded with a Devices M2 pen-recorder/preamplifier system in conjunction with a Bell & Howell CEC pressure transducer.

It was found that intrauterine pressure could be monitored in unrestrained guinea-pigs for periods of a week or more. The effects of treatment with progesterone or MPA on intrauterine pressure were studied as follows:

(a) Three animals were given seven injections of progesterone (1 of 10 mg.; 5 of 25 mg.; and 1 of 40 mg. either s.c. or i.m.). (b) Three animals were each given single injections (s.c. or i.m.) of 25 mg. MPA. (c) Five animals were given s.c. injections of progesterone for 5 days (i.e. 2·5, 5·0, 10·0, 25·0 and 20·0 mg./day respectively). The recording balloons were implanted on day 3 of treatment and intrauterine pressure was recorded on day 5. (d) Three animals received four intrauterine injections: two of 5·0, and one of 8·0 mg. progesterone (in 0·2 ml. arachis oil) and one of 7·5 mg. MPA (in 0·15 ml. aqueous suspension).

Intrauterine pressure was monitored for not less than 12 hr. after each injection, so that at least 12 hr. elapsed between successive injections into the same animals. In all cases intrauterine pressure was also recorded continuously for 1 hr. before each treatment and, in addition, for 12 hr. in four untreated animals to obtain control readings.

The records were analysed as follows: (i) For frequency of pressure cycles: all pressure cycles exceeding 5 mm. Hg amplitude were counted for 2 hr. periods at 0, 4, 8, 12 and 20 hr. after treatment. (ii) Since the primary effect of progesterone on uterine activity in the rabbit is one of decreasing the amplitude of intrauterine pressure cycles (Csapo & Takeda, 1965) an attempt was made to estimate quantitatively the changes in amplitude which occurred in the present experiments. An arbitrary amplitude was selected for each experiment (n mm. Hg) which was approximately equivalent to 50% maximum amplitude of pressure cycles recorded during 1 hr. before treatment. All pressure cycles exceeding this value (H) were then counted in each of the 2-hr. periods which had also been analysed for frequency.

Normal term was defined according to the criteria of Goy, Hoar & Young (1957) who found in 1411 guinea-pig pregnancies, that the length of gestation varied inversely with litter-size and that normal delivery occurred between days 62 and 74 depending on the number of foetuses.
RESULTS

Pregnant animals

Systemic administration of progesterone (Group I)

The four animals with s.c. pellets of progesterone delivered an average of 3·5 foetuses at 70·3 ± 1·9 (s.e.) days post coitum. The average uptake of progesterone from the pellets was 0·9 ± 0·11 mg./24 hr.

Intrauterine administration of progesterone (Group II)

None of the 24 animals in this group carried their litters beyond term. Two animals (in Group IIc) aborted soon after the implantation of progesterone, and five animals from subgroup (d) were killed before term to recover progesterone pellets for reweighing. The results of the remaining 15 animals in subgroups (a, b and c) are summarized in Table 1. These animals delivered 3·6 foetuses on average, 67·0 ± 0·8 days post coitum (range 63–70 days). Of the 45 foetuses delivered 26 were alive. Subgroup (a) was excluded since the foetuses were dead as was anticipated after the injection of oil into the amniotic cavity.

Table 1. The effect of treatment with intrauterine progesterone and medroxy-progesterone (MPA) on the length of pregnancy in the guinea-pig

<table>
<thead>
<tr>
<th>Group</th>
<th>Serial no. of animals</th>
<th>Treatment</th>
<th>Total dose (mg.)</th>
<th>Day of treatment</th>
<th>Day of delivery</th>
<th>Foetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Πa</td>
<td>7</td>
<td>Progesterone crystals in oil (intra-amniotic)</td>
<td>800</td>
<td>64</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>1000</td>
<td>63</td>
<td>69</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td></td>
<td>200</td>
<td>65</td>
<td>67</td>
<td>-</td>
</tr>
<tr>
<td>Πb</td>
<td>9</td>
<td>Aqueous suspension MPA (intra-amniotic)</td>
<td>100</td>
<td>64</td>
<td>67</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>250</td>
<td>66</td>
<td>65</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td></td>
<td>200</td>
<td>64</td>
<td>65</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td>250</td>
<td>64</td>
<td>67</td>
<td>3</td>
</tr>
<tr>
<td>Πc</td>
<td>2</td>
<td>Progesterone pellet (one per uterine horn)</td>
<td>50</td>
<td>34</td>
<td>68</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>50</td>
<td>57</td>
<td>68</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>100</td>
<td>64</td>
<td>70</td>
<td>-</td>
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<tr>
<td></td>
<td>5</td>
<td></td>
<td>100</td>
<td>63</td>
<td>65</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Progesterone pellet (one per amniotic sac)</td>
<td>100</td>
<td>65</td>
<td>68</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td></td>
<td>100</td>
<td>61</td>
<td>63</td>
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<tr>
<td></td>
<td>24</td>
<td></td>
<td>150</td>
<td>62</td>
<td>69</td>
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<tr>
<td></td>
<td>26</td>
<td></td>
<td>150</td>
<td>55</td>
<td>63</td>
<td>-</td>
</tr>
</tbody>
</table>

In addition two animals from Group IIc, not shown, aborted before day 63.

Pellets were recovered from the seven animals in subgroup (d) (i.e. from five killed before term and two after delivery), dried and reweighed. The uptake of progesterone had been at the rate of 2·3 ± 0·2 mg./24 hr.

Non-pregnant animals

The intrauterine pressure cycles recorded from the guinea-pig uterus closely resembled in shape and frequency those described for the rabbit (Fuchs, 1964; Csapo & Takeda, 1965; Porter, 1968) and in women (Pose & Fielitz, 1959; Csapo & Pinto-Dantas, 1966), but differed in that the maximum amplitude recorded was
Progesterone and guinea-pig myometrium

only about 50 mm. Hg compared with over 100 mm. Hg recorded in the other species. There was a tendency for 10–20 pressure cycles to occur as discrete bursts of activity separated by quiescent periods of 5–10 min. No significant changes in activity were found among animals in different stages of the oestrous cycle.

Systemic progesterone and MPA (subgroups a, b and c)

Intrauterine pressure recordings from one animal obtained during 18 hr. after treatment with progesterone are illustrated in Fig. 1. Numerical data (frequency of high amplitude contractions, H, and maximum amplitude of contractions) are shown in Table 2. Changes in mean frequency of pressure cycles in seven animals treated with progesterone and three animals treated with MPA are shown in Fig. 2.

Table 2. Changes in mean maximum amplitude of contractions (Max) and frequency of high amplitude contractions (H) after treatment with progesterone (P) and/or medroxyprogesterone (MPA). (Means ± s.e.)

(Maximum amplitude expressed in mm. Hg and frequency as number of high amplitude contractions/2 hr.)

<table>
<thead>
<tr>
<th>Time after treatment (hr.)</th>
<th>(a; 7 obs.)</th>
<th>(b; 3 obs.)</th>
<th>(d; 4 obs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systemic P</td>
<td>Systemic MPA</td>
<td>Intrauterine P and MPA</td>
</tr>
<tr>
<td>Max</td>
<td>H</td>
<td>Max</td>
<td>H</td>
</tr>
<tr>
<td>0</td>
<td>34.3 ± 3.9</td>
<td>41.8 ± 9.6</td>
<td>33.7 ± 3.7</td>
</tr>
<tr>
<td>4</td>
<td>32.3 ± 3.3</td>
<td>45.0 ± 7.6</td>
<td>31.7 ± 4.3</td>
</tr>
<tr>
<td>8</td>
<td>35.7 ± 3.7</td>
<td>50.4 ± 11.1</td>
<td>30.7 ± 4.4</td>
</tr>
<tr>
<td>12</td>
<td>34.2 ± 5.5</td>
<td>46.0 ± 9.6</td>
<td>30.3 ± 4.8</td>
</tr>
<tr>
<td>20+</td>
<td>32.3 ± 7.2</td>
<td>47.5 ± 18.5</td>
<td>32.7 ± 4.2</td>
</tr>
</tbody>
</table>

No consistent or significant change in either frequency of high amplitude cycles, maximum amplitude of cycles, or total frequency of contractions was observed up to 20 hr. after the courses of treatment with progesterone or MPA. This was so despite the use of doses of progesterone up to 40 mg. and MPA up to 50 mg. There was no obvious change in shape of the pressure cycles (Fig. 1) indicating that the rate of rise of pressure (Csapo & Sauvage, 1968) was unaffected. Uterine activity in animals in which balloons had been inserted after the administration of progesterone treatment had been started (subgroup c) was not significantly different from that in other groups.

Two animals which had received single doses of 25 mg. progesterone (i.m.) were injected with 25 m-u. oxytocin (i.m.) at 0 hr. and again 8 hr. later and the intrauterine pressure response was recorded on each occasion. No significant difference in amplitude or frequency between the responses at the two different times was found.

Intrauterine progesterone and MPA (subgroup d)

The results of the four experiments in which progesterone and MPA were injected into the uterine lumen are summarized in Table 2 and changes in total frequency are shown in Fig. 2. They were similar to those in the preceding groups in that neither consistent nor significant variations in uterine activity followed the instillation of these substances into the uterine lumen.
Fig. 1. Intrauterine pressure recordings from a non-pregnant guinea-pig (1 day after oestrus) at intervals from 0–18 hr. after treatment with 25 mg. progesterone (s.c.) (arrow). Balloon volume: 0·25 ml. Full-scale pen deflexion: 0–50 mm. Hg.
DISCUSSION

The failure of animals with subcutaneous implants of progesterone (Group I) to carry their litters beyond normal term confirms the earlier reports of Zarrow et al. (1963) and Schofield (1964) that systemic progesterone administration does not prevent delivery in the guinea-pig. Weighing of progesterone pellets confirmed that the hormone was absorbed and indicated that the average rate of absorption was 0·9 mg./24 hr., a figure comparable to that (1·5 mg./24 hr.) reported by Heap & Deanesly (1967) in ovariectomized pregnant guinea-pigs.

The intrauterine administration of progesterone and/or MPA also failed to prolong pregnancy and in two cases did not prevent abortion. These results contrast with those in rabbits, in which systemic administration of 1·5–2·0 mg. progesterone daily prolonged pregnancy in nine out of ten animals (Heckel & Allen, 1937). The treatments with progesterone did not affect foetal mortality significantly, since the findings of Goy et al. (1957) suggest that at least 30% foetal mortality occurs in untreated guinea-pigs. This finding differs from the high foetal mortality which followed progesterone treatment in sheep (Bengtsson & Schofield, 1963).

Although these results suggest that progesterone may not exert a blocking action on the guinea-pig myometrium, several alternative explanations for these results must be considered before such a conclusion is reached. These include the possibility that: (1) the hormone was not absorbed from the uterus; (2) the distribution of the hormone was such that insufficient muscle was blocked to prevent parturition;
(3) the guinea-pig produces an antagonist to progesterone at term; and (4) that the rate of absorption was inadequate.

Possibility (1) may be rejected since it was found that progesterone had been absorbed from the intrauterine pellets at an average rate of 2-3 mg./day (i.e. over twice the rate compared with that from subcutaneous pellets). In the pilot experiments cited above (Porter, 1969) and in Group IIa and four animals of Group IIc, progesterone was administered to only one amniotic sac in each uterine horn through small flank incisions. Since possibility (2) might therefore explain the lack of effect of this treatment, in subsequent experiments all amniotic sacs were injected through a mid-line incision, to ensure uniform distribution of the steroids throughout the uterus. However, the results were unchanged.

The possibility that a progesterone antagonist may be produced by the guinea-pig at term (3), or, that the rate of absorption of the hormone was below that necessary to exert an effect (4) acquires relevance only if it can be shown that progesterone can block myometrial activity in this species, at least under some circumstances.

Failure of exogenous progesterone to affect myometrial activity in the pregnant animal is not good evidence that progesterone is without effect on the myometrium, since the progesterone requirement of the muscle may be higher than in the non-pregnant animal and can only be met by direct passage of the hormone from the placenta. It may not be possible to produce the necessary concentrations by systemic (or intrauterine?) administration of progesterone. In the non-pregnant animal the normal source of progesterone is extraterine (i.e. the corpus luteum) and hence is distributed to the uterus via the blood stream. Therefore if endogenous progesterone affects myometrial activity in the non-pregnant animal, then exogenous progesterone should also affect it (Csapo & Pinto-Dantas, 1966; Csapo, 1969). Earlier work on uterine activity in the non-pregnant guinea-pig is sparse, and was conducted either in vitro or in situ, with equivocal results. Bell & Robson (1936) found that progesterone (total dose 6 mg. administered over 3 days) had no effect on the uterine response to oxytocin, but Adler & Bell (1943) using larger amounts (up to 30 mg.) claimed that progesterone reduced uterine sensitivity to oxytocin. Stewart (1949) also reported that in vitro the sensitivity to oxytocin was less in uteri from progesterone-treated, than from stilboestrol-treated guinea-pigs.

The results of the present study indicate that neither progesterone nor medroxy-progesterone blocked uterine mechanical activity in vivo in the non-pregnant guinea-pig. The contrast with the rabbit is striking since in the latter species intra-uterine pressure cycles recorded in the same manner are completely abolished within 12 hr. of a systemic injection of 5 mg. (Csapo & Takeda, 1965) or an intrauterine injection of 0.8 mg. progesterone (Porter, 1968). It is unlikely that the lack of effect in the guinea-pig is due to too low a dose, since on the basis of body weight the doses employed were up to 50 times the effective dose in the rabbit.

The results of the experiments in which progesterone was administered directly into the uterine lumen of non-pregnant guinea-pigs, suggest that systemic inactivation of the hormone (e.g. by protein binding, rapid clearance, etc.) does not account for its lack of effect. This conclusion seems permissible since it has been shown (Porter, 1968) that progesterone administered by this route can reach the myometrium without entering the general circulation.
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The lack of effect of progesterone on spontaneous and oxytocin-induced uterine activity in the non-pregnant guinea-pig is thus consistent with the results in pregnant animals. It would seem therefore that the failure of the hormone to delay parturition was neither due to the manner of administration nor to interaction with other substances, but to the fact that progesterone is not a myometrial blocking agent in the guinea-pig.

Studies in progress suggest that the guinea-pig uterus is, nevertheless, quiescent during pregnancy and refractory to oxytocin. Although the results reported indicate that this condition is not due to progesterone, they do not rule out that it may be due to progesterone analogues.

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REFERENCES


