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Biochemical modifications involved in the maturation of the ovine fetal adrenal gland in late gestation : their modalities and regulation.

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Summary. During the last month of intra-uterine life, the steroidogenic response of the ovine fetal adrenal glands to ACTH increases and becomes maximal at the time of birth. This development involves modifications at different steps of the adrenocorticotrophic hormone (ACTH) action mechanism. It has been shown that the enhanced capacity of the cells to produce cAMP is related to at least three factors : an increased number of ACTH receptors, increased activity of the α subunit of adenylate cyclase, and enhancement of guanosine 5'-triphosphate (GTP) availability. The ability to produce pregnenolone and the activity of both 3β -hydroxy steroid dehydrogenase/isomerase and 17α -hydroxylase are mainly enhanced in the steroidogenic pathway. The infusion of ACTH for 5 days into 115 to 120-day old fetuses results in the development of most of these biochemical process. Similarly, ACTH can induce maturation of cultured fetal adrenal cells and some other proopiomelanocortin (POMC)-derived peptides can potentiate its acute steroidogenic activity *in vitro*. However, even in the absence of ACTH, the adenylate cyclase system and the steroidogenic potency of cultured cells increase but to a lesser extent than when ACTH is present in the culture medium. It is suggested that ACTH is the main trophic hormone of the ovine fetal adrenal during the last month of gestation, even if other stimulatory factors may also be important. The *in vivo* maturation of ovine fetal adrenal is blocked by the presence of some unknown inhibitory factors in the fetal circulation which are of likely extrapituitary origin.

Introduction

During the last 3 weeks of intrauterine life, the weight of the ovine fetal adrenals increases strikingly (Comline and Silver, 1961). This results mainly from the growth of the cortical zona and expresses both cellular hyperplasia and hypertrophy (Durand *et al.*, 1978 ; Robinson *et al.*, 1979). The steroidogenic response of fetal adrenal glands to adrenocorticotrophic hormone (ACTH) develops simultaneously (Basset and Thorburn, 1973 ; Madill and Basset, 1973 ; Wintour *et al.*, 1975) and becomes maximal at the time of delivery. In this survey, attention will be focused on results from our laboratories regarding the biochemical steps involved in the enhancement of ovine fetal adrenal cell responsiveness to ACTH between 115 to 120 days of gestation and birth (145 ± 1 day in our experiments).

Moreover, since ACTH infused into the ovine fetus for several days is able to make the adrenal cells responsive to this hormone, at a time when they are normally unresponsive (Liggins, 1968), we can report the effect of such a treatment on the enzyme activities we have studied during spontaneous maturation. Finally, recent data on the *in vitro* effects of proopiomelanocortin (POMC)-derived peptides or of pituitary extracts and of fetal hypophysectomy on the ability of fetal adrenal cells to produce both cAMP and corticosteroids *in vitro* are presented.

1. Development of the ACTH-receptor adenylate cyclase system.

The number of ACTH receptors and the adenylate cyclase (AC) activity of adrenal crude membranes from ovine fetuses and newborn lambs is shown in figure 1. The number of ACTH binding sites remains low until 140 days of gestation and increases 3-fold during the last 5 days of gestation. At 124 days,

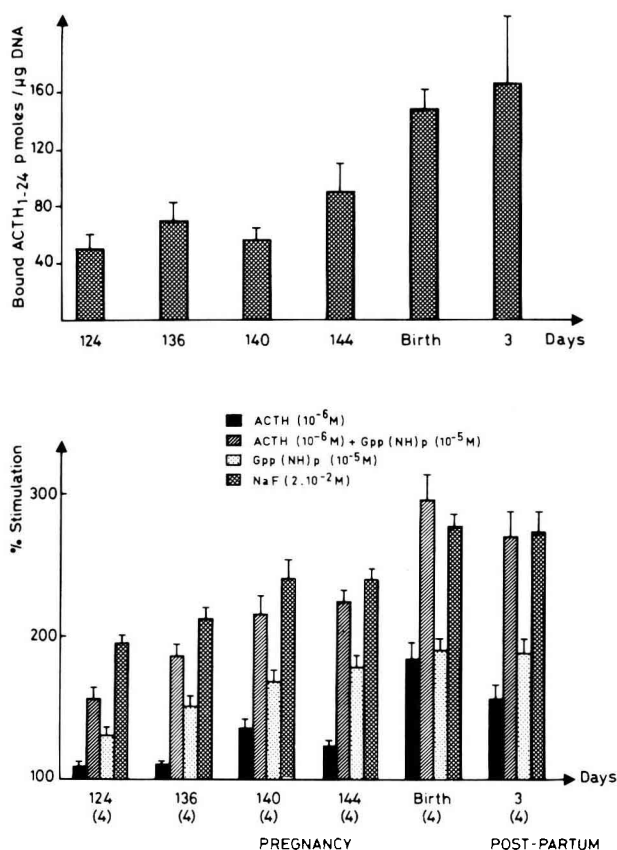


FIG. 1. — ACTH binding (top) and adenylate cyclase (bottom) activities in crude membranes prepared from ovine fetuses at late gestation and from newborn lambs. Results of adenylate cyclase activity are expressed as the percentage of stimulation over basal activity (from Durand *et al.*, 1981b).

ACTH (1-24) alone cannot increase AC activity, while the addition of a guanosine 5'-triphosphate (GTP) analog to ACTH (1-24) results in a significantly higher response than that observed with Gpp (NH)p alone. However, although there is no increase in the number of ACTH receptors at 140 days, the AC responds to ACTH (1-24) alone. Hence, the relative insensitivity of AC before 140 days of gestation is related primarily to a defect in GTP availability. Secondly, since the response to Gpp (NH)p alone or NaF is lower on day 124 than on day 140, the activity of the catalytic subunit of the cyclase (subunit C) must be lower and/or the guanine nucleotide-binding regulatory components (subunit Ns and/or Ni) altered. Conversely, the increasing responsiveness to ACTH (1-24) observed between day 140 of gestation and birth correlates closely with the concomitant increase in the number of ACTH receptors since during this period the response to both Gpp (NH)p and NaF remains unchanged. In order to determine which subunit (s) of the AC system was responsible for the enhancement of the response to Gpp (NH)p or NaF, we recently carried out « reconstitution » experiments (Durand *et al.*, 1985b). In these experiments, human erythrocyte membranes were used as a source of Ns (Johnson *et al.*, 1978). The possible involvement of a Ni subunit was investigated by studying the effects of high GTP concentrations on both the basal and forskolin-stimulated AC activity of fetal and neonatal membranes. The addition of erythrocyte ghosts to fetal adrenal membranes enhanced NaF or Gpp (NH)p stimulation of AC which became similar to that observed in neonate membranes. On the other hand, AC activity displayed a biphasic response to GTP, and the magnitude of the inhibition achieved with 10^{-3} M GTP was similar in fetal and neonatal adrenal membranes. These data show that the « maturation » of the AC system of the ovine fetal adrenal gland involves enhancement of the number of ACTH receptors and development of the Ns subunit, whereas the Ni and C subunits are hardly, if at all, involved in this process. Figure 2 shows the *in vitro* production of cAMP and corticosteroids by

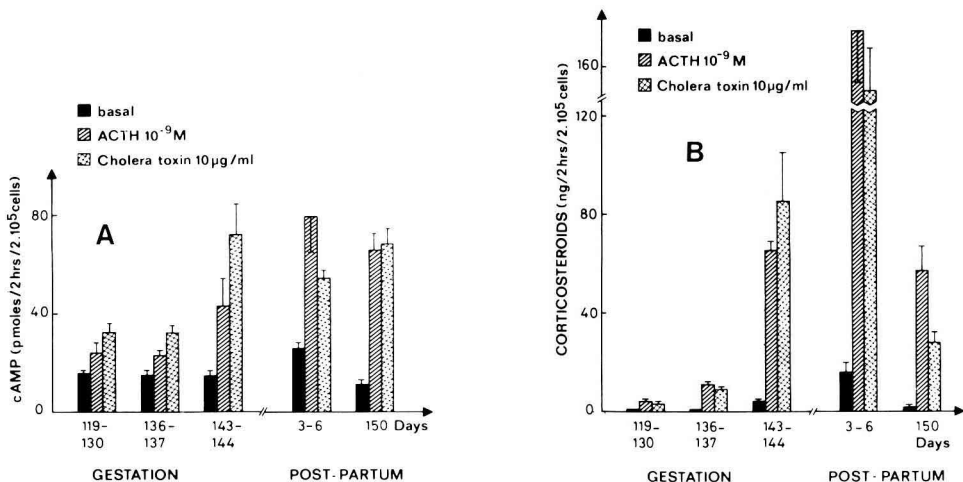


FIG. 2. — Effect of ACTH-(1-24) (10^{-9}) or cholera toxin ($10 \mu\text{g/ml}$) on cAMP (A) and corticosteroid (B) production by adrenal cells from fetal, neonatal and adult sheep (from Durand *et al.*, 1981a).

isolated adrenal cells from ovine fetuses and lambs. The cAMP response to ACTH (1–24) remains low until 137 days of gestation then increases to reach a peak in newborn lambs. Response to cholera toxin is also developed during the same period, but in that case, maximal stimulation was already obtained in prepartum animals. It can be concluded from the preceding results that the relative unresponsiveness of the fetal adrenal to the steroidogenic effect of ACTH is at least partly related to a defect in the amount of ACTH-induced cAMP production. Such a conclusion has received support from Manchester and Challis (1982) who observed that cAMP, but neither Gpp(NH)p nor ACTH, could stimulate progesterone production by adrenal cells from 130-day old ovine fetuses, whereas those three factors were equipotent with cells from term animals. However, figure 2 shows that the increasing stimulation of steroidogenesis by both ACTH (1-24) and cholera toxin, which occurred between 119-130 and 136-137 days of gestation, was achieved without any detectable change in cAMP output. Also, cholera toxin at 143 days fully stimulated a response in cAMP but not in steroidogenesis. Therefore, modifications beyond cAMP must be involved in the maturation process.

2. Modification of the steroidogenic pathway

The *in vitro* metabolism of labelled pregnenolone by isolated adrenal cells from 120-day old ovine fetuses and from newborn lambs is shown in figure 3. Newborn cells metabolize pregnenolone more rapidly than fetal cells. Moreover, at the end of the incubation period, 80 % of the pregnenolone used by fetal cells

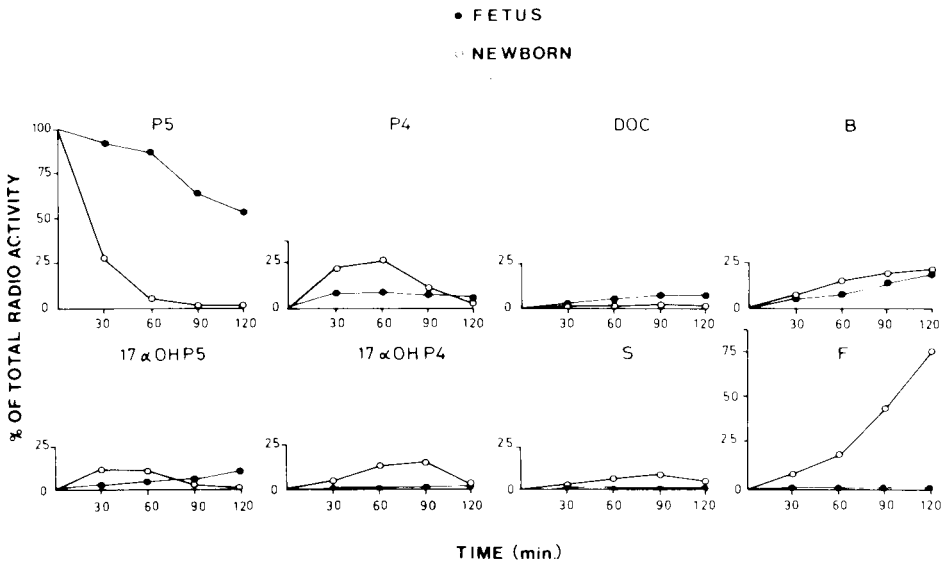


FIG. 3. — Time-course of (^{14}C)-pregnenolone (5.10^{-5}M) metabolism by adrenal cells isolated from 120-day-old fetuses (●) and 3-day-old newborn lambs (○) (from Durand *et al.*, 1982a).

was metabolized through the 17-deoxy pathway while the 17-oxy pathway was preferentially used by newborn cells. These results demonstrate deficient 17 α -hydroxylase activity in fetal cells. In addition, the slow rate of progesterone formation and the accumulation of 17 α -hydroxypregnenolone by fetal cells demonstrate the lower activity of 3 β -hydroxysteroid dehydrogenase/isomerase in fetal as compared to newborn lambs. Lastly, the accumulation of progesterone and 11-deoxycorticosterone suggests a partial deficiency of 21-hydroxylase and 11 β -hydroxylase activities in fetal cells. It should be noted that the amount of cortisol plus corticosterone produced under maximal ACTH (1-24) stimulation is always several times lower than that formed in the presence of exogenous pregnenolone, but that the difference is much more pronounced for fetal than for newborn adrenal cells (Durand *et al.*, 1982a, 1984a; Cathiard *et al.*, 1985a). Hence, it can be concluded that a defect in pregnenolone availability is another very important factor causing fetal adrenal cells to produce less corticosteroid than newborn cells.

3. Effect of in vivo ACTH (1-24) treatment on the ACTH-receptor adenylate cyclase system.

The infusion of 115-day old ovine fetuses with 0.1 mg of ACTH (1-24) per day for 5 days resulted in a striking increase in the number of ACTH receptors (fig. 4), and the responsiveness of AC to ACTH (1-24), Gpp (NH)p or NaF, higher in ACTH (1-24)-treated fetuses than in control twin fetuses, was similar to that of newborns. Also the responsiveness of adrenal cells isolated from ACTH (1-24)-

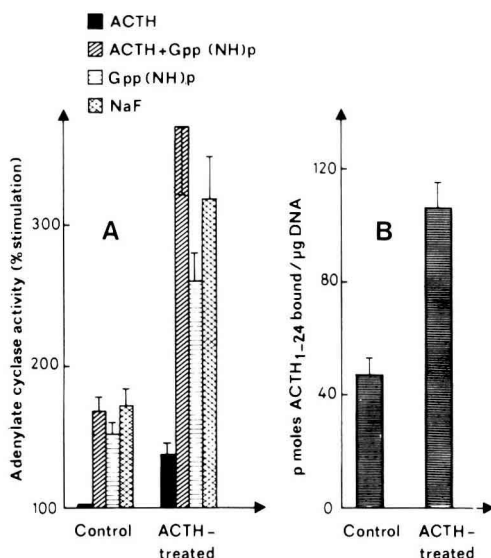


FIG. 4. — Adenylate cyclase (a) and ACTH binding (b) activities of crude adrenal membranes from 120-day-old twin fetuses, one of which had been perfused for five days with ACTH (0.1 mg/day) (from Durand *et al.*, 1981c).

treated fetuses to *in vitro* stimulation by ACTH (1-24) or cholera toxin was significantly greater than that of cells from control fetuses in terms of both cAMP and corticosteroid production (Durand *et al.*, 1981c).

4. Effect of *in vivo* ACTH (1-24) treatment on the steroidogenic pathway.

The results presented in figure 5 show that *in vivo* ACTH (1-24) treatment of fetuses greatly enhanced the rate of *in vitro* metabolism of ^{14}C -pregnenolone

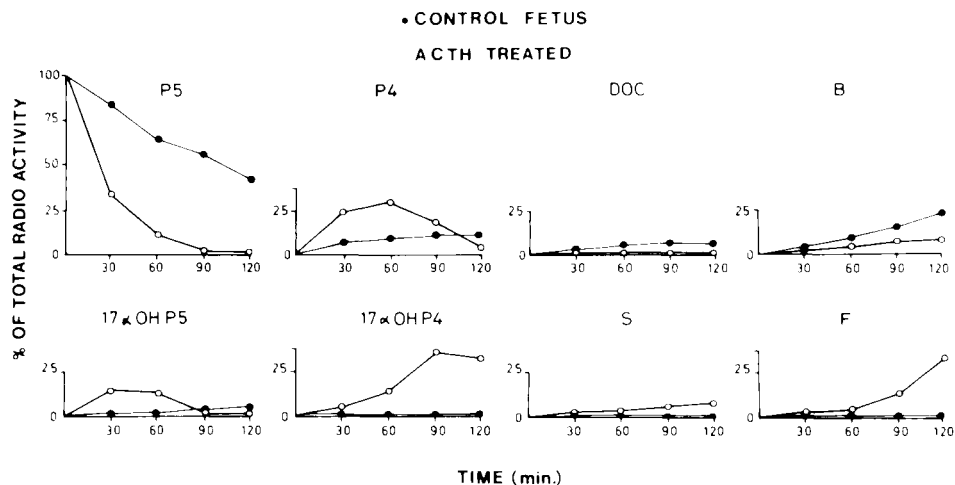


FIG. 5. — Time-course of (^{14}C) pregnenolone metabolism by adrenal cells isolated from 120-day-old control (●) and ACTH-treated fetuses (○) (from Durand *et al.*, 1982a).

which became similar to that of newborn animals. Moreover, cells from ACTH (1-24)-treated fetuses preferentially used the 17-oxy pathway as opposed to the control animals. However, there was a progressive accumulation of both 17 α -hydroxyprogesterone and, to a lesser extent, of 11-deoxy cortisol in cells from ACTH (1-24)-infused fetuses. These data suggest that 21 and 11 β hydroxylations are the main rate-limiting steps in ACTH (1-24)-treated fetuses and not 3 β -hydroxysteroid dehydrogenase/isomerase and 17 α -hydrolase as in control fetuses. It should also be noted that the difference between the amounts of cortisol and corticosterone produced under maximal ACTH (1-24) stimulation or in the presence of exogenous pregnenolone was much less with cells from ACTH (1-24)-treated fetuses than with cells from control fetuses (Durand *et al.*, 1982a). It is therefore very likely that ACTH (1-24) treatment enhances pregnenolone synthesis to about the same extent as in newborn lambs.

5. *In vitro* development of the ACTH-sensitive adenylate cyclase system.

Adrenal cells from fetuses were cultured for 6 days in the absence or presence of ACTH (1-24) (10^{-8}M). Basal cAMP output remained fairly constant

throughout the experiment (fig. 6). However, the response to ACTH (1-24) of cells cultured in the absence of this hormone for 5 days was about 25-fold higher on day 6 than on day 1. When cells were exposed to ACTH (1-24) for 2 h per day from day 1 onwards, the response to this hormone increased in a striking way during the course of the experiment and cAMP production on day 6 was almost

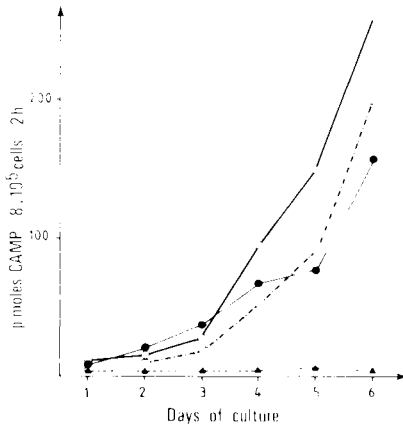


FIG. 6. — cAMP production by cultured adrenal cells from 120-day-old fetuses. Cells were cultured in the absence (▲, □) or presence of ACTH-(1-24) (10^{-8} M) either 24 hrs (●) or 2 hrs (○) per day. Basal (▲) or ACTH-(1-24) (10^{-8} M) (●, ○, □)-stimulated cAMP production was assessed daily for 2 hrs. (adapted from Durand *et al.*, 1982c).

40-fold higher than that observed on day 1. However, after 4 days, continuous ACTH (1-24) treatment resulted in an acute response to ACTH (1-24) which was weaker than for cells treated only 2 h per day. Hence, the development observed seemed to involve a combination of at least three processes :

- I) increased cAMP response to ACTH (1-24) when cells were cultured in the absence of this hormone for several days ;
- II) ACTH (1-24)-induced acceleration of cAMP production when ACTH (1-24) was given 2 h per day ;
- III) desensitization to ACTH (1-24) when the hormone was continuously present in the culture medium.

The exact causes of the increased responsiveness of AC *in vitro* are unknown. However, the preliminary results presented in table 1 show that the response to ACTH + Gpp (NH)p, Gpp (NH)p or NaF of membranes prepared from cells maintained for 6 days in ACTH-free media was much higher than that of cells cultured for only 24 h, whereas the response to forskolin was less altered. This would indicate modifications of some properties of Ns and perhaps an increase in the number of ACTH receptors and/or better coupling of the receptor and the AC catalytic subunit.

TABLE 1
Adenylate cyclase activity (pmoles/mg protein/min) in membranes from ovine fetal (115-130 day-old) adrenal cells cultured for either 1 or 6 days in the absence of ACTH.

Number of days in culture	Stimulatory factors				
	none	ACTH 1-24 (10^{-6}) + Gpp (NH)p (10^{-5} M)	Gpp (NH)p (10^{-5} M)	NaF ($2 \cdot 10^{-2}$ M)	Forskolin (10^{-4} M)
1	3.26 \pm 1.50	3.41 \pm 0.47	4.88 \pm 1.38	13.69 \pm 0.42	10.40 \pm 1.29
6	4.04 \pm 0.58	37.48 \pm 1.55	34.11 \pm 1.32	80.71 \pm 5.37	34.72 \pm 1.64

6. In vitro development of the steroidogenic pathway

The steroidogenic capacity of fetal adrenal cells maintained in ACTH-free medium increases during culture. Basal and ACTH (1-24)-stimulated corticoid output are respectively 5 and 12-fold higher on day 6 than on day 1. The main corticosteroid produced in both cases is corticosterone (Durand *et al.*, 1984a). This increased steroidogenic production is due to an enhancement of pregnenolone production (30-fold) and to a rise (2-fold) in the activity of 3 β -hydroxysteroid dehydrogenase/isomerase (Cathiard *et al.*, 1985a). Continuous ACTH (1-24) treatment from the first day of culture onwards further increases steroidogenic response to the hormone, resulting in a steroid secretion which is more than 110-fold higher on day 6 than on day 1 (Durand *et al.*, 1984a) ; but in this case the main steroid secreted is cortisol. This increase in cortisol production is due not only to a development of 17 α -hydroxylase activity but also to the enhanced activation of 3 β -hydroxysteroid dehydrogenase/isomerase and 21-hydroxylase. Moreover, pregnenolone production is 110-fold higher on day 6 than on day 1 (Cathiard *et al.*, 1985a). These results show that the development of 17 α and 21-hydroxylase activities are ACTH-dependent, while the development of 3 β -hydroxysteroid dehydrogenase/isomerase and that of some steps involved in pregnenolone biosynthesis are only partially dependent on the hormone. Since even with freshly isolated fetal adrenal cells the enzyme activities involved in the conversion of pregnenolone to corticoids (cortisol plus corticosterone) do not seem to be the major limiting step in the biosynthesis of these steroids (see above), it is likely that the increased ability to produce pregnenolone is the main factor responsible for the enhancement of the capacity of fetal adrenal cells to produce corticosteroids.

All the foregoing data indicate that ACTH is one of the main factors responsible for the maturation of ovine fetal adrenals in late gestation both at the membrane level and at the steroidogenic pathway. In addition, the results of our *in vitro* experiments suggest that the maturation of ovine fetal adrenal *in vivo* is blocked by the presence of some inhibitory factors in the fetal circulation. However, data available from most laboratories studying the ovine fetus suggest that the prepartum increase in glucocorticoid secretion occurs prior to any large increase in fetal plasma ACTH levels (Ress *et al.*, 1975 ; Jones *et al.*, 1977 ; Wintour *et al.*, 1980 ; Hennessy *et al.*, 1982 ; Rose *et al.*, 1982). Hence, it is now of primary importance to determine whether the sharp, prepartum increase in the fetal adrenocortical function involves only ACTH (« automagnifying phenomenon ») or whether it is due to a decrease of the inhibitory factors or even to an increase of some synergistic factors on ACTH action. Recently, several authors have shown that some peptides derived from the N-terminal fraction of POMC could have a biological activity in adrenal steroidogenesis in both adult rats and humans (Pedersen and Brownie, 1980 ; Al Dujaili *et al.*, 1981 ; Lis *et al.*, 1981).

Therefore, we examined the effects of some peptides, derived from the non-ACTH part of POMC, and the influence of acid pituitary extracts from fetal or newborn lambs on the *in vitro* maturation of ovine fetal adrenal cells. In addition,

we determined the influence of fetal hypophysectomy on the spontaneous and ACTH-induced maturation of cultured fetal adrenal cells.

7. Effects of POMC-derived peptides and of acid pituitary extracts on the maturation of ovine fetal adrenal cells in culture.

The following POMC-derived peptides were used : porcine POMC N-terminal (1-80) [N-POMC-(1-80)], amidated porcine POMC N-terminal (1-61) [N-POMC-(1-61)], porcine β -LPH, γ 2-MSH and γ 3-MSH. We tested the effect of these peptides, alone or in combination with ACTH (1-24), on the development of the ability of adrenal cells from 125-day old ovine fetuses to produce both cAMP and corticosteroids (Durand *et al.*, 1984b). N-POMC-(1-61) amide, γ 2-MSH and γ 3-MSH behaved similarly in our system :

- I) they slightly increased corticosteroid production without modifying cAMP output when added alone to the incubation medium, but this effect was seen only after 3 days of culture ;
- II) they potentiated steroidogenic response to 10^{-11} M ACTH (1-24) but also only from day 3 onwards ;
- III) a 5-day peptide treatment induced fetal adrenal cell maturation resulting in the same enhancement of the corticosteroid production stimulated by 10^{-8} M ACTH (1-24) without modifying the response of either cAMP or pregnenolone.

N-POMC (1-80) and β -LPH shared several common features because :

- I) each one alone stimulated corticosteroid production significantly more than was obtained with the other POMC-derived peptides (except ACTH (1-24)) ;
- II) they did not potentiate the steroidogenic action of 10^{-11} M ACTH (1-24) ;
- III) when cells were cultured in their presence for 5 days, the response to ACTH (1-24) was enhanced in respect to cAMP and pregnenolone outputs as well as to cortisol + corticosterone production.

In a second series of experiments, we assessed the daily response of cultured adrenal cells repeatedly stimulated by either ACTH (1-24) or by acid pituitary extracts from 124-day old fetuses or 5-day old newborns and observed that both cAMP and corticosteroid outputs induced by ACTH (1-24) or by fetal or newborn pituitary extracts developed identically to become 7 and 40-fold higher, respectively, on day 4 than on day 1. Moreover the response to ACTH (1-24) on day 5 was also identical in both cAMP and corticosteroids whether the cells had been previously treated with ACTH (1-24) or with fetal or newborn pituitary extracts (Durand *et al.*, 1985a).

The above data show that N-POMC-derived peptides can potentiate the acute steroidogenic activity of ACTH (1-24) on ovine fetal adrenal cells after several days in culture. They also demonstrate that some of these peptides, including β -LPH, have a trophic effect which enhances the response of fetal cells to the steroidogenic role of ACTH (1-24). It should be emphasized that such a behaviour appears to be specific to fetal cells since it is not observed in adult cells

(Cathiard *et al.*, 1985b). Finally, it appears that the pituitary from 124-day old ovine fetuses contains enough trophic (and steroidogenic) substances to allow *in vitro* adrenal maturation. However, very little is known of the ability of fetal pituitary cells to release adrenocorticotrophic substance(s) in the fetal circulation under physiological conditions (Durand *et al.*, 1982b).

8. Effect of fetal hypophysectomy on *in vitro* maturation of cultured ovine fetal adrenal cells.

The adrenal cells from 124-day old ovine fetuses (intact or hypophysectomized at 118 days of gestation) were cultured for 6 days in the absence or presence of 10^{-8}M ACTH (1-24). Figure 7 shows that with ACTH (1-24) treatment, cAMP and corticosteroid responses to the hormone developed more slowly in cells from hypophysectomized fetuses than in cells from control fetuses during the first 3 days of culture. Likewise, both cAMP and corticosteroid responses to the ACTH (1-24) of adrenal cells from hypophysectomized fetuses cultured for 1 to 3 days in the absence of ACTH were lower than those of cells from control fetuses cultured under the same conditions (Durand *et al.*, 1985a). At the beginning of culture, adrenal cells from hypophysectomized fetuses also showed lower pregnenolone metabolism than that of cells from intact fetuses. The addition of ACTH (1-24) to the culture medium increased the activity of 3β -hydroxysteroid dehydrogenase/isomerase, 17α and 21 -hydroxylases within 5

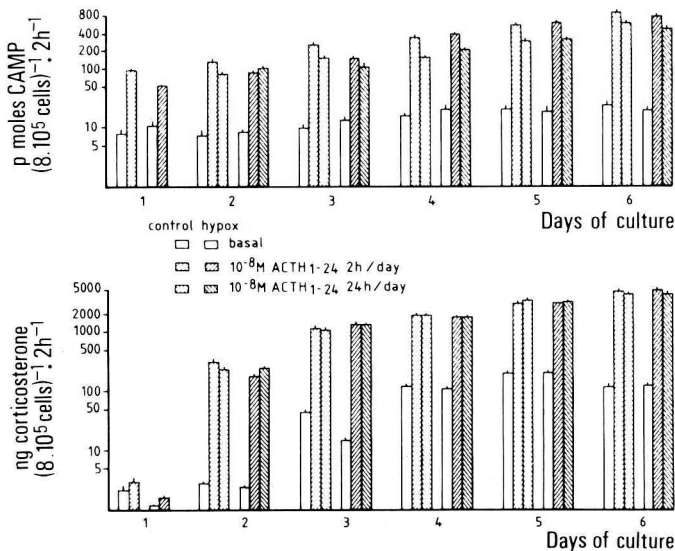


FIG. 7. — cAMP and corticosteroid productions by cultured ovine adrenal cells from 124-day-old control (□) and Hx (▨) ovine fetuses. Cells were cultured in the absence (□, ▨) or presence of ACTH 1-24 (10^{-8}M) either for 2 hours (▨, ▩) or 24 hours (▨, ▩)/day. Basal (□, ▨) or ACTH (1-24)-stimulated (▨, ▩, ▨, ▩) cAMP and corticosteroid productions were assessed daily for 2 hrs. (from Durand *et al.*, 1985a).

days, but the activity of 17α -hydroxylase in adrenal cells from hypophysectomized fetuses remained lower than that of cells from intact fetuses (Cathiard *et al.*, 1985b). These data demonstrate that removal of pituitary hormones *in vivo* decreases the ability of fetal adrenal cells to respond *in vitro* to ACTH (1-24). Moreover, since no development at all occurs *in vivo* in the absence of the pituitary gland (Durand *et al.*, 1985a), the putative factors inhibiting fetal adrenal maturation may still be present after hypophysectomy.

9. Conclusion

The results presented here define some of the biochemical steps involved in the maturation of ovine fetal adrenal cells during late gestation. They also demonstrate that ACTH (1-24) can induce fetal adrenal cells to respond to ACTH in a way similar to that of cells from newborn lambs. Moreover, they suggest that, in addition to ACTH, two kinds of factors are needed for the regulation of this maturation :

- I) stimulatory factors, some of which are probably derived from POMC and can act either alone or by potentiating ACTH action ;
- II) inhibitory factors of likely extrapituitary origin.

Obviously, there are many gaps in our understanding of this process. For example, at the biochemical level it is now important to determine whether the increased activity of Ns results from a larger number of subunits or a modification of their properties. Another problem is understanding the causes of the lower capacity of fetal adrenal cells to produce pregnenolone as compared to newborn cells. In addition, nothing is known of the development of the steps which allow the hormonal signal to be transmitted from the plasma membrane to the intracellular structures which synthesize corticosteroids.

Finally, it is not clear yet whether the changes observed *in vivo* result from the progressive appearance in the adrenal gland of cells responsive to ACTH and/or development of the steroidogenic capacity of pre-existing cells. As for the hormonal « cocktail » which regulates maturation, it now appears essential to determine the « corticotropic activity » released in the fetal circulation during the last 3 weeks of gestation and to identify the different molecules responsible for that activity. In a second step, the regulation of the secretion of these different hormones will have to be investigated.

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Résumé. *Modifications biochimiques impliquées dans la maturation de la fonction corticosurrénalienne du fœtus ovin : modalités et régulation.*

Au cours du dernier mois de la vie intra-utérine, la réponse stéroïdogénique à l'ACTH des glandes surrénales du fœtus ovin augmente, et devient maximale au moment de la naissance. Ce développement traduit des modifications situées à différentes étapes du mécanisme d'action de l'ACTH. Au niveau de la membrane plasmique, nous avons montré que l'augmentation de la production d'AMPc implique au moins trois processus : une augmentation du nombre de récepteurs de l'ACTH, une activité accrue de la sous-unité Ns de l'adénylate cyclase, une augmentation du GTP disponible. Au niveau de la chaîne de biosynthèse des corticostéroïdes, on observe principalement un accroissement de la capacité de synthèse de la pregnénolone, et des activités augmentées pour les 3β -hydroxystéroïde déshydrogénase/isomérase et 17α -hydroxylase. Une infusion d'ACTH de 5 jours à des fœtus âgés de 115-120 jours est capable d'induire la plupart de ces modifications. De même, l'ACTH peut induire la maturation des cellules surrénales fœtales en culture, et certains peptides dérivés de la POMC potentialisent son effet stéroïdogénique aigu *in vitro*. Cependant, même en absence d'ACTH, le système adénylate cyclasique et les potentialités stéroïdogéniques des cellules en culture se développent, mais à un degré moindre que lorsque l'ACTH est présente dans le milieu de culture. Ces résultats suggèrent que l'ACTH est la principale hormone trophique de la surrénale fœtale ovine au cours du dernier mois de la gestation, même si d'autres facteurs stimulants sont également requis. La maturation surrénalienne fœtale est bloquée *in vivo* par la présence dans la circulation fœtale de facteurs inhibiteurs dont l'origine est vraisemblablement extra hypophysaire.

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