PARTURITION IN THE COW: ENDOCRINE CHANGES IN ANIMALS WITH CHRONICALLY IMPLANTED CATHETERS IN THE FOETAL AND MATERNAL CIRCULATIONS

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SUMMARY

Intravascular catheters were placed in the umbilical, uterine and maternal peripheral circulations of 16 Jersey cows between 240 and 260 days of gestation. Foetal plasma cortisol, blood gases and pH, and maternal plasma oestrogen, progesterone and cortisol were measured in ten animals during late pregnancy and throughout spontaneous parturition; all delivered live foetuses although parturition was earlier than normal and the placentas was generally retained. The gradual pre-partum rise in foetal plasma cortisol during the last week of gestation (from 10–20 ng/ml 7 days before parturition to 51 ± 5 ng/ml in the last 3 h before delivery) was much less marked than the abrupt increase immediately after birth when the cortisol concentration invariably doubled. Maternal plasma oestrogen rose from 0·35 ± 0·04 ng/ml to 1·20 ± 0·11 ng/ml during the week before parturition. Progesterone concentrations remained stable until a sudden fall 1–2 days before delivery. The slight alterations in maternal plasma cortisol during this period were not statistically significant. The maternal plasma oestrogen levels were higher in the uterine vein than in the periphery, whereas uterine venous progesterone concentrations were significantly lower than in the peripheral circulation.

The hormonal changes associated with the artificial induction of labour were investigated by the administration of cortisol, dexamethasone or corticotrophin to the foetus (four animals). In each case premature delivery occurred 5–6 days later; the maternal hormonal changes were very similar to those in untreated catheterized cows. These findings contrasted sharply with the known, rapid effect of dexamethasone given directly to cows in late pregnancy. This action of dexamethasone was confirmed in two catheterized cows (at 257 and 260 days of gestation); one dose of 25 mg (i.m. to the cow) was sufficient to cause delivery within 40 h. No changes in foetal plasma cortisol concentration occurred until the increase im-
mediately after delivery but the usual maternal hormonal changes were telescoped into a sudden rise in oestrogen and fall in progesterone. The changes in foetal and maternal hormone levels during induced and naturally occurring parturition are discussed in relation to findings in other species.

INTRODUCTION

It is now recognized that the administration of dexamethasone to the pregnant cow can induce premature delivery; a single dose may be effective when given within 20 days of term (Adams, 1969; Adams & Wagner, 1970; Edqvist, Ekman, Gustafsson, Jacobsson, Johansson & Lindell, 1972). This effect is often compared with the premature parturition produced by the administration of cortisol or dexamethasone to the sheep foetus. There is now a considerable amount of evidence which indicates that, in the sheep, enhanced secretion of cortisol from the foetal adrenal plays an integral part in the induction of normal parturition (Bassett & Thorburn, 1969; Liggins, Grieves, Kendall & Knox, 1972; Nathanielsz, Comline, Silver & Paisley, 1972). However, doses of dexamethasone equivalent to those which produce delivery when administered to the foetus do not produce parturition when given to the ewe (Liggins, 1969 a), although large doses are effective (Adams & Wagner, 1970; Fylling, 1971). It may not, therefore, be valid to compare the sheep and cow directly even when the route and dose of administered steroid are comparable. In fact the evidence for the role of corticosteroids in the induction of parturition in the cow is indirect and often conflicting. Genetic cranial defects of the foetus prolong gestation when associated with a low foetal adrenal weight (Holm, Parker & Galligan, 1961); on the other hand the abrupt preparturient adrenal hypertrophy so characteristic of the foetal lamb is not found in the normal calf (Comline & Silver, 1966).

The present experiments were carried out on cows with indwelling foetal and maternal vascular catheters implanted within the last 20–40 days of gestation. This technique enabled hormone changes in foetal and maternal plasma to be followed towards the end of pregnancy and throughout parturition in conscious animals. In addition the hormonal changes associated with the artificial induction of parturition have been compared in both catheterized and non-operated animals. A preliminary account of some of these results has been given previously (Comline, Silver, Nathanielsz & Hall, 1973).

METHODS

Animals

Sixteen Jersey cows (355–457 kg) of known gestational ages were used for implantation of catheters between 240 and 260 days. They were fed hay ad libitum and 1 kg concentrate twice daily. Food but not water was withheld for 24 h before the operation. Procaine penicillin (1 500 000 i.u.) with streptomycin (1 250 000 i.u.; Distavone, Dista Products Ltd) were given i.m. 1 day before and for 3 days after the operation.

Four non-catheterized cows were used for the induction of premature parturition by steroid administration.
Anaesthesia

The majority of animals received 7.5–10 mg acepromazine (Acetylpromazine, Crookes Veterinary Ltd) 90 min before the operation. Anaesthesia was induced by the i.v. injection of 40–50 g chloral hydrate (May & Baker Ltd) diluted in sterile distilled water, followed by 0.5 g methohexitone sodium (Bristol Sodium, Lilly) given immediately before the insertion of auffed endotracheal tube. Intermittent positive pressure ventilation was carried out using a large animal respirator (designed by C. M. Monahan) and to-and-fro carbon dioxide absorption. Anaesthesia was maintained with a mixture of N₂O : O₂ (50 %) and with increments of 200 mg methohexitone sodium when required. Calcium borogluconate (500 ml 40 %, Veterinary Drug Co. Ltd) was given subcutaneously at the end of the operation in most animals. The animals were usually standing within 2 h of the end of the operation and had recovered their normal appetite within 24 h.

Operative procedures

These were adapted from methods which were developed for the sheep (Comline & Silver, 1970, 1972). The uterus was exposed through a paramedial incision immediately dorsal to the mammary vein with the cow on its right side. In the first three experiments a catheter was inserted into the medial or metatarsal vein of the hind limb of the foetus. It was, however, often dislodged by the movement of the foetus and in subsequent animals catheters were inserted into the umbilical vein through a placetome selected near the junction of the uterine horns. Sterile vinyl catheters (o.d. 0.97 mm) with a total length of 2.5–3 m (V3 tubing, Bolab Inc., Derry, New Hampshire) were inserted about 45–50 cm into the umbilical vein and into a large uterine vein through the branch draining the maternal side of the same placetome. Previous tests in anaesthetized preparations had shown that the ends of the catheters were usually within a main vessel of the umbilical cord and the uterine vein when this method was used. Maternal arterial and caval blood samples were taken from catheters inserted into the circumflex iliac artery and vein (Yelverton, Henderson & Dougherty, 1969).

The placetome used for the insertion of intravascular catheters was removed by shelling it away from the foetal membranes and a balloon filled with 2–4 ml 0.9 % (w/v) NaCl solution was then inserted into the allantoic cavity or between the allantois and the uterine wall. Tetracycline hydrochloride (125 mg Achromycin; Lederle) was injected into the foetal fluids before closure of the uterine incision. All catheters were then sealed, threaded through the abdominal cavity, brought out on the flank through a stab wound in the skin and placed in a waterproof bag attached to the animal. The duration of the operation until the final closure of the incision in the abdominal wall was usually about 1.5–2 h.

Maternal arterial blood gas tensions and pH were measured throughout the operation; measurements on umbilical and uterine venous blood were made immediately after insertion of the catheters. Mean blood gas and pH values in the foetus recorded during twelve operations were as follows: pH, 7.31 ± 0.02; P O₂, 41.7 ± 2.8 mmHg; P CO₂, 44.0 ± 2.7 mmHg.
Sampling procedures

After the operation strict aseptic precautions were always observed when samples were taken (see Comline & Silver, 1972). The catheters had a dead space of 1·5–2·0 ml and were regularly flushed with 2·0 ml heparin–saline solution (1000–2000 i.u. heparin in 0·9 % (w/v) NaCl) at operation and after all subsequent routine sampling. When a number of blood samples were taken over a short period the catheters were flushed with a more dilute heparin solution (100 i.u./ml).

Routine observations

In addition to daily blood samples (2–3 ml) which were routinely measured for blood gas tensions, packed cell volume (PCV) and pH, the intra-uterine pressure was measured daily by connecting the saline-filled balloon to a pressure transducer and polygraph. The onset of rhythmical uterine contractions provided the first indication of impending parturition. Once these had begun the intra-uterine pressure recording was continued for long periods and the frequency of sampling was increased to 1 h intervals during the first stages of labour and 15–30 min intervals during the second stage. Umbilical vein samples were taken immediately before delivery; if possible, jugular vein samples were taken from the calf immediately after birth followed by sampling at 15–30 min intervals. Jugular samples were then taken daily from cow and calf for some days after birth.

Premature induction of parturition

This was investigated in four unoperated control animals and six animals with foetal and maternal vascular catheters. Treatment was begun between 250 and 260 days of gestation in all catheterized animals, and between 253 and 268 days in non-catheterized cows.

In four operated animals premature labour was induced by injection into the foetal circulation of cortisol (EFC cortilan; Glaxo), dexamethasone (Decadron; Merck Sharpe & Dohme) or corticotrophin (ACTH, Synacthen; CIBA). Injections were given four times daily at 09.00, 14.00, 18.30 and 23.00 h; details of the doses are given later. The effects of i.m. injections of either dexamethasone or cortisol to the cow were investigated in the remaining six animals; further details are given in the Results.

Field observations

Jugular blood samples were taken from 40 pregnant Jersey cows of known gestational age, ranging from 200 days to term. All samples were chilled immediately and centrifuged when brought into the laboratory. The plasma was used for the estimation of progesterone and total oestrogen concentrations.

Treatment and analysis of samples

$Po_2$, $Pco_2$, pH and PCV were measured on about 0·5 ml blood while the remainder (2·0–2·5 ml) was immediately cooled and centrifuged at 0 °C, and the plasma was stored at –20 °C for hormone estimations.

Total plasma corticosteroids were measured by competitive protein-binding
Parturition in the cow (Murphy, 1967). The sensitivity of the assay was 0.5 μg/sample. Foetal plasma samples (100 μl) were extracted with ethanol and measured directly in the assay without chromatographic separation. Maternal samples, usually 400 μl, were extracted twice with ether to remove progesterone and then treated as the foetal samples. Recovery of added unlabelled cortisol in this system at about 30 ng/ml was 93.7 ± 2.1 (S.E.M.) % (n = 12). No correction was made for recovery in the values given here and all results are expressed as plasma cortisol concentrations. The justification for this procedure was shown in six maternal and foetal plasma samples, in which values for cortisol concentration obtained after LH-20 separation on 55 cm LH-20 columns, using a system described by Malinowska & Nathanielsz (1974), did not differ significantly from those found after simple extraction only. Corrections for losses after chromatography were made by the addition of tracer amounts of labelled cortisol. The percentage of total steroid, measured as cortisol, after column separation was 89.5 ± 3.1 % for maternal plasma and 86.2 ± 3.5 % for foetal plasma. These findings, and the fact that in the newborn calf maximal ACTH stimulation of the adrenal results in a rise in peripheral plasma steroids of which 91% is cortisol (P.W. Nathanielsz, unpublished observations) led us to adopt the simple extraction procedure as a routine method for the detection of changes in plasma cortisol in the present experiments.

Maternal total plasma oestrogens and progesterone were routinely measured in arterial or peripheral venous plasma. No significant differences between the concentrations of either hormone in blood from these two sources were found and, therefore, values from maternal arterial or peripheral venous plasma have been pooled; unless otherwise stated ‘maternal plasma concentrations’ refer to peripheral blood. Oestrogen and progesterone concentrations in uterine venous plasma were estimated in five cows for comparison with corresponding peripheral levels.

Both oestrogen and progesterone were estimated by radioimmunoassay. The antisera used were S 52/5 for oestrogens and S 257/2 for progesterone, kindly provided by Dr G. Abraham (Abraham, 1972; Abraham, Swerdloff, Tulchinsky & Odell, 1971). Plasma was extracted twice with 10 volumes of ether for both oestrogen and progesterone estimations. For progesterone the extract was washed twice with distilled water to remove 17-hydroxyprogesterone.

The major cross-reactions of the oestrogen antiserum are oestradiol-17β 100 %, oestradiol-17α 40 %, and oestrone 35 % (Abraham, 1972). Solvent blanks did not differ from the assay tube containing no oestrogen. The concentrations of oestrogen estimated correspond to total ether-extractable unconjugated immunoreactive oestrogens: the sensitivity of the assay was 10 pg/sample. In a preliminary investigation of hormone concentrations in some of these cows only 100 μl were extracted and assayed for total oestrogen. Oestrogen concentrations in plasma samples containing less than 0.1 ng/ml were therefore not measurable (Comline et al. 1973). Subsequently, up to 400 μl of maternal plasma were extracted from those samples in which a low plasma oestrogen concentration was expected.

The progesterone antiserum cross-reacts with some adrenal steroids (Symons, 1973) but in view of the low maternal plasma concentrations of cortisol, it is very unlikely that any ether-extractable adrenal steroids will contribute to the nanogram quantities of progesterone measured.
The extraction of labelled and unlabelled oestradiol-17β and oestrone was 79% and that for progesterone was 85%. These values were used to correct the plasma concentrations reported.

*Presentation of data in relation to time of parturition*

When data for individual animals during the last week of gestation are given, time 0 in hours refers to the time of delivery and all values, both ante- and post-partum, are plotted on a time scale related to this point. Group means presented more of a problem. Daily samples were always taken between 09.00 and 10.00 h and in all group comparisons means from these samples are used, up to the day before parturition (Day −1). On Day 0, the day of birth, four mean values are generally given; (1) 1–3 h before birth, (2) 5–30 min before birth, (3) 5–15 min after birth, and (4) 1–4 h after birth. When only two Day 0 samples are given they have been calculated from samples taken from 0·5 to 3 h before and 0·5 to 5 h after birth. Obviously the exact time relationship between samples taken on Day 0 and those from Day −1 and Day 1 will vary between animals, depending on the time of delivery. However, the mean time interval between 09.30 h on Day −1 and the time of delivery in the ten catheterized control cows was 25·3 ± 1·6 h.

**RESULTS**

*Assessment of normality of preparation*

**Daily resting values**

In the ten cows used in this study live foetuses were retained until parturition and daily measurements of foetal blood gas tensions, pH and PCV showed that throughout the latter part of gestation conditions in the foetus were stable and there was no significant variation in any of these parameters. Mean daily values for $P_{O_2}$, $P_{CO_2}$ and pH ranged between 38 and 40 mmHg, 43–45 mmHg and 7·384–7·395 respectively. At no stage was there a fall in foetal blood $P_{O_2}$ and pH nor a rise in $P_{CO_2}$ or PCV as term approached.

*Parturition in untreated catheterized animals*

While observations made during late gestation indicated that the pregnant cow and its foetus could provide a stable preparation for investigation, the mean gestational age at parturition in this series of cows was 264 ± 5 days which is outside the 95% confidence limits for normal delivery in this breed (Comline & Silver, 1966). Placental retention was also a feature of all but two of these calvings irrespective of the degree of prematurity. In all other respects parturition appeared to be normal. The first stage of labour lasted 12 h or more and the uterine contractions which began at a low frequency and were small in magnitude (5–10 mmHg), increased up to 20–30 mmHg within an hour or so of birth (Fig. 1). The second stage was very variable in duration and delivery of the calf was in some instances delayed until the cow was left undisturbed and the intra-uterine recording was discontinued.

Live calves were born to all animals investigated in these experiments. In those animals in which frequent blood samples were taken throughout parturition there was no evidence of any foetal hypoxia or acidaemia before birth. Both blood gas
tensions and pH remained stable to within a few minutes of delivery and only in the immediate postnatal period was there a fall in blood pH and a rise in $P_{\text{CO}_2}$; an example is shown in Fig. 2. It was difficult to ascertain the degree of neonatal hypoxia when the umbilical cord was severed, just before respiration was established, because in these animals sampling before birth was normally from the umbilical vein and after birth the only available vessel was the jugular vein.

![Graphs showing changes in uterine pressure during labour in the cow](image)

*Fig. 1. Changes in uterine pressure during labour in the cow, recorded from a balloon in the allantoic cavity, (a) 9½, (b) 2 and (c) 10 min before parturition. FV, umbilical vein.*

The subsequent condition of the calf seemed to depend upon the gestational age. Three calves born at 253, 255 and 261 days of gestation had dyspnoea, a high $P_{\text{CO}_2}$ when measured after birth, and lactacidaemia which appeared to be associated with inefficient distension of the lungs and atelectasis. In the remaining calves born after 260 days of gestation, stable jugular $P_{\text{O}_2}$ values were generally attained within 10–15 min of birth and blood pH was restored by 0·5–1 h in most animals. Subse-
quently these calves suckled normally and gained weight from the day of birth. These findings are similar to those reported for parturition in the sheep (Comline & Silver, 1972).

Fig. 2. Sequential changes in uterine (○) and umbilical (●) venous Po$_2$ in late gestation and during parturition in the cow. Changes in foetal and neonatal blood pH (×) are also shown. The 1st stage of labour (I) was monitored from $-7$ h; the 2nd stage of labour (II) lasted about 2 h. Sampling from the uterine vein was continued after birth and jugular blood samples were taken from the calf after delivery (arrow).

**Hormone levels during gestation and parturition: catheterized animals**

**Plasma cortisol concentrations**

Foetal plasma cortisol concentrations were measured in ten animals. Figure 3 shows mean values obtained from all daily samples taken before and after parturition. Seven animals were sampled for 7–20 days and the remaining animals calved within 5, 4 or 3 days of the operation. Between 240 and 250 days of gestation foetal plasma cortisol levels were often below the limits of the assay (5 ng/ml). In foetuses 10–7 days before birth plasma cortisol levels were about 10 ng/ml but during the week before delivery there was a gradual rise in concentration. The maximum values found in these
foetuses within 1 h of birth (61 ± 11 ng/ml) were significantly lower (P < 0.05) than values found in the newly born calf within 15 min of birth (94 ± 9 ng/ml). In seven calves in which hormone changes during parturition were followed more closely, no significant alterations in plasma cortisol occurred during the second stage of labour but there was invariably an abrupt increase 5 min after delivery. The fall in plasma cortisol concentration 24–48 h after birth confirms previous observations in this species (Nathanielsz et al. 1972).

Maternal (peripheral) plasma cortisol concentrations varied from 5 to 20 ng/ml in different individuals, but there was no consistent change as gestation proceeded and no significant rise before or during parturition in these animals. Mean values for seven cows are given in Fig. 3. The differences between maternal and foetal cortisol concentrations were statistically significant from Day −2 until parturition.

![Figure 3. Mean changes in foetal (●) and maternal (○) plasma cortisol concentrations during the perinatal period in the cow. Vertical bars indicate ± s.e.m. (n = 4–10 foetuses and 4–7 mothers).](image)

**Maternal oestrogen and progesterone concentrations: peripheral plasma**

Mean values for eight cows with indwelling catheters are given in Fig. 4. Both oestrogen and progesterone concentrations varied widely between individual animals during late gestation but in all cows the plasma oestrogen concentration rose within 4–5 days of birth and reached a peak on the day of parturition. In order to compare the time course of the hormonal changes, mean foetal plasma cortisol is also shown in Fig. 4. Within 24 h after delivery the maternal plasma oestrogen concentration had fallen to below 0.1 ng/ml. Little overall change in progesterone levels occurred until within 1–2 days of parturition when the concentration fell precipitously.
Maternal oestrogen and progesterone concentrations: uterine venous plasma

In five animals in which uterine venous samples were routinely obtained the oestrogen and progesterone concentrations in the plasma leaving the uterus were compared with those in the peripheral circulation (Fig. 5). The concentration of oestrogen in uterine venous blood was significantly higher than that in the peripheral plasma during the last week of gestation. The increase in peripheral plasma oestrogen within 4–5 days of birth was closely paralleled by a relatively greater rise in concentration in uterine venous plasma, such that the ratio of peripheral:uterine plasma oestrogen concentration remained between 0.5 and 0.6 during the last 7 days of gestation (0.56 ± 0.20). After parturition there was a further increase in oestrogen concentration in uterine vein samples taken within 15 min of occlusion of the umbilical cord.

The data for progesterone concentrations in the two blood streams present a complete contrast, since the level in the peripheral plasma was consistently higher than that in plasma draining the uterus. The differences in concentration were statistically significant until the day before parturition when progesterone concentrations fell (Fig. 5).
Hormone levels during gestation and at parturition: field observations

The wide range of maternal plasma oestrogen seen in the experimental series, and the fact that the mean gestational age of this group at parturition was 2 weeks earlier than normal, prompted an investigation to compare the hormonal changes in pregnant Jersey cows under field conditions with those in the catheterized animals. Forty cows were sampled at random during late gestation and four of these known to be near term were sampled every 1 or 2 days until parturition occurred.

![Graph showing changes in oestrogen and progesterone concentrations](image)

**Fig. 5.** Mean changes in uterine venous (○) and peripheral (●) plasma oestrogen and progesterone concentrations in five catheterized cows. Vertical lines indicate ± S.E.M.

**Maternal plasma oestrogen concentrations**

The mean values for both field and experimental animals are shown in Fig. 6. Baseline concentrations of 0.15–0.30 ng/ml were found up to 3 weeks before term in the field group. These values were comparable to those found in the experimental animals 10–20 days before delivery. There was however a marked difference between the two groups near term. Up to 9 days before parturition this difference was not statistically significant but thereafter the oestrogen concentrations in the field animals rose continuously to reach a maximum at or even a day or two before parturition. In contrast the values attained in the catheterized cows at the corresponding stages were significantly lower and a most dramatic rise in maternal oestrogen occurred immediately before birth.
Maternal plasma progesterone

There were no significant changes in plasma progesterone in the 8 week period of observation until the day of birth when the concentration dropped suddenly to a mean of $1.6 \pm 0.3$ ng/ml ($n = 6$). The corresponding mean figures for the experimental group were similar at all stages (Fig. 6).

![Graph showing maternal peripheral plasma oestrogen and progesterone concentrations in 40 pregnant Jersey cows sampled between 70 and 1 days before parturition (●, field trial). Means from 3–6 observations; vertical lines indicate ± s.e.m. when $n > 3$. Comparable data for the experimental series are also shown (○). Horizontal lines above the abscissa indicate the gestational age range for each mean.](image)

Artificial induction of parturition

Premature induction of labour in the cow was investigated by three methods. First, cortical hormones were administered to the foetus, secondly endogenous foetal cortisol production was stimulated by the administration of ACTH to the foetus, and thirdly dexamethasone or cortisol was given directly (i.m.) to the mother. Treatment of the catheterized animals was begun on or about 255 days of gestation after a 4–7 day control period to ensure that the animals were in a stable condition.
Administration of cortical hormone to the foetus

In the first experiment an injection of 100 mg cortisol was given four times/day, i.v., to the foetus (gestational age 252 days) until parturition occurred 5½ days later. This dose of cortisol was calculated from the data of Liggins (1969a) who obtained premature induction of labour after 2–3 days in sheep with 25–50 mg/day. In a second cow the effect of the synthetic glucocorticoid, dexamethasone, was investigated (1-25 mg, four times/day, i.v. to the foetus at 259 days of gestation). This dose was comparable, on a weight for weight basis, with the lower dose range used by Liggins (0-1–0-5 mg/day), but in this cow labour also began 5½ days later (at 265 days of gestation).

![Graph](image)

Fig. 7. Changes in maternal peripheral plasma oestrone and progesterone concentrations in two cows (●, ○) in which premature parturition was induced by either cortisol (●) or dexamethasone (○) administered to the foetus. Details of doses are given in the text; horizontal bar indicates duration of treatment.

The changes in maternal plasma oestrone and progesterone in these two animals are shown in Fig. 7. Both the fall in progesterone and rise in oestrone concentrations were within the range for catheterized, untreated cows in the perinatal period. Foetal plasma cortisol values were extremely high in the cortisol-injected foetus (1–10 µg/ml), but these fell to 6–0–18 ng/ml after birth when the injections had been stopped. In the dexamethasone-treated animal foetal plasma cortisol concentrations ranged from 4–13 ng/ml in the 2–3 days before treatment, but foetal plasma samples could not be obtained during the injection period: after birth the characteristic rise in plasma cortisol was absent and the values of 8 and 18 ng/ml obtained 10 and 30 min after birth were entirely comparable to those found in the cortisol-treated calf.
Thus in both animals endogenous adrenal function appeared to have been suppressed by the exogenous steroid. Adrenal weights of the cortisol-treated calf, examined 12 h after delivery, were 850 and 930 mg, i.e. lower than the mean value of \(1.11 \pm 0.09\) g normally found in foetuses of comparable age (Comline & Silver, 1966). The dexamethasone-treated animal was allowed to suckle and after an initial 24 h period of respiratory distress, probably associated with lack of cortisol production, the animal recovered.

![Graph showing changes in foetal plasma cortisol, maternal plasma oestrogen, and maternal plasma progesterone](image)

Fig. 8. Changes in foetal plasma cortisol, maternal plasma oestrogen, and maternal plasma progesterone, in two cows (○, ●) in which premature parturition was induced by ACTH (Synacthen) injections into the foetus. Duration of treatment indicated by horizontal bars; high dose (thick bar); low + high dose (thin + thick bar). Further details are given in the text.
Administration of ACTH to the foetus

Two dose levels of synthetic ACTH (Synacthen) were investigated for their effect on parturition. In both animals the foetuses were injected four times daily; foetus (a) (253 days) with a dose of 0·5 mg/day and foetus (b) (255 days) with 0·25 mg/day for the first 4 days followed by 0·5 mg/day. In both animals parturition occurred after 5½–6 days of treatment. The changes in foetal plasma cortisol and maternal plasma oestrogen and progesterone concentrations are shown in Fig. 8. After parturition induced by ACTH the two calves, although small, were vigorous and showed little obvious signs of immaturity. Their condition contrasted markedly with those delivered after foetal injections of either cortisol or dexamethasone which showed obvious signs of muscular weakness and respiratory distress.

Although the higher dose of ACTH induced a rapid response from the foetal adrenal, shown by the high foetal plasma cortisol levels after 2–3 days of injection, parturition occurred no earlier in this animal than in the other. The maternal plasma oestrogen and progesterone changes in both cows (Fig. 8) were very similar to those observed in the untreated, catheterized animals before parturition.

Administration of dexamethasone to the mother

Reports on the effectiveness of a single i.m. dose of dexamethasone for the premature induction of labour in the cow (Adams, 1969; Adams & Wagner, 1970) led us to investigate the hormonal changes produced in both foetus and mother by such treatment. Dexamethasone (25 mg, i.m.) was given on day 260 and day 257 to two animals which in the previous 7–10 days had low stable foetal plasma cortisol levels. In both cows labour was precipitated 36 and 40 h later. The changes in maternal and foetal hormone levels are shown in Fig. 9. In one animal relatively high peripheral plasma oestrogen concentrations were present before the treatment began, so that the large increase after dexamethasone injection might not have been due solely to this treatment. However, cortisol concentrations in the foetal plasma just before parturition were similar to those found before the dexamethasone injection was given, but immediately after birth a typical rise in cortisol concentration occurred in the calf (Fig. 9).

All three methods for induction of premature parturition in the cow resulted in apparently normal first and second stages of labour as judged both by their duration and by the type and frequency of uterine contractions but in every case the placenta was retained. In the foetus, both during hormone administration and parturition itself, there was no disturbance of acid/base balance, blood gas exchange or PCV. In fact the conditions observed in these foetuses were indistinguishable from those seen in the catheterized control animals (see Fig. 1).

Induction of parturition in four non-operated cows

While the effect of dexamethasone on the artificial induction of parturition in the cow is well known, this does not appear to have been compared with the action of naturally occurring steroids injected into the mother in a similar manner. The effects of doses of cortisol equivalent to or greater than a dose of 25 mg dexamethasone were investigated for their ability to induce premature labour. A single dose of 0·7 g
cortisol (i.m.) did not trigger parturition when given at 268 days of gestation: this cow subsequently calved at 283 days (Fig. 10, I). No changes in maternal plasma progesterone followed the cortisol injection and the gradual rise in oestrogen concentration which began at this time might well have occurred naturally (Fig. 10, I). In a second cow two larger doses of cortisol (1 g/dose, i.m.) were also ineffective when given at 267 and 269 days of gestation and these were accompanied by slight increases in oestrogen levels but no change in progesterone concentrations (Fig. 10, II), although dexamethasone as a single injection (25 mg) given 5 days later (274 days) induced parturition within 30 h. In two other animals the effects of dexamethasone alone (25 mg, i.m.) were investigated. One dose of this hormone did not induce labour although a rise in oestrogen concentration occurred in both cows after injec-

Fig. 9. Changes in foetal plasma cortisol, maternal plasma oestrogen and progesterone concentrations in two cows (○, ●) in which premature parturition was induced by a single dose of dexamethasone (arrow) administered i.m. to the mother.
tion at 253 and 263 days respectively (Fig. 10, III and IV). A second dexamethasone dose was given to one of the animals 5 days later at 268 days of gestation and parturition followed 36 h later with the characteristic abrupt rise in maternal oestrogen and fall in progesterone (Fig. 10, IV).

Fig. 10. Changes in maternal peripheral plasma oestrogen (●) and progesterone (○) concentrations in four non-operated cows (I, II, III and IV) after injection of dexamethasone (D) or cortisone (C). Further details of the doses are given in the text.
DISCUSSION

The endocrine changes in both foetus and mother which precede parturition in the cow are neither as abrupt nor as large as those reported in the sheep or goat. Consequently it is more difficult to draw definitive conclusions from the results of the present experiments and in large part their interpretation depends on a comparison with data from the other species of ruminants.

Foetal changes

The very low concentrations of plasma cortisol found in the foetal calf before 240–250 days were at the limits of the present assay and may well represent a basal secretion from the foetal adrenal cortex with minimum stimulation from the hypothalamo-pituitary system. In fact the values for the calf were very similar to those found in the foetal lamb after hypophysectomy (Nathanielsz et al. 1972). The rise in foetal plasma cortisol which started 6–7 days before birth was, moreover, small and variable in comparison with the values found under similar conditions in the sheep and goat. The concentration rose more rapidly during the first stage of parturition but even then the mean maximum levels in the calf foetus at or within 3 h of birth were 51 ± 5 ng/ml whereas the concentration in foetal sheep or goat plasma at this time may be as high as 250 or 150 ng/ml respectively (Bassett & Thorburn, 1969; Nathanielsz et al. 1972; Thorburn, Nicol, Bassett, Shutt & Cox, 1972). These differences in total plasma cortisol concentrations may well be more apparent than real if there are species differences in plasma binding capacity for cortisol. In the sheep there appears to be a rise in both free and bound plasma cortisol during the last 15 days of gestation (Liggins, Fairclough, Grieves, Kendall & Knox, 1973). Preliminary experiments in the calf suggest that there is no change in the percentage of free (diffusible) cortisol during the last 2 weeks of gestation (P. W. Nathanielsz & A. L. Thomas, unpublished observations); the mean value over the period was 4·36 ± 0·4 % (n = 19).

A further difference between the sheep and calf foetus was observed when premature parturition was induced with exogenous cortisol (or dexamethasone). In the cow 5–6 days of injections into the foetus were required before the onset of parturition, as compared with only 2–3 days of treatment in the foetal sheep (Liggins, 1969a). Even if it is argued that the dosage used in the present experiments was excessive, the injection of ACTH raised the endogenous foetal cortisol values within 2–3 days to pre-partum levels. Yet again, delivery did not occur until 5–6 days after the start of the treatment.

The pituitary-adrenal system of the foetal calves in the present experiments was not apparently stimulated maximally by conditions in utero. The foetal blood Po2, Pco2 and pH values remained stable both before and during parturition until rupture of the cord; the values were similar to those found in the foetal lamb (Comline & Silver, 1972). The sudden increase in the cortisol concentration which occurred immediately after delivery was in marked contrast to the gradual rise during the preceding 6–7 days in utero. Invariably the values obtained within 5 min after birth were double those found in the umbilical blood during labour in calves born as early as 255 days as well as those nearer term. The reasons for this sudden rise in cortisol
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concentration are by no means clear. A possible explanation might be a change in the utilization of cortisol due to loss of the placenta. Alternatively a rapid reaction by the hypothalamo-pituitary-adrenal system to the stimuli at birth could also occur; if so, many factors such as tactile stimuli, temperature, pH or changes in the circulation, to which the newly born calf is exposed might be responsible for the increased cortisol concentration. There is no doubt that with such a highly sensitive system, values found in the newborn animal, even those obtained from the umbilical cord at delivery, may be quite unrepresentative of conditions in utero: this conclusion probably applies to all species in which neonatal plasma cortisol concentrations are highest immediately after birth.

The present evidence on the role of foetal cortisol secretion in the initiation of parturition in the cow seems to be contradictory and less convincing than that in the sheep or goat. On the other hand, prolonged gestation in certain breeds of cattle is associated with hypoplastic foetal adrenals (Holm et al. 1961) and cortisol can, given time, induce delivery when administered directly to the foetus. Whether it acts as the initial trigger to parturition, which is the obvious inference from the elegant experiments in the sheep (Liggins, 1969b), is another matter. Further data on the binding capacity and secretory rate of cortisol may help to answer this question.

The only other species in which the foetus has been examined directly under chronic conditions immediately before parturition is the horse. Here, although the observations were limited, no evidence for a pre-parturient rise in plasma cortisol was found although the absolute levels during the last 7 days of pregnancy (30–58 ng/ml) were higher than those found in foetal calves (Comline et al. 1973).

Maternal changes

In spite of considerable variations between individuals the general differences between the cow and sheep in the endocrine climate towards the end of gestation have become more obvious within the last 2 years. In the cow there is no real counterpart to the abrupt rise in oestrogens from a very low level in the peripheral plasma which occurs in the sheep 24–48 h before parturition (Bedford, Challis, Harrison & Heap, 1972). Instead the maternal peripheral plasma oestrogen concentrations are relatively high during the last quarter of pregnancy and there is a gradual increase during the last 2 weeks so that the final pre-parturient rise occurs against a background of much higher oestrogen levels. As a result, the maximum concentrations found in the cow during labour are about four times those in the sheep at this stage. In contrast to these differences in oestrogen, the peripheral plasma progesterone concentrations are remarkably similar in both species of ruminant and indeed a marked fall in progesterone concentration seems to be a common feature of parturition in many animals (Ash, Challis, Harrison, Heap, Illingworth, Perry & Poyser, 1973). This comparison between the sheep and cow has been made on the basis of total immunoreactive plasma oestrogen and progesterone concentrations. At present there is no information about the possible species differences in the binding of these hormones.

One of the more striking findings in the cow is shown by a comparison of the oestrogen and progesterone in the uterine and the peripheral circulations. In both the cow and sheep (Bedford et al. 1972) the oestrogen concentration in uterine venous plasma
is higher than that found in the peripheral circulation. With progesterone, however, the position is reversed in that the concentration in the uterine vein of the cow is lower than that in the periphery, while in the sheep it is 2–5 times greater. This suggests an apparent uptake of progesterone by the bovine uterus from the peripheral circulation which would seem to confirm previous suggestions (Gorski, Erb, Dickson & Butler, 1958; Erb, Estergreen, Gomes, Plotka & Frost, 1968) that progesterone production during late pregnancy in the cow is not entirely derived from the placenta or even the ovaries. An obvious alternative source is the maternal adrenal cortex which is known, in the cow, to secrete progesterone or at least a compound with very similar properties (Balfour, Comline & Short, 1957). It is possible that the immunoassay used in the present study, although very sensitive, may be unable to distinguish between progesterone itself and close derivatives. Nevertheless, the apparent differences in progesterone concentration of uterine and peripheral blood between the sheep and cow confirm a different role of the placenta as a source of compounds antigenically similar to progesterone, in late pregnancy in the two species.

The possibility that maternal cortisol secretion might also act as a stimulus to parturition does not appear to be supported by the evidence available at present. Peripheral maternal plasma cortisol levels in the cow appear to alter very little close to parturition in contrast to the large changes in oestrogen and progesterone. Adams & Wagner (1970) and Hoffmann, Schams, Giménez, Ender, Herrmann & Karg (1973) both report slight rises in maternal plasma cortisol preceding birth which were similar in magnitude to those found in the present experiments. In the catheterized cows in which maternal as well as foetal cortisol could be estimated from samples taken without disturbance to the animal, the changes over the last few days of pregnancy were not statistically significant.

One of the major questions raised by the present findings is how far the results from the experimental animals reflect the position in the normal animal in its usual environment and in the absence of surgery. The relative prematurity at birth and the high incidence of retained placentae in animals with catheters suggest that premature parturition is liable to follow the insertion of catheters and interference with the uterus. Parturition occurred at a mean gestational age of 278 ± 1.3 days in a random sample of ten cows in the field series and at 264 ± 5 days in the experimental group. In the catheterized animals the pre-parturient changes in maternal oestrogen concentration were smaller and of relatively short duration (4–6 days) in comparison with those found in the normal control cows (9–15 days). The results from the present observations in Jersey cows were similar to those reported for other breeds of cattle (Edqvist, Ekman, Gustafsson & Johansson, 1973; Symons, 1973).

**Action of dexamethasone**

A number of recent reports suggest that the efficacy of dexamethasone in inducing early delivery is not quite as great as originally thought (Adams & Wagner, 1970; Edqvist et al. 1972). However, there is little doubt that premature parturition can be induced provided one, two or more injections are given after 260 days of gestation. The expected rise in oestrogens and fall in progesterone are then telescoped into 48 h if the treatment succeeds; if it does not cause parturition there appears to be little change apart from slight variations in the progesterone concentration, of which the
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fall in peripheral values reported by Schams, Hoffmann, Fischer, Marz & Karg (1972) may be taken as an example. The present findings in both catheterized and non-catheterized animals support these findings.

The mode of action of dexamethasone in the cow is still unknown. An explanation must take into account the finding that the hormone has a rapid and effective action when injected into the cow but appears to be relatively inefficient, as is cortisol, when given to the foetus. It may well be that the placental permeability to dexamethasone is greater than that to cortisol in the maternal to foetal direction. In the sheep the position seems to be virtually reversed; dexamethasone will induce parturition when infused into the foetus for 2–3 days but only very large doses are effective in the ewe (Adams & Wagner, 1970; Fylling, 1971). Evidence from other species is even more conflicting; maternally administered dexamethasone does not induce premature delivery in man (Warren & Cheatum, 1967), monkey (Bosu, Johansson & Gemzell, 1973), horse (Adams & Wagner, 1970) or guinea-pig (Ash et al. 1973) whereas cortisol appears to cause premature parturition in the rabbit when given to the mother or foetus (Nathanielsz, Abel & Smith, 1973). The cow may be unique among all these species in that maintenance of pregnancy, at least during the last 30 days, may in part depend on an extra-uterine-ovarian source of progesterone and it is during this period premature parturition, after 1 or 2 i.m. doses of dexamethasone to the cow, occurs most readily (Adams & Wagner, 1970).

Dexamethasone has been shown to have a depressant effect on cortisol secretion by the adrenal gland of the cow (Adams & Wagner, 1970; Hoffmann et al. 1973) and it is possible that if the cortex is a source of progesterone then this secretion might also be reduced. Direct evidence for a gestational role of the bovine adrenal cortex in late pregnancy is lacking and only further experiments can resolve the question of whether dexamethasone acts specifically on the placenta, or indirectly through the adrenal cortex or elsewhere.

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