THE EFFECT OF PROGESTERONE ON RECORDED PARTURITION AND ON OXYTOCIN SENSITIVITY IN THE SHEEP

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SUMMARY

Intra-uterine pressure changes were recorded at the end of pregnancy and during parturition in eight ewes by means of endo-radiosondes implanted in one uterine horn some weeks previously. Recordings were made at frequent intervals so that the progress of parturition could be carefully monitored. When the early stages of parturition were established, a single injection of 80 mg. progesterone in oil was made in six ewes. As a result, parturition was delayed and the intra-uterine pressure waves declined or were abolished. Delivery of the lambs occurred up to 7 days later. In two ewes the injection was given somewhat later during parturition and, in these animals, the depressant effect of progesterone was much less. The increased intra-uterine pressure waves which followed digital examination of the cervical canal were abolished for more than 20 hr. in all eight ewes. Determination of threshold doses of oxytocin showed wide variations and revealed no more than a general trend of increasing myometrial sensitivity with approaching parturition, a trend which was temporarily reversed in five ewes by the injection of progesterone. The eight ewes delivered 13 viable lambs and one dead lamb.

INTRODUCTION

The importance of progesterone in the maintenance of pregnancy is now accepted for species such as the rabbit and rat. In these species the main source of progesterone is the ovary, i.e. outside the uterus, and therefore it is a relatively simple procedure to augment or remove the supply of progesterone. In species such as the sheep, the main source of progesterone is the placenta, a situation which is much more difficult to investigate. Up to the present time, the evidence suggests that, in this species, a decline in the influence of progesterone is not a necessary prelude to parturition. For example, Short & Moore (1959) were unable to detect any changes in the

* The Editor and the Society regretfully record the untimely death of Brenda Schofield on 2 December 1968.
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concentration of progesterone in the blood of ewes before the delivery of the placenta. Bengtsson & Schofield (1963) showed that normal parturition could not be delayed by the daily administration of progesterone from a week before term. Some of these experiments were repeated by Hindson, Schofield & Turner (1968a) in their attempt to reproduce the condition of ring-womb (non-dilatation of the cervix); intra-uterine pressure changes were recorded at the same time so that parturition was carefully monitored. Although all the ewes delivered their lambs spontaneously at term, labour was protracted in half of the ewes, and, in general, the intra-uterine pressure waves recorded were significantly weaker than in normal ewes. In the same study, a single injection of progesterone was mistakenly given to a control ewe in which, fortuitously, parturition had already started. This injection appeared to depress the myometrium and parturition was suppressed. It was decided, therefore, in the next season to explore the possibility of delaying parturition by a single injection of progesterone, appropriately timed, and this paper describes such experiments. Control recordings in normal ewes have already been reported (Hindson, Schofield, Turner & Wolff, 1965; Hindson, Schofield & Turner, 1967, 1968b). All the ewes came from the same local flock and their average gestation period was 145 days.

It was thought appropriate also to investigate the sensitivity of the myometrium to oxytocin and to discover whether an inverse relationship of the influence of progesterone and the sensitivity to oxytocin could be demonstrated in this species, as it has been in the rabbit.

METHOD

A group of eight cross-bred ewes (Suffolk x Clun), known to have had at least two normal lambing seasons, were purchased in the early autumn and put with the ram in November. Tupping dates were obtained in the usual manner by colouring the underside of the ram. The experimental procedures and the method of recording intra-uterine pressure changes were the same as those described previously (Hindson et al. 1965, 1967). Several weeks before term, pressure-sensitive endo-radiosondes were implanted in one horn of the uterus through an incision in the left flank. Recordings of intra-uterine pressure were made once daily from 2 weeks before term and, when the ewe was within a week of term, at least five times in 24 hr.

In order to provide a channel for the administration of oxytocin, polythene tubing was inserted into the jugular vein and led under the wool, around the neck and along the back of the ewe, being sutured to the skin at appropriate intervals (Ward, 1968). Since the ewes were kept in a paddock, it was found necessary to provide extra security for the retention of the tubing in the neck region. This was done by wrapping pieces of sticky tape around the tubing so as to provide a flange which could be sutured to the skin. Following the adoption of these procedures, all the tubing remained in situ. Oxytocin sensitivity was then measured once or twice daily by determining the threshold dose of oxytocin required to increase myometrial activity above the resting level—as reflected by the recording of pressure changes. The capacity of the polythene tubing was 0·5 ml., therefore the doses of oxytocin were injected in volumes less than this and washed in with 2 ml. glucose-saline containing heparin. Synthetic oxytocin, Syntocinon, was used, and, in general, the
doses were 25, 50, 100, 200 and 400 m-u., respectively. In one ewe the dose was dissolved in 11 ml. glucose-saline as well as in less than 0·5 ml. in order to compare the effects of volume.

When it was established that parturition had begun, a single intramuscular injection of 80 mg. progesterone in oil was given. This stage was considered to have been reached when the following criteria were met: (1) Small discrete pressure waves of uterine origin occurring at least twice in 10 min. and clearly distinguishable from pressure changes produced by the recurrent body movements of the ewe; (2) digital examination of the os showing that the cervix would admit one finger to the depth of at least one ring; (3) digital examination producing a period of increased myometrial activity, as described previously (Hindson et al. 1968b).

Six ewes were injected at this stage and two ewes (36 and 37) at a slightly later stage, as judged by the fact that the pressure waves were of greater amplitude. In ewe 37 regular small waves had been recorded 3 hr. previously at a frequency of 1 in 2 min.

After the injection of progesterone, recordings were continued at regular intervals and the state of the cervix was monitored at least twice daily by vaginal exploration. No attempt was made specifically to stretch the vagina or cervix in these explorations. When the cervix had reached full dilatation, the lambs were delivered manually so that the relative positions of the lambs and the radiosonde could be established. This was considered necessary information since it has been shown previously (Hindson et al. 1968b) that the horn carrying the presenting lamb (dominant horn) develops pressure waves which have approximately double the amplitude of those in the secondary horn.

Oxytocin thresholds were also determined in three ewes which were used in another experiment (H. E. Christensen, J. C. Hindson, B. M. Schofield & W. R. Ward, in preparation). In these, 20 mg. stilboestrol were injected 2 weeks before term in an attempt to reproduce the condition of ring-womb.

RESULTS

It was found that the single injection of progesterone had a marked depressant effect on the myometrium, abolishing or considerably diminishing the pressure waves in most ewes and delaying delivery of the lambs. Furthermore, the response to digital examination of the os was abolished for varying lengths of time. The results obtained in the eight ewes are shown in Table 1. In order to make the figures comparable with those of previous experiments, the time of completed ‘spontaneous’ delivery is given. An estimate of this was arrived at by adding half an hour to the time at which full dilatation of the cervix was reached, the actual extrusion process having been recorded in the first series (Hindson et al. 1965). The state of the cervix was assessed in the same way as previously (Hindson et al. 1967). Since the cervical canal in the ewe is convoluted, the cervix, on palpation, gives the impression of ‘rings’. Hence the state of the cervix has been designated according to the extent to which a finger could be inserted into the cervical canal—one ring, two rings and through to the allantoi (one finger). The table includes information on the site of the radiosonde.
In six ewes (42, 43, 35, 39, 67 and 31) pressure waves were suppressed for periods of time varying from 34 to 118 hr. after the progesterone injection. The response to vaginal examination was abolished in all the ewes and, except in ewe 35, re-appeared many hours later. Figure 1 illustrates the effect of progesterone in ewe 67. Thereafter responses were obtained up to the time of delivery except in ewes 35 and 43 in which there was no response during the last 18 hr. The injection of progesterone also halted the opening of the cervix—for approximately the same

Table 1. The effect of progesterone on recorded parturition in the sheep

(Results in eight ewes which were injected with a single dose of progesterone. The day of pregnancy, the amplitude of the recorded waves and the state of the cervix at the time of injection are given. Likewise the times after the injection when responses to vaginal examination were present or absent, when the waves began to increase in amplitude above the previous level and when the cervix started to dilate further. Finally, the delay due to progesterone and the gestation period are recorded.

The position of the sonde in each ewe is indicated by D (dominant horn) and S (secondary horn). Ewes 35 and 67 had single lambs. In ewe 31 the radiosonde failed 96 hr. after the injection; its position relative to the lambs is not known.)

<table>
<thead>
<tr>
<th>Ewe</th>
<th>Day of prog.</th>
<th>Amplitude of waves (cm. H2O)</th>
<th>State of cervix*</th>
<th>Response to vaginal exploration</th>
<th>Waves as large as before injection</th>
<th>Further dilatation of the cervix</th>
<th>Delivery</th>
<th>Gestation period (days)</th>
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<td>67D</td>
<td>144</td>
<td>8</td>
<td>1 ring</td>
<td>Absent</td>
<td>34-41</td>
<td>42</td>
<td>53</td>
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<td>33</td>
<td>18</td>
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<td>39S</td>
<td>140</td>
<td>8</td>
<td>1 ring</td>
<td>20</td>
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<td>42D</td>
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<td>36D</td>
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<td>11</td>
<td>1 ring</td>
<td>12</td>
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<td>No decline</td>
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* For definition see p. 209.
period of time as the pressure waves were suppressed. Once it had become re-established, labour in all these ewes was protracted in comparison with control ewes (Hindson et al. 1965, 1967, 1968b). In ewes 36 and 37, which had been injected at a later stage in the parturition process, there was no decline in the amplitude of the waves after the injection of progesterone. Spontaneous delivery occurred 28 and 13 hr. respectively after the injection. Thirteen viable lambs and one dead lamb were delivered from the eight ewes.

In seven of the ewes a large number of observations were made of the response to oxytocin injections. Subsequently, the records were carefully analysed in order to decide which doses could be accepted as threshold. The variation between ewes, and even in a single ewe, was wide. There appeared, however, to be a general trend of decreasing thresholds with approaching parturition, a trend which was temporarily reversed in five ewes by the injection of progesterone. Consistent responses were obtained repeatedly in one ewe to the same dose of oxytocin, whether contained in a large or a small volume of saline.

When supra-threshold doses of oxytocin were injected, the response was similar to that following digital examination of the os, namely, a distinct phase of intruterine pressure waves. With both these responses, the latent period varied from 1 to 7 min. and there was considerable variation even in the same ewe. Figure 2 shows the responses in ewe 36 to 100 m-u. oxytocin and examination of the os 6 hr. before the injection of progesterone. At this stage small isolated spontaneous waves were being recorded and the threshold dose of oxytocin was 50 m-u. The latent period following the stimulus was, on this occasion, about twice as long after the vaginal exploration as after the injection of oxytocin. However, there was no consistent difference in the respective latent periods, either in this ewe, or in the series generally.

![Fig. 1. Recording of intra-uterine pressure waves in ewe 67 at the time of the progesterone injection (upper tracing) and 6 hr. later (lower tracing). Read from right to left. At E the pen recorder was stopped and the state of the cervical canal was assessed by digital examination. Before this, waves are underlined which are considered to be uterine in origin. The continuous small rapid pressure changes resulted from the body movements of the ewe. The large waves after E in the top tracing are uterine.](image-url)
In the three ewes injected with stilboestrol, the threshold dose was 400 m-u. oxytocin at the time of the injection. Uterine activity followed within 24 hr. In two ewes the development of pressure waves was rapid and they aborted single foetuses 28 and 29 hr. respectively after the injection. After stilboestrol the oxytocin threshold appeared, from a few observations, to decrease to less than 100 m-u. In the remaining ewe, the pressure waves reached a maximum about 36 hr. after stilboestrol and then declined until true labour occurred 2 weeks later, terminating in the delivery of twin lambs. In this ewe changes in sensitivity to oxytocin followed the changes in uterine activity, the threshold dose falling to 50 m-u. 36 hr. after the stilboestrol and increasing to 400 m-u. thereafter. With approaching delivery, the threshold dose again diminished, but the sensitivity just pre partum could not be determined as the radiosonde failed on the day before delivery.

![Recording of intra-uterine pressure waves](image)

**DISCUSSION**

The results of these experiments demonstrate that progesterone has a depressant effect on myometrial activity in the sheep and that a single dose of 80 mg., appropriately timed, delays parturition for up to 7 days. Results already reported (Hindson et al. 1968a) demonstrate clearly that repeated daily injections of such a dose do not delay the onset of parturition and that the delivery of the lambs is delayed in some cases only as a result of protracted labour. The difference from normal ewes in the gestation period is, therefore, of the order of a day or less and both this and the diminished amplitude of the pressure waves would be missed unless the parturition process was being monitored carefully. The difference between the effect of repeated doses and a single dose is striking. It seems that the regulatory mechanisms of the uterus can adapt to continued high doses of progesterone and pregnancy is successfully terminated in spite of them. If, however, parturition has already started, then
a sudden change in blood progesterone has a significantly greater effect and labour is suppressed for several days. Absolute amounts of progesterone appear to be of less importance than abrupt variations in concentration and this suggests that, at a given time, the myometrium develops resistance to a stable level of circulating progesterone and that it is only affected significantly by an abrupt change at a time when some component of the uterine complex is undergoing a basic change relative to progesterone. However, the period in which this resistance can be decreased is apparently very limited, for in ewes 36 and 37, which were injected with progesterone slightly later in the evolution of maximum intra-uterine pressure waves, the single injection had much less effect and there was no decline in the recorded waves. Ewe 36 had a long labour (more than twice the average of the normal duration of 12 hr.) but the effect in ewe 37 was much less, the total length of labour being 19 hr. This general situation is in contrast to that in species, such as the rabbit and rat, which are dependent mainly upon ovarian progesterone and in which a change in the output of progesterone can be clearly demonstrated before parturition. In these species normal parturition is delayed by the daily administration of progesterone.

In the twin pregnancies, the radiosonde was found sometimes in the dominant, sometimes in the secondary, horn. However, this information is not of particular importance for assessing the pressure waves in the early stages of parturition since the differentiation in the amplitude of the pressure waves did not appear to develop until parturition was further advanced and a pattern of regular pressure waves was established (Hindson et al. 1968b).

The period of increased pressure waves following digital examination of the cervical canal can be assumed to be due to release of oxytocin from the posterior pituitary gland, arising from Ferguson’s reflex (1941). Debackere, Peeters & Tuyttens (1961) showed that stimulation of the birth canal of the ewe leads to prompt release of oxytocin, so it seems likely that the latent period after both the injection of oxytocin and cervical examination is due to a delay in the myometrium itself. This is puzzling since the half-life of oxytocin in the blood of non-pregnant ewes was found to be 45–50 sec. (Fitzpatrick, 1961).

The effect of progesterone in abolishing the response to digital examination of the os is presumably due to its depressant action on the hypothalamus. Such a depressant effect has been demonstrated in rats by Barraclough & Cross (1963) who showed that increased electrical activity recorded in the hypothalamus after cervical stretching was absent after slow intravenous injection of progesterone. This assumption is supported by the fact that after administration of progesterone to the ewes, there was no major change in the sensitivity of the myometrium to exogenous oxytocin. Furthermore, the response was abolished in ewe 37, although there was no apparent diminution in spontaneous myometrial activity.

During pregnancy in the sheep, the cervical canal is effectively closed by a seal. At the end of pregnancy, the cervix opens by dissolution of the seal and, at the same time, intra-uterine pressure waves develop which gradually efface and dilate the cervix. These two processes normally take place concurrently, are not causally related, but are presumably under a common (hormonal) control. The fact that in the present experiments a single injection of progesterone suppressed, for approximately the same length of time, both the amplitude of the pressure waves and the
opening of the cervical canal, suggests that a decline in the influence of progesterone is the basis of this common control.

As regards the measurement of oxytocin sensitivity, the results obtained did not show a regular correlation with the approach of labour and only general trends were indicated. The determination of threshold doses in the intact animal is a method which was used for assessing oxytocin sensitivity also in the rabbit (Schofield, 1960; Porter & Schofield, 1966). Neither investigation demonstrated more than general trends over days rather than hours at the end of pregnancy and attempts to predict the imminence of delivery by an ‘oxytocin sensitivity test’ (Fuchs, 1966) have proved to be very frustrating (Porter & Schofield, 1966). The situation would undoubtedly have been similar in the present experiments had there been any deviation from the policy of constant vigilance and regular recordings of intra-uterine pressure changes, regardless of oxytocin sensitivity. Smyth (1958a) claimed to have demonstrated a correlation in women between the magnitude of the threshold dose of oxytocin and the number of days (up to 7) before the onset of labour, and has constructed a table (1958b) for the guidance of obstetricians who were uncertain whether to send their patients home. However, the published observations upon which this procedure is founded are somewhat limited and other workers, using the same test, have done no more than demonstrate a general trend of increasing sensitivity at the end of pregnancy, with considerable variation between individual patients (Göltner, 1959; Sas, Kovacs & Uhlarik, 1963).

In general, there is ample evidence of marked differences between mid and late pregnancy in the sensitivity of the myometrium to oxytocin (Schofield, 1960; Fitzpatrick, 1960; Theobald, 1961; Caldeyro-Barcia & Sereno, 1961). Moreover, in the present experiments, when the changes which normally precede delivery of the lambs were telescoped into a short period of time by injecting stilboestrol 2 weeks before term, a more exact relationship between oxytocin sensitivity and the development of intra-uterine pressure waves became apparent in the few experiments reported. It seems appropriate to conclude, therefore, that oxytocin sensitivity as indicated by the threshold dose, is a good yardstick but a poor inch-tape with which to measure the potential performance of the intact myometrium. (For comment on the sensitivity recorded in vitro see Coutinho & Csapo, 1959.) The results of the present experiments suggest that in the sheep, as in the rabbit, there is an inverse relationship between the influence of progesterone and the sensitivity of the myometrium to oxytocin.

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