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DEVELOPMENT OF FETAL PITUITARY–ADRENAL FUNCTION

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SUMMARY

The factors responsible for the growth of the fetal adrenal, and its increase in cortisol secretion during late pregnancy have been examined in experimental studies in sheep. The fetal pituitary is necessary for these changes to occur. However, the increase in cortisol secretion at term is not preceded by an increase in ACTH in fetal plasma. Prolactin increases in parallel with fetal cortisol. ACTH is elevated in fetal plasma during late pregnancy, despite the demonstration of a negative feedback relationship with cortisol, suggesting an overriding stimulus to ACTH. Comparison of fetal ACTH levels during Synacthen (β1–24 ACTH) infusion into intact and hypophysectomized fetal lambs demonstrates endogenous ACTH release at this time. The possible mechanisms by which negative feedback may be overridden are discussed.

Studies *in vivo* and *in vitro* show that there is a maturation of fetal adrenal sensitivity during late pregnancy. *In vivo* at days 120–130, ACTH provokes little or no increase in the fetal adrenal secretion of cortisol, whereas intra-fetal infusion of prostaglandin E_2, but not $PGF_{2\alpha}$, provokes a rapid 300% increase in the cortisol concentration in fetal plasma. These findings indicate that the fetal adrenal has the pathway necessary for cortisol production by day 130 p.c., and suggest that adrenal maturation is a key factor in the release of cortisol which precedes parturition.

It is now generally accepted that in domestic ruminants, maturation of the fetal hypothalamo-hypophyseal-adrenal axis during late pregnancy is responsible for the onset of parturition and for the development of some of the organ systems necessary for extra-uterine life. In sheep, it has been shown experimentally that removal of the fetal pituitary or fetal adrenal *in utero* leads to prolongation of gestation, whereas infusion of ACTH or cortisol to the fetal lamb causes premature labour [see 1–3]. Comparable infusions to the mother, or the infusion of mineralocorticoids to the fetus do not provoke premature parturition. However, whilst it is accepted that maturation of the fetal pituitary-adrenal axis leads to parturition, the rate-limiting factors for this process have not been identified.

In this review we examine some of the factors responsible for the increase in size and steroidogenic activity of the fetal adrenal during late pregnancy. In particular, we shall consider whether the changes in fetal cortisol production before birth are due to either an alteration in the trophic stimulus applied to the fetal adrenal gland, or to 'inherent' maturational changes in the adrenal's responsiveness to trophic hormones. The present discussion will be largely confined to studies using the sheep. It should be remembered that in primates there is a greater degree of transplacental transfer of cortisol from mother to fetus, and the fetus may be less autonomous with respect to its regulation of cortisol production [see 1, 2].

IS THERE A CHANGE IN TROPHIC DRIVE TO THE FETAL ADRENAL GLAND IN LATE PREGNANCY?

Measurements of cortisol in blood samples taken daily from chronically catheterized fetal lambs show a progressive increase from basal values of 10–20 ng/ml at 25 days *prepartum*, to values of 20–50 ng/ml by 7–15 days *prepartum*, followed by a more rapid increase to 150—greater than 200 ng/ml during the last 1–2 days of intrauterine life [4–7]. The predominant corticosteroid in the plasma of the fetal lamb is cortisol, with smaller quantities of corticosterone. The major increase during late pregnancy is in cortisol. Cortisone and 11-deoxycortisol have been detected in only small amounts in fetal lamb plasma [7, 8]. The changes in fetal plasma cortisol concentration largely reflect an increase in the production rate of cortisol by the fetal adrenal gland [3–5], although there is also an increase in the cortisol binding capacity of fetal plasma [9]. The increase in cortisol at term is dependent on an intact fetal pituitary. However, at earlier times in pregnancy, the concentration of cortisol in the plasma of hypophysectomized fetuses is not consistently different from that

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in unoperated fetuses and the necessity for an intact pituitary at these times has not been established.

Most of the above measurements have been made on samples of fetal plasma, generally taken at a similar time each day during the weeks before parturition. More recent studies have indicated that this sampling regime may be inadequate, because the fetal plasma cortisol concentrations may vary during 24 h periods; the values at night generally being somewhat higher than those during the day [10]. In addition, in individual animals, there appear to be occasional random increases in fetal cortisol [10, 11], which are not necessarily related temporally to fetal plasma ACTH.

Early measurements of ACTH in fetal lambs, and estimates in the human may have suffered from the failure to recognise that this peptide is readily released in response to a variety of stimuli, including hypoxaemia (see below). The values must be interpreted with caution. More recent estimates have shown that ACTH levels in chronically catheterized fetal lambs are generally lower than 50–200 pg/ml up to 15–20 days *prepartum* and do not increase before the parturient rise in cortisol [8, 12]. Thus an increase in the circulating ACTH concentration *per se*, at least as measured by current radioimmunoassay and biological assay procedures, would not appear to be responsible for the initial stimulus to the preparturient increase in fetal plasma cortisol (Fig. 1). The highest concentrations of ACTH in fetal plasma are found during the last 1–2 days of intrauterine life [8, 12], at a time when cortisol is elevated. The high ACTH concentrations may be associated with the stress of labour [13].

ACTH in the plasma of the fetal lamb is probably derived largely from the fetal pituitary, and the concentration of ACTH in fetal plasma falls to undetectable levels after fetal hypophysectomy or fetal pituitary stalk section [10, 14]. Despite the evidence for various forms of corticotrophin in the fetal pituitary and plasma the quantitative importance of these in fetal blood compared to maternal blood remains to be established [11]. The rate of conversion of the less active to the more active ACTH forms is slower in fetal than in maternal blood [15]. In sheep, there is little evidence for the transplacental transfer of [^{125}I]-ACTH from mother to fetus [16], nor is there yet evidence for a placental source of ACTH, as in man [17, 18].

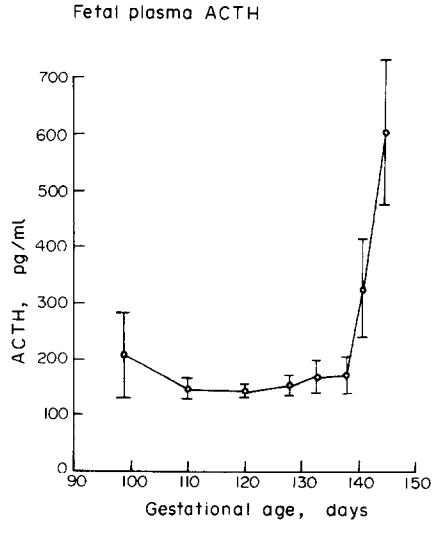

Fig. 1. The concentration of ACTH in the carotid arterial plasma of fetal sheep during the latter half of pregnancy. Carotid arterial blood samples were collected from 42 undisturbed fetal sheep with chronically implanted vascular catheters. Blood samples were collected daily for up to 4 days, and at least 7 days after surgery. The results are expressed as means ± S.E.M.

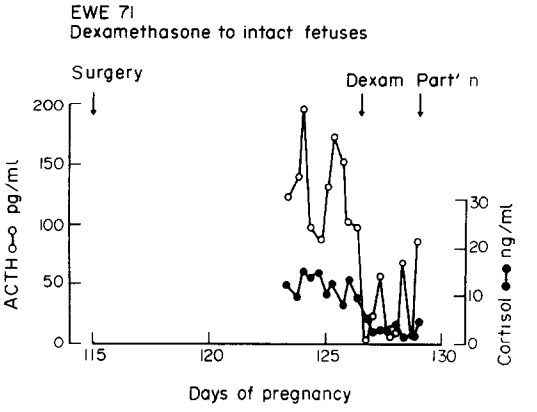

Fig. 2. The changes in the concentrations of cortisol (●—●) and ACTH (○—○) in fetal plasma during the continuous intrafetal infusion of dexamethasone. Fetal vascular catheters were implanted on day 115 p.c. Dexamethasone infusion (1 mg/24 h) was started on day 126 p.c., and premature delivery occurred 45 h later.

FEEDBACK RELATIONS BETWEEN ACTH AND CORTISOL IN THE FETAL LAMB *IN UTERO*

The parallel increase in ACTH and cortisol in fetal lamb plasma at term is of interest, because it would appear to defy the general concept of negative feedback, and would imply some alternative regulatory mechanism in the fetus. However, negative feedback clearly does operate in the fetal lamb during late pregnancy. The intra-fetal infusion of dexamethasone at amounts which precipitate premature labour (1 mg per 24 h infusion) results in a progressive and significant decrease in the concentrations of both ACTH and cortisol in fetal plasma (Fig. 2). Administration of pharmacological amounts of cortisol (1 mg/ml) as a single bolus injection intra-arterially to the fetus, lowers the ACTH concentration in fetal plasma within 15 min [19], but at physiological amounts of cortisol the suppression is not complete [10]. It would therefore appear that there is some mechanism for increasing pituitary ACTH release in late pregnancy, and overcoming the normal negative feedback mechanism exerted by cortisol. This possibility has been examined as follows.

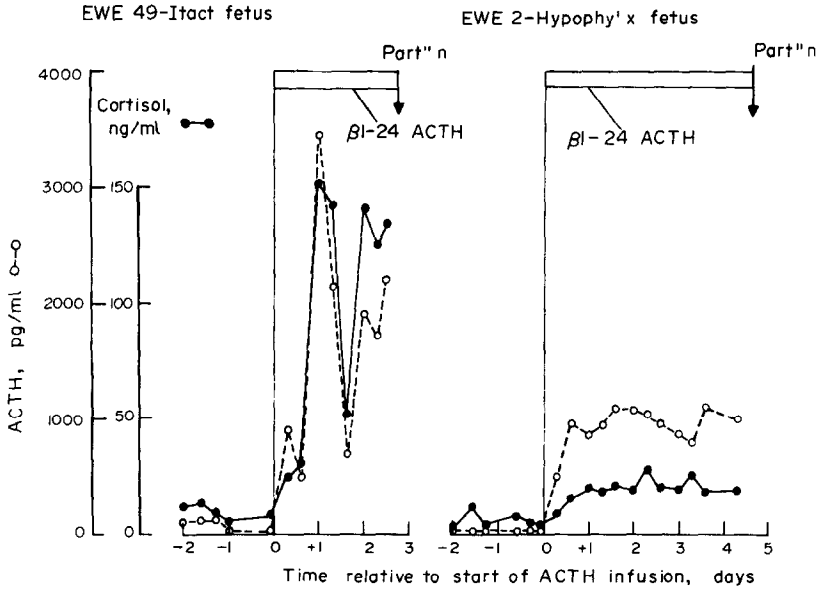

Fig. 3. Representative animals to show the effects of continuous Synacthen (β1–24 ACTH) infusion into an intact and hypophysectomized fetus on the fetal plasma concentrations of ACTH (o—o) and cortisol (●—●). Note the large fluctuations in these hormones measured in the plasma of the intact fetus compared to the more stable levels in the hypophysectomized fetus.

Synacthen (β1–24 ACTH) was infused intravenously at a constant rate into 4 intact and 4 hypophysectomized fetal lambs. ACTH was subsequently measured in fetal plasma in a radioimmunoassay system employing an NH_2-terminal antibody, which would detect both endogenously released, and exogenously infused ACTH. The ACTH concentrations were also measured by bioassay, in which the capacity to stimulate cortisol secretion by isolated guinea-pig adrenal cortical cells was determined. During the infusion of Synacthen into the intact fetus, the ACTH concentration in the fetal plasma rose, but fluctuated considerably at concentrations between 500 and 3000 pg/ml, despite the constant rate of infusion (Fig. 3). However, during Synacthen infusion to hypophysectomized fetuses, lower (500–1000 pg/ml) concentrations of ACTH were measured in samples of fetal plasma, and these values remained relatively constant throughout the infusion. (Fig. 3)[20]. These data indicated that endogenous ACTH is released during the administration of Synacthen to the intact fetus, and this release may resemble that seen at normal term. Whether there is a direct positive effect of Synacthen on endogenous ACTH release, or whether the positive factor relates to other stimuli to ACTH release, remains to be ascertained.

It is of note that during the infusion of Synacthen to hypophysectomized fetuses, there was an increase in the concentration of cortisol in fetal plasma, but only to values of 20–40 ng/ml. These are similar to the concentrations found before the *prepartum* cortisol surge in intact normal fetuses, and only 10–20% of the values achieved during Synacthen infusion to intact fetuses. Despite these low cortisol concentrations delivery of the hypophysectomised fetus occurred [14]. During Synacthen infusion, the percentage of bound cortisol is similar in the plasma of hypophysectomized and intact fetuses, thus the absolute concentration of free cortisol is much lower in the former group. Whether the ability to induce delivery with these very low cortisol levels relates to the absence of other pituitary hormones is an intriguing question.

One stimulus to ACTH release could be vasopressin (AVP), which acts as a corticotrophin-releasing hormone *in vitro* and *in vivo* [21]. The concentration of vasopressin in the plasma of the fetal sheep increases during late pregnancy [22] and the peptide is released in response to hypoxaemia [23]. Infusions of vasopressin to fetal lambs provoked a significant increase in the fetal plasma ACTH concentration [24], but the increment in ACTH was less than that achieved during fetal hypoxaemia [25]. Because it was possible to block the systemic rise in AVP in response to hypoxaemia without changing the rise in ACTH [24] it has been argued that circulating AVP may not be involved in the stimulation of ACTH release. However, in view of the proposed direct pathway from the paraventricular and supra-optic nuclei to the hypophyseal tract [26] the relationship between peripheral levels of AVP and ACTH release remains to be elucidated.

The possibility that trophic hormones other than ACTH (or its fragments, including αMSH) might have a role in stimulating fetal adrenal function is also worthy of consideration. In man, Winters *et al.* [27] have demonstrated a relationship between fetal plasma prolactin concentrations, and the increase in

total weight of the fetal adrenal gland during pregnancy. They have argued that prolactin, oestrogen and ACTH may interact in controlling the fetal adrenal cortex. In the fetal sheep, a significant correlation (coefficient of correlation = 0.90) has been demonstrated between the mean concentrations of prolactin and cortisol in plasma from chronically catheterized fetuses during the last 30 days of gestation [28]. Prolactin became consistently detectable in fetal plasma for the first time between days 109 and 115 of gestation. Its concentration increased significantly to term, reaching a mean maximum value of about 40 ng/ml. The sheep placenta is relatively impermeable to prolactin, and in hypophysectomized sheep fetuses the prolactin concentration remained low (< 3 ng/ml) during the period 120–145 days, suggesting that there is little transfer of prolactin from the mother to fetus.

After fetal pituitary stalk section, the concentration of ACTH in fetal plasma decreased to undetectable values, but prolactin was still present [10]. The concentrations of both hormones fell after fetal hypophysectomy [10, 14]. After hypophysectomy, the fetal adrenal atrophies, but this does not occur after stalk-section [10], perhaps suggesting a role for prolactin in the growth of the fetal adrenal. It is of interest that in intact fetuses, prolactin release was not significantly stimulated by the intra-fetal infusion of PGE_2 or $PGF_{2\alpha}$ at 125–130 days or at term ([29] see below).

The possibility that other pituitary hormones may influence fetal pituitary-adrenal function requires consideration, particularly in the interpretation of hypophysectomy experiments. For example, GH has been shown to have both short-term and long-term effects on the steroidogenic actions of ACTH [30]. In this regard, the high levels of GH in the plasma of the fetal lamb may be of importance [31].

MATURATION OF THE FETAL ADRENAL RESPONSE

In vitro incubation or perifusion studies with adrenal tissue from fetal lambs have suggested that the increase in cortisol secretion by the fetal adrenal may be associated with the induction of 17α-hydroxylase and 11β-hydroxylase activities, possibly under the influence of ACTH (32–34). Thus in a perifusion system, the mean secretion of corticosteroids by sheep fetal adrenal tissue in response to a fixed amount of synthetic (β1–24) ACTH increased with gestational age. There was also an increase in the ratio of cortisol:corticosterone produced [32]. This 'maturational' change in the ACTH-induced steroidogenic response parallels the increase in fetal adrenal weight, and the fetal plasma cortisol concentration *in vivo* [34, 35]. Other incubation studies with adrenals taken from sheep fetuses at day 110–140 of gestation suggested that 11-deoxycortisol was the major steroid formed from radioactive pregnenolone or progesterone. More cortisol was produced with adrenals taken from term fetuses, or after the intrafetal infusion of ACTH, implying an induction of 11β-hydroxylating activity [32, 33]. However, quantitative interpretation of *in vitro* experiments with tissue homogenates is fraught with problems such as satisfactory maintenance of enzyme co-factors requirements, and, in contrast, Davies and Ryan [36] have demonstrated significant 11β and 17α-hydroxylase activity in fetal adrenal tissue taken from lambs as early as day 100 of gestation.

A maturational change of some nature in the fetal adrenal was implicit from the *in vivo* studies of Bassett and Thorburn [35]. These workers showed that the short term intravenous infusion of Synacthen (10 μg/h for 2 h) into fetal lambs before 140 days of gestation, failed to increase the fetal plasma corticosteroid concentration appreciably, whereas in later gestation, the response increased. Similar observations have been made by Liggins *et al.* [37] who measured the increment in the fetal plasma cortisol concentration *in vivo* in response to an intrafetal infusion of Synacthen (10 μg/min for 60 min) at various times during the last 60 days of pregnancy. These workers made the further interesting observation that the increase in cortisol in response to ACTH was reduced in fetal lambs during the final 24 h before delivery, suggesting that at term the fetal adrenal may be producing cortisol at near its maximum secretion rate.

The change in fetal adrenal sensitivity to endogenous ACTH has been examined by comparing the increment in fetal ACTH and cortisol in response to experimentally induced fetal hypoxaemia over a period of 1 h [8, 25] (Fig. 4). Fetal hypoxaemia is a potent stimulus to the release of ACTH from the fetal pituitary (maternal ACTH does not cross the ovine placenta to the fetal side) [16], and a mean increase of 5–10 fold over basal levels may be achieved. Between day 100–135 of gestation, the increase in fetal plasma ACTH stimulates only a small rise in fetal plasma cortisol, whereas a smaller increment in ACTH in the mother produces a much larger increment in cortisol [8, 25]. After day 135 of gestation, fetal hypoxaemia provokes a similar increase in the concentration of fetal ACTH, but at this stage of pregnancy there is a marked increase in the release of cortisol from the fetal adrenal gland, implying a change in its 'sensitivity' to trophic stimulation.

These studies support the concept that in the sheep during late pregnancy there is a maturation of the fetal adrenal secretory response to ACTH. The relative insensitivity of the fetal adrenal at earlier stages of pregnancy may be of importance as a protective mechanism against premature labour. The maturational changes occurring before the onset of spontaneous delivery require the presence of ACTH as a permissive or trophic agent.

The *in vitro* studies of Madill and Bassett [34] and Bassett and Thorburn [35] indicated that whilst the maximum sensitivity of the fetal adrenal to ACTH occurred around the time of birth, there was a de-

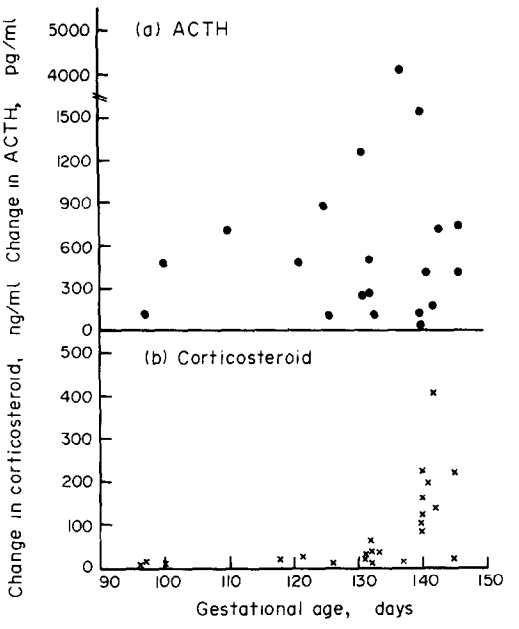

Fig. 4. The hypoxaemia-induced changes in the concentration of ACTH and corticosteroid in plasma of fetal sheep during the latter half of pregnancy. Twenty six pregnant sheep with chronically implanted fetal and maternal vascular catheters were given 9%O_2 and 3%CO_2 in N_2 to breathe for 60 min. The changes in hormone concentration represent the difference between the control concentration and that seen after 60 min of hypoxaemia.

crease in this response during the *postpartum* period. We have investigated this possibility further by injecting hyperphysiological amounts of Synacthen (100 μg) as a bolus to newborn lambs at 4 h, 7 days, 1–2 months of life, and to adult ewes. The results are summarized in Fig. 5. At four hours of life, 100 μg Synacthen provokes a relatively small increase in the mean fetal plasma cortisol concentration, consistent with the suggestion that the fetal adrenal may be secreting cortisol at close to its full capacity in the immediate *post-partum* period. At 7 days or 1–2 months the response in plasma cortisol resembles more that seen in the adult ewe both quantitatively and qualitatively. It is of interest that at 4 h the rate of disappearance of cortisol from the vascular compartment is somewhat slower than that seen in older lambs. This may be due to the increase in the corticosteroid-binding-globulin binding capacity in the plasma of the fetal lamb during late pregnancy [9], CBG production appears to decrease after birth to the low levels found in the adult [see 38].

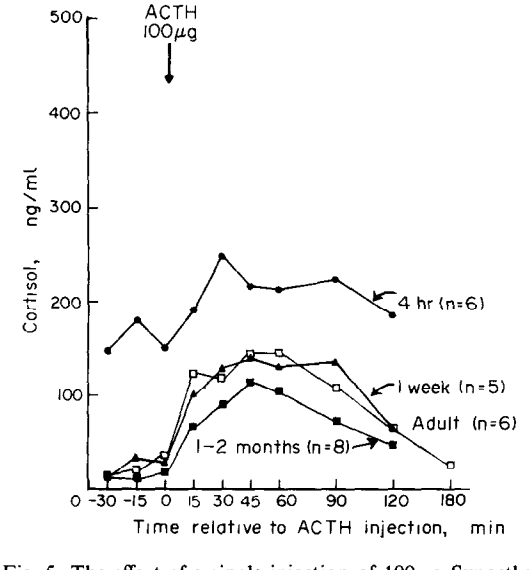

Fig. 5. The effect of a single injection of 100 μg Synacthen (β 1–24 ACTH) i.v. at time 0 on the cortisol concentration in the plasma of lambs at 4 h (●—●), 1 week (▲—▲) and 1–2 months (■—■) of life, and adult sheep (□—□). The values are means for the numbers of experiments indicated in each group. For clarity S.E.M.'s have not been drawn.

THE EFFECT OF INTRAFETAL PROSTAGLANDINS ON FETAL PLASMA CORTICOSTEROIDS

Whilst the above studies point to the importance of maturational changes in fetal pituitary adrenal function during late pregnancy, the rate limiting factors of this process have not been identified. In adult animals, prostaglandins (and especially PGE_2) are found in the adrenal gland and are known to increase adrenal steroid production and release *in vitro* [39, 40]. In adult feline adrenocortical cells it has been suggested that prostaglandins may mediate the effects of ACTH on steroidogenesis [40, 41]. We have found an increase in the concentration of primary prostaglandins, especially PGE_2, in the plasma of fetal lambs during late pregnancy [6], and therefore examined the possibility that prostaglandins might influence fetal adrenal production of corticosteroids by infusing PGE_2 and $PGF_{2\alpha}$ into fetal lambs on days 125–130 of pregnancy. At this time of gestation, the fetal adrenal does not respond or responds only poorly to exogenous ACTH [42].

Whereas PGE_2 provoked a 3-fold increase in fetal plasma cortisol within 30 min, a similar amount of $PGF_{2\alpha}$ did not influence the concentration of cortisol in fetal plasma (Fig. 6). Thus the response was not due to the sampling protocol. There was no change in the fetal blood pO_2, pCO_2, arterial pressure, fetal heart rate, haematocrit or plasma prolactin concentrations attributable to the prostaglandin during either PGE_2 or $PGF_{2\alpha}$ administration. PGE_2 infusion also increased fetal cortisol levels in sheep in labour, although the percentage increase was lower than at 125–130 days. This is consistent with the earlier observations on the effects of ACTH administration to fetal lambs in labour. These results suggest that there may be a specific response of the fetal adrenal to PGE_2, at a time when the fetal adrenal does not respond to ACTH, raising the possibility that the capacity to produce prostaglandins may be associated with maturation of the fetal adrenal gland. At present,

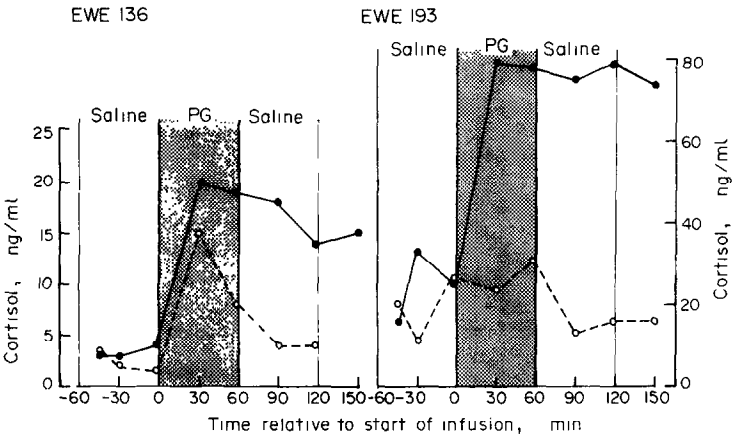

Fig. 6. The change in fetal plasma cortisol concentration during the infusion of PGE_2 (●—●; 1.6 μg/min) and $PGF_{2\alpha}$ (○—○; 1.6 μg/min) into the carotid artery of two representative fetal lambs between 125 and 130 days of gestation. Saline was infused at the same rate (0.156 ml/min) during the h before, and the h after the prostaglandin infusion.

it is difficult to say conclusively whether the effects of ACTH and PGE_2 on adrenal function are exerted through separate mechanisms or a common pathway. However, these studies indicate that the adrenal steroid biosynthetic pathway is functional, and other interpretations are required to explain the insensitivity of the fetal adrenal gland to ACTH.

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DISCUSSION

Naftolin. Because blood levels may be ignoring some very important metabolism at tissue levels can we assume that the blood levels are sufficient or should we be doing production rates?

Challis. With respect to the cortisol concentrations in fetal sheep blood, clearance rates have been measured, and the production rates calculated using continuous infusion isotope kinetics techniques. When one looks at changes in fetal plasma cortisol levels, one is looking largely at an increase in the secretion rate of cortisol by the fetal adrenal gland itself, although there is also an increase in the concentration of corticosteroid binding globulin in fetal sheep plasma during the last seven days or so of the pregnancy.

Naftolin. And the things you showed us today?

Challis. Those increases in cortisol concentrations reflect an increase in the production rate of cortisol by the fetal adrenal gland and that I think is now fairly well established. There are some data appearing on the metabolic clearance rate of ACTH in the fetus. There are differences depending on which group you follow, because of the problems I referred to, and the type of experiment that is being done. In fact, we just do not know enough about the way that ACTH is being released in the fetus. Certainly it is our feeling that with daily samples or twice daily samples one could be missing significant changes in ACTH if it is released in little pulses or with a marked diurnal rhythm.

Swaab. I should like to make a short comment on your hypothesis that prolactin might be an adrenotropic hormone. We have measured prolactin in anencephalics and normal controls, in amniotic fluid and in cord blood. The levels in both groups were similar (Honnebier, 1974) while the fetal adrenal is aplastic in anencephaly. So prolactin seems not of great importance as an adrenotropic factor in the fetus.

Challis. What I showed was a temporal relationship between prolactin and cortisol. We know there are published data demonstrating prolactin receptors in the adult sheep adrenal; we do not yet know whether there are prolactin receptors in the fetal sheep adrenal. The only other piece of evidence we have, is that when one hypophysectomizes the sheep fetus, the prolactin concentrations decrease to levels below the sensitivity of assay (<0.5 ng/ml) and the fetal adrenal atrophies. At present we have not established, nor are we stating, that a cause and effect relation-

ship exists between the prolactin and the cortisol production. All I wanted to try to do was to stress that we should not think of the maturation of fetal adrenal function simply in terms of a relationship between ACTH and cortisol. There are a lot of other trophic hormones that could be involved. We have talked about some of them, such as CLIP, αMSH, growth hormone, etc.

Naftolin. Let us not forget Dr Challis's important caveat. The sheep is an animal which doesn't have the same adrenocortical anatomy as humans. We had better not go back and forth too easily.

Challis. Can I make just one other point. In the sheep at term, there does not appear to be the sort of feto-placental unit for estrogen biosynthesis that has been described in primates. This is really a terribly important difference between the sheep model on the one hand and the other models about which we shall be hearing later.

Silman. We have recently looked at sheep fetal pituitary to try and identify the fetal ACTH, and it does appear to be different to the adult and there also does appear to be a change over as you approach term. The unfortunate thing is that both these forms of ACTH would be picked up by the immunoassay. Certainly the immunoassay would not have distinguished the two so really if one is getting a change in ACTH accompanying that early rise in cortisol it would not have been picked up by any of our immunoassays at the moment.

Challis. We are aware of those reservations. We should like to have seen some changes in "ACTH", but one can only present the data that one has, and develop assays for the other peptides.

Tabei. In relation to the trigger of labour, you pointed out the fetal adrenal function. I also noticed that maternal progesterone level was going down with gestational age which was prevented by fetal hypophysectomy. How do you explain the correlation of this phenomena and the fetal adrenal function?

Challis. In the sheep, fetal cortisol appears to induce or activate in the placenta both 17α hydroxylase and 17–20 lyase activities. Normally the sheep placenta metabolizes progesterone to 20α-dihydro progesterone because it has a very active 20α hydroxy steroid dehydrogenase activity. With the additional 17α-hydroxylase activity, one finds the formation of a further metabolite, 17α, 20α-dihydroxy progesterone. This has been demonstrated *in vitro* and *in vivo*. In sheep prepared with chronic utero-ovarian vein catheters, there is an increase in the 17α 20α-dihydroxy progesterone levels which coincides with the fall in progesterone. We believe that this is perhaps the way fetal cortisol brings about the decrease in progesterone.

Tabei. Does this mean that these placental enzymes are controlled by fetal pituitary gland to decrease the progesterone level?

Challis. Well, indirectly, by the fetal pituitary, yes.