HORMONAL CONTROL OF MYOMETRIAL FUNCTION DURING PREGNANCY IN THE SHEEP

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Summary. In a series of thirteen sheep, experiments were carried out during the last month of pregnancy to assess the extent of progesterone influence on the myometrium. Staircase effects were recorded from the longitudinal fibres of the myometrium in the intact ewe and also in vitro, and the threshold dose of oxytocin required to elicit a contraction was determined. The results, both in vivo and in vitro, demonstrate that the progesterone influence declines before parturition and it is concluded that the extrusion of the foetus is accomplished by an oestrogen-dominated myometrium. This finding is discussed in relation to the fact that no fall in the progesterone level in blood can be detected before parturition. The oxytocin thresholds determined in vivo suggest that the sensitivity of the myometrium increases with the approach to parturition.

INTRODUCTION

There has been much discussion about the importance of progesterone in the maintenance of pregnancy ever since 1929, when Allen & Corner made an extract of corpus luteum and demonstrated its pregnancy-maintaining effect in the ovariectomized rabbit. Controversial evidence and difficulties in assessing the hormonal condition of the myometrium, from the hormone levels in the blood and the excretion of the hormones and their metabolites, have led to widely differing opinions as to the factors concerned with the maintenance and termination of pregnancy in different species. Only in the rabbit has hormonal control of gestation and the mechanism by which ovarian hormones exert their effect on the myometrium been carefully analysed (Csapo & Corner, 1952; Csapo, 1955, 1956 a, b, 1959a; Bengtsson, 1957; Schofield, 1955, 1957; etc.). During pregnancy, the myometrium is under strong progesterone influence and this has a blocking effect which depresses activation and conduction in the muscle. Thus, a stimulus reaching the myometrium elicits only a local response and no propagated, co-ordinated, effective contractions. Some 24 hr before parturition, this progesterone block is withdrawn and the myometrium becomes dominated by oestrogen, and is reactive to intrinsic and extrinsic stimuli. Parturition can then take place.

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Hormone Control of Myometrial Function

It is unlikely that, among Eutherian mammals, there are widely differing mechanisms for the control of pregnancy and parturition and it is possible that the mechanisms obtaining in the rabbit may, with minor modifications, apply also to other species. In the sheep, chemical tests have shown that the level of progesterone in the blood during pregnancy is no higher than that found during the luteal phase of the oestrous cycle and that this level does not fall until after parturition, there being no demonstrable change prior to this act (Short & Moore, 1959; Neher & Zarrow, 1954). It is not known, however, what is the significance of the progesterone levels and whether the uterine cells are necessarily influenced by these levels. The biological tests worked out by Csapo (1955, etc.) on isolated uterine strips are a more reliable indication of ovarian hormone influence. For this reason, those biological tests that were used for exploring hormonal dominance in the intact rabbit (Schofield, 1957) have been employed for a similar exploration of hormonal dominance in the sheep during the last few weeks of pregnancy: the findings are reported in this paper.

METHODS

Breeds and tupping technique

Four breeds of sheep were used in these experiments, Kent, Kerry Hill × Welsh Mountain, Cheviot × Border Leicester and Clun, and the tupping dates were determined and spaced out by the use of a vasectomized and a normal ram, respectively. The ewes were kept in a paddock with the vasectomized ram which was marked on the underside so as to colour each ewe it mounted and thus indicate when the ewe was in oestrus. Each day one or two oestrous ewes were placed with the normal ram and pregnancy dated from that day.

Selection of 'parturient' sheep

The average length of gestation is 148 days, but there may be a variation of several days either side of this mean. For this reason, the immediately prepartum sheep were selected from the group, the udder pressure and the conformation of the sheep serving as indications of the comparative imminence of parturition. This was found to be more accurate than assuming a constant gestation length. In the case of twin pregnancies when one foetus remained in utero after the experiment, the date of birth was noted. It is, however, impossible to exclude the effect of experimental interference on the onset of delivery.

Anaesthetic

In pilot experiments, several types of anaesthesia were tried, but, in the final experimental series, the sheep were either intubated and maintained with halothane (Fluothane, I.C.I.) or intubated under thiopentone and maintained with cyclopropane. As regards maternal mortality, cyclopropane proved to be the more satisfactory when the experiments were finally reduced to about 2 hr.
Operative technique

Sterile conditions were maintained throughout these experiments. A longitudinal incision a few inches from the mid-line was made in the lower abdominal wall. Single foetuses were delivered by Caesarian section, whereas in the case of twins only one of the foetuses was delivered. The cotyledons were left in situ and the uterine incision was sutured together. This made available one uterine horn with myometrium which was no longer distended. A longitudinal segment about 3 cm in length was cut out of the uterine wall and placed in oxygenated Krebs' solution for in-vitro experiments. A suitable part of the empty horn was then selected, being opposite the mesometrium and away from cotyledons. The blood vessels run at right angles to the longitudinal fibres and hence the nutrition of a longitudinal segment was not interrupted by the in-vivo recording technique. The method used for recording the staircase effect in the intact ewe was essentially similar to that used in the experiments on the rabbit (Schofield, 1960). The apparatus is illustrated in Text-fig. 1. Instead of single platinum electrodes, there were two such electrodes 2 mm apart at the end of each electrode arm. The two pairs of electrodes were implanted through the uterine wall about 2 cm apart along the longitudinal fibres and sewn in position. The perspex chamber was then lowered over the electrode arms. There was considerable abdominal pressure, and since the recording segment had to be raised to avoid close contact with the rest of the abdominal contents, the abdominal wall could not be sutured together over the flange of the chamber, as in the experiments on the rabbit. Instead, the segment and the chamber were kept slightly raised above a small portion of the empty horn which protruded through the abdominal incision. The exposed uterus was carefully covered with gauze soaked in saline solution and the internal temperature of the chamber kept at 39° C. The condition of the uterus remained good, the recordings were

Text-fig. 1. Showing the apparatus used for recording myometrial responses to direct electrical stimulation. The two electrode arms, A and B, carry platinum electrodes which are implanted in the uterine wall and secured in place by thread. A is held in a rack-and-pinion support which may be moved to adjust to resting length of the segment. One end of B is attached by thread to the short arm of an isometric lever which writes on smoked paper. The perspex chamber is lowered over the electrode arms to complete the preparation. (Slightly modified from J. Physiol. 1960, 151, 578.)
repeatable and the segment appeared quite normal at the end of the experiment. When the recording apparatus was removed, the abdominal muscle and skin, separately, were carefully sutured together with isolated nylon sutures and the animal injected with penicillin, streptomycin and lamb-dysentery serum.

**Recording technique**

*In vivo.* The segment was set at resting length, as in the previous experiments on the rabbit, and the segment stimulated with 15 or 20 volts (60 c/s a.c.) of 5 sec duration at a frequency of one stimulus per \( \frac{1}{4}, 1 \) and 2 min. The tension developed was recorded on a smoked drum by an isometric lever and staircase effects were elicited by changing the frequency of stimulation. When a repeatable staircase effect had been observed, the stimulator was switched off; and, after a few minutes rest, oxytocin was injected intravenously in order to find the threshold dose that would produce a contraction of the recording segment.

*In vitro.* Parallel experiments were carried out *in vitro* on the segment that had been removed during the operation, by the method described for the rabbit (Csapo, 1956c). The endometrium was trimmed off and a strip of myometrium about 2 x 20 mm in size was suspended in an isolated-organ bath containing Krebs’ solution at 39°C through which a mixture of 95% oxygen and 5% carbon dioxide was bubbled. After the strip had been set at resting length, the optimal voltage was determined (varying between 5 and 10 volts). This stimulus at 5 sec duration was used to elicit the staircase effect as *in vivo.* After a repeatable staircase effect had been observed, the stimulator was switched off and the strip allowed to rest for a few minutes. Oxytocin was then added to the bath of 34-ml capacity in order to find the threshold dose that would produce a contraction of the myometrium.

Due to technical difficulties at the beginning of this study it was not possible to perform both *in-vivo* and *in-vitro* recordings in every case.

**Table 1**

**Showing the staircase effects and oxytocin thresholds obtained *in vivo* and *in vitro* in sheep towards the end of pregnancy and post-partum**

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Days before term</th>
<th>Staircase effect</th>
<th>Oxytocin threshold (m.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In theory</td>
<td>In practice</td>
<td>In vivo</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>8</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Due the previous day</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>1.5</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Several hours post-partum</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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RESULTS

The staircase effect and oxytocin thresholds from this series of thirteen sheep are shown in Table 1. The expected date of parturition is stated and also the actual date in those cases where a twin lamb remained in utero after the experiment.

Text-fig. 2. Tracing showing the negative staircase effect recorded on the intact uterus in Sheep 1. Stimulus once every ½, 1, 2 or 4 min.

Text-fig. 3. Tracing showing the negative staircase effect recorded on an isolated strip of myometrium taken from Sheep 3. Stimulus once every ½, 1 or 2 min.

Text-fig. 4. Tracings showing the positive staircase effects recorded on the uterus of Sheep 10, A in vivo, B in vitro. Stimulus once every ½, 1, 2 or 4 min.

The material can be divided into two groups, comprising: (1) sheep, Numbers 1 to 4, in which the staircase effect was negative indicating progesterone domination, and (2) sheep, Numbers 5 to 13, in which the staircase effect was positive indicating oestrogen domination. In Group 1, the ewes were more than
7 days from the expected date of parturition, whereas, in Group 2, they were within 3 days of term, except for sheep Number 5 which produced a dead lamb 8 days after the experiment.

The in-vitro results confirm the in-vivo observations; staircase effects obtained both in vivo and in vitro are illustrated in Text-figs. 2 to 4.

It was noted that the uteri in the two groups also differed markedly in response to handling and exposure to air. Those in Group 1, with the negative staircase and presumed progesterone dominance, were very flaccid and unreactive. Those in Group 2, on the other hand, were very reactive and contracted readily. It was more difficult to record from the latter group of uteri, particularly the post-partum uterus, because of their tendency towards spontaneous activity which interfered with the staircase effect.

Table 1 gives the oxytocin thresholds in m.u. per bath of 34-ml capacity. It will be noted that in Group 1 the in-vivo thresholds for Sheep 1 and 4 were 1800 m.u. and between 20 and 200 m.u., respectively: no values are available for Sheep 2 and 3, though the in-vitro threshold for Sheep 3 was 5 m.u. In Group 2, however, all the sheep recorded had in-vivo thresholds of 60 m.u. or less and in-vitro thresholds of 2 m.u. or less, with the exception of Sheep 10 which had a threshold of 5 m.u.; this sheep was due to deliver on the previous day but at the time of the experiment showed no immediate signs of parturition.

**DISCUSSION**

These experiments indicate that although progesterone dominates the myometrium in late pregnancy in the sheep, this progesterone influence declines before parturition so that the extrusion of the foetus is accomplished by an oestrogen-dominated myometrium. The staircase effect is a reliable qualitative biological test for estimating the hormonal dominance of the myometrium and also for showing major changes in hormonal influence. Its value lies in the fact that it makes an assessment at the target organ, that is, by estimating the influence of progesterone on cell function. It has been shown by Csapo (1955, 1956a) on rabbit myometrium that the staircase phenomenon is based upon biochemical and biophysical properties within the cells, and these are changed in a typical way by the ovarian hormones. Since the basic components involved in muscle function are the same for a variety of muscles (Csapo, 1959b), one would expect these properties to be similar in all myometrial cells regardless of species. It was not surprising, therefore, to find that the staircase effect could be observed on the uterine muscle of the sheep, as in the rabbit, and that changes in hormonal dominance could be recorded in this way. Observations made in vitro and in vivo on rabbit myometrium gave closely similar results (Csapo & Corner, 1952; Csapo, 1955, etc., Schofield, 1954, 1955). So also in the experiments reported here, the in-vitro and in-vivo results were confirmatory. This is an added assurance that the in-vivo recordings were not to any extent influenced by superimposed regulations which might be different in the sheep from those in the rabbit.

In the rabbit, the influence of progesterone on the general behaviour of the myometrium has declined some 24 hr before parturition (Bengtsson, 1957;
Schofield, 1957). Since the gestation period in the sheep is nearly five times longer, one might expect this influence to decline some days before term. The experiments reported here suggest that this is, in fact, the case, but the exact date of the hormonal change could not be determined accurately due to the small number of experiments and the difficulty in calculating the exact date of delivery.

The oxytocin thresholds have a wide scatter, particularly in the in-vitro experiments, and the number of observations is too few to draw any definite conclusions. However, the in-vivo thresholds suggest an increase in sensitivity of the myometrium as parturition approaches, reaching a maximum a few hours after parturition. The blocking effect of progesterone on the oxytocin response has been established in the rabbit (Csapo, 1955, 1956a) which, during most of pregnancy, is not responsive to oxytocin in doses as high as 4 units (Schofield, 1957, 1960). Thus, in the rabbit, progesterone exerts an absolute block on myometrial sensitivity to oxytocin. Perhaps in the ewe, the block is only relative and although the myometrium will respond to oxytocin throughout pregnancy, the sensitivity increases with the approach to parturition. Such would seem to be the case in the human female (Caldeyro-Barcia & Poseiro, 1959; Smyth, 1958). This tentative conclusion agrees with similar conclusions drawn by Fitzpatrick (1960) from a study of the increase in uterine pressure that occurs in response to given doses of oxytocin in pregnant and post-partum sheep. The validity of regarding as quantitative the tension developed in response to oxytocin has, however, been questioned by Coutinho & Csapo (1959).

It is interesting that, in the sheep, the influence of progesterone on the myometrial cells as estimated by the staircase effect disappears before parturition, despite the fact that the progesterone levels in systemic blood are, at this stage, unaltered. Thus, new questions are posed as to the site and nature of the change that causes the decline of progesterone influence at the end of pregnancy. If the change is quantitative, then possibly an alteration occurs in the quantity of progesterone influencing the myometrium which is not reflected in the systemic blood; this may be related to the 'local' effect of progesterone postulated by Goto & Csapo (1959). On the other hand, it may be that a change occurs in the availability of progesterone to the target organ — either an alteration in the metabolism of progesterone (Bengtsson, 1959) or an alteration in the end organ and its ability to respond to the presence of progesterone.

It is clear at least that in the sheep there is no correlation between the level of progesterone in the blood (Short & Moore, 1959; Short, 1960) and the hormonal dominance of the myometrium as assessed by the staircase effect.

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REFERENCES


