

## Heterogeneity of adrenocorticotrophin in the pituitary gland and plasma during the perinatal period

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### Summary.

1) Three immunoreactive forms of ACTH are characterized by their apparent molecular weight (MW) and bioactivity when acid extracts of the anterior or of the neurointermediate lobe of fetal and newborn rat pituitary glands are subjected to gel filtration on Sephadex G50 fine columns.

The « big » ACTH form which shows an apparent MW close to 44 000 hardly stimulates the *in vitro* release of corticosterone by perfused fetal adrenals. In contrast, the « intermediate » (MW :  $\leq$  13 000) and « little » (MW :  $\leq$  4 500) forms show high biological activity by eliciting corticosterone secretion which is log-dose-dependent.

During the perinatal period, the relative proportions between these different molecular forms of ACTH change in both the anterior and neurointermediate lobes. « Big » ACTH is the main form in the neurointermediate lobe of the fetal rat pituitary. After birth, the « big » ACTH/total ACTH ratio regularly decreases until postpartum week 4 ; it is not very different then from that of pregnant adult females. The three immunoreactive forms of ACTH are present in the anterior lobe throughout the perinatal period. The gradual increase of the « intermediate » and « little » forms is accompanied by a correlative decrease in the « big » form.

2) The anterior lobes of 17, 19 and 21-day old fetuses, stimulated *in vitro* by an acid extract of adult hypothalamus, release the three immunoreactive forms of ACTH in the same proportions as those observed in corresponding extracts of anterior lobes.

3) On days 17, 19 and 21 of gestation, fetal plasma contains all the immunoreactive forms of ACTH previously observed in pituitary glands. The proportion of the « little » ACTH form gradually increases, whereas that of the « big » form decreases as gestation progresses. At term, the relative proportion of « little » ACTH is greater in the plasma than in the fetal pituitary.

4) Controlled trypsin digestion of pituitary « big » ACTH results in a conversion to the « intermediate » and « little » immunoreactive forms. When « intermediate » ACTH is submitted to tryptic digestion under the same conditions, there is continuous loss of immunoreactivity but no change of hormonal form. These findings strengthen the hypothesis that « big » ACTH is a precursor of « intermediate » and « little » ACTH ; in contrast, the « intermediate » form is not a precursor of the « little » one. The high molecular-weight form of ACTH might be converted endogenously into lower MW forms in the fetal circulation at term as well as in newborns.

5) In fetal plasma, immunoreactive ACTH levels reach peak values on day 19 of gestation and decrease thereafter until day 21. This profile of plasma ACTH levels could explain that of corticosterone concentration which also reaches a peak on day 19 in the adrenals as well as in the plasma. During the first postnatal days, the sharp decrease in

plasma ACTH levels is in agreement with the low corticosterone concentrations reported in both the adrenals and the plasma of newborns.

During fetal life, the corticostimulatory activity of the pituitary gland is modulated both by the rate of ACTH release and by the biological potency of the different molecular forms of released ACTH.

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## Introduction

Less than ten years ago, ACTH was still considered as a polypeptide hormone with 39 amino acid residues. The presence of other forms of ACTH was first reported in a cell line of mouse pituitary carcinoma (Orth *et al.*, 1970, 1973). High molecular-weight immunoreactive ACTH was further observed in human plasma as well as in extracts of pituitary glands and ectopic tumors (Yalow and Berson, 1971). ACTH polymorphism was confirmed in the pituitary of several mammals, including man (Coslovsky and Yalow, 1974 ; Eipper and Mains, 1975 ; see review in Orth and Nicholson, 1977). Moreover, the high molecular-weight forms of ACTH were glycoproteins (Eipper *et al.*, 1976). More recent studies on rat pituitary glands suggest that ACTH originates from a larger precursor molecule called Pro-ACTH/endorphin (Eipper and Mains, 1978 ; Mains and Eipper, 1979) which also contains the amino acid sequences of both the  $\beta$ LPH and 16-K fragments located near the N-end of the precursor (Lowry *et al.*, 1976 ; Eipper and Mains, 1978 ; Nakanishi *et al.*, 1979).

While the polymorphism of ACTH has been investigated thoroughly over the last few years in both adult pituitary gland and plasma, few studies have examined the heterogeneity of ACTH in the fetus and newborn.

The aim of this paper is to review recent progress on the evolution of ACTH polymorphism in the pituitary gland and the plasma during perinatal development.

### I. ACTH polymorphism in the fetus and neonate

A. *ACTH in the pituitary gland.* — The presence of the adrenocorticotrophic hormone (ACTH) in the neurointermediate and anterior lobes of the pituitary gland of fetal rats was demonstrated by using immunocytological (Chatelain *et al.*, 1979), biological and radioimmunological (Chatelain and Dupouy, 1981 a, b) methods. The ACTH content of the fetal neurointermediate lobe from days 18 to 21 of gestation is 10 to 20-fold less than that of the pars distalis (Chatelain and Dupouy, 1980a). Three forms of ACTH are characterized by their apparent molecular weight, bioactivity and immunoreactivity when acid extracts of whole fetal pituitary gland are subjected to gel filtration on Sephadex G50 fine (Chatelain and Dupouy, 1980b). The apparent molecular weight (MW) of the « big » form of ACTH, eluted as Blue Dextran 2000 and proteins in the void volume of the columns, is 44 000 ; « little » ACTH, eluted in the same fractions as human synthetic ACTH<sub>1-39</sub>, has an apparent MW of 4 500. The third molecular form, designated as « intermediate » ACTH, elutes between the first two ; its apparent MW is almost 13 000. Between days 17 and 21 of gestation, a sharp rise in the

bioreactive ACTH content of the pituitary (Dupouy, 1976) is associated with an increase in the proportion of « intermediate » and « little » forms of ACTH and a correlative decrease in the « big » form (Chatelain and Dupouy, 1980b). These three immunoreactive forms of ACTH are also found in the lobes of the fetal and neonatal pituitary glands as well as in the adult pituitary (Chatelain and Dupouy, 1985).

The « big » form of ACTH is mainly found in the pars neurointermedia of the rat fetus between days 18 and 21 of gestation, while the three immunoreactive forms are observed in newborns from weeks 1 to 4. The « big » ACTH/total ACTH ratio, which is very high during fetal life, decreases regularly after birth (fig. 1). In 4-week old newborns, it is as low as in pregnant females (fig. 1). This ratio is always less in the pars distalis than in the pars neurointermedia. In the former it decreases during gestation and the first two weeks after birth (fig. 1). Between days 14 and 28 postpartum, this ratio is similar to that found in pregnant females. The ratio of « intermediate » + « little » ACTH/total ACTH evolves in a contrary way (fig. 1).

Similar changes occur (Silman *et al.*, 1979) in the pituitary gland of the sheep fetus and the lamb which contains abundant material of high molecular weight (Silman, 1979 ; Silman *et al.*, 1979 ; Roebuck *et al.*, 1980). The ratio of pituitary concentration between the high and low molecular-weight forms of ACTH was higher in 120 to 145-day fetuses (mean : 1.66) than in a 3-month old lamb (mean : 1.22) or pregnant ewes (mean : 0.46). Moreover, as two of the three immunoreactive forms of high molecular-weight ACTH contained amino acid sequences similar to both ACTH and  $\beta$ -LPH (Silman, 1979 ; Silman *et al.*, 1979), the common precursor for ACTH and lipotrophin observed in the adults of several species could be present in the sheep fetus. These observations suggest that there is a progressive change in the proportions of the different molecular forms of pituitary ACTH in sheep as well as in rats during perinatal development.

In contrast to rats and sheep, the pituitary gland of fetal rhesus monkeys was not reported to contain a macromolecular form of ACTH (Silman *et al.*, 1978).

The ACTH<sub>1-39</sub> and  $\beta$ -LPH present in the pars distalis of the adult pituitary of several species are considered to arise from the proteolytic cleavage of a common « pro-opiomelanocorticotrophin » precursor (Liotta *et al.*, 1978 ; Lissitsky *et al.*, 1978 ; Jackson and Lowry, 1980). During the perinatal development of rats, the progressive disappearance of the « big » form of ACTH coupled with the appearance of the « little » form could mean that the enzyme systems involved in such cleavage at the cellular level are becoming functionally mature. The evolution of various molecular forms of ACTH are reported to be different in adult pars neurointermedia, i.e. the proteolytic cleavage of ACTH<sub>1-39</sub> produces several peptides of lower molecular weight, such as  $\alpha$ -MSH (ACTH<sub>1-13</sub> and CLIP (ACTH<sub>18-39</sub>) (Gianoulakis *et al.*, 1979 ; Mains and Eipper, 1979, 1980 ; Eipper and Mains, 1980) ; the adult pars neurointermedia contains little ACTH<sub>1-39</sub> (Crine *et al.*, 1979 ; Gianoulakis *et al.*, 1979) but a lot of  $\alpha$ -MSH, CLIP and  $\beta$ -endorphin (Scott *et al.*, 1974a, b ; Kraicer, 1977 ; Lissitsky *et al.*, 1978 ; Gianoulakis *et al.*, 1979 ; Jackson and Lowry, 1979 ; Chretien and Seidah, 1981). This cleavage could be primed in late pregnancy or early postnatal life and might account for

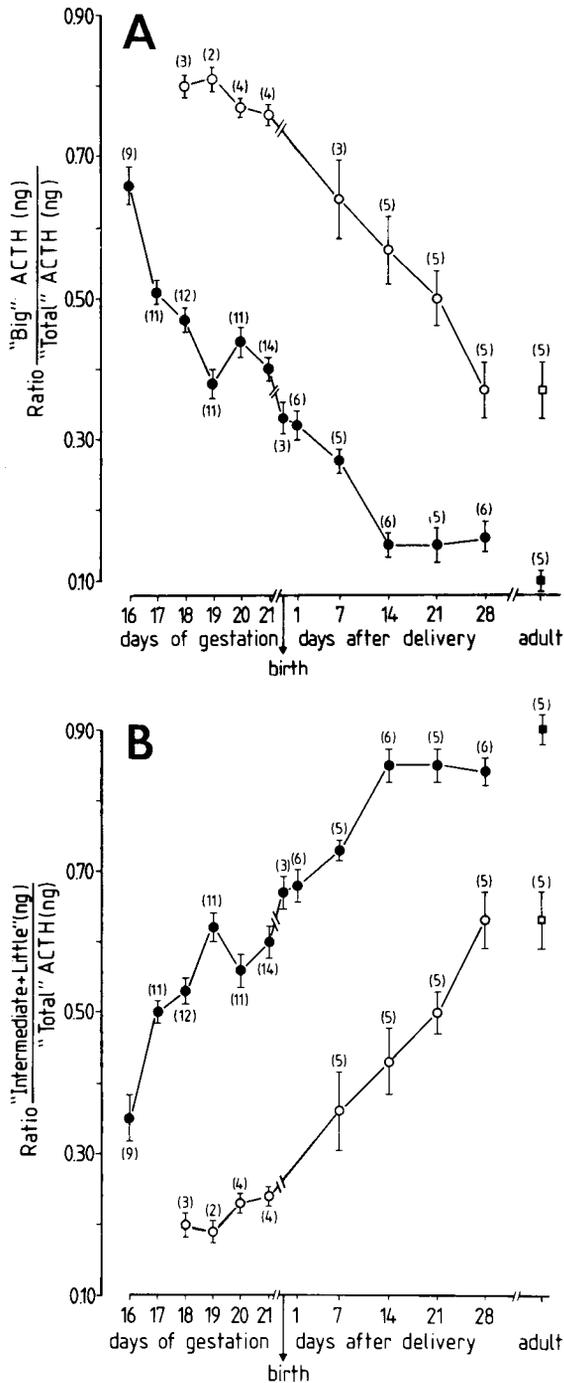


FIG. 1. — Variations in the anterior (●—●) and neurointermediate (○—○) lobes of the « big » ACTH/« total » ACTH ratio (A) and the « intermediate » and « little » ACTH/« total » ACTH ratio (B). Each point represents the mean  $\pm$  SEM ; number of chromatographs in parentheses.

Acid extracts (0.1 N HCl) of fetal rat hypophyses were subjected to gel filtration at 4 °C on Sephadex G 50 fine columns (Pharmacia K9/60, 60  $\times$  0,9 cm) equilibrated and further eluted with acetic acid 0.1 M containing 0.3 % HSA. The flow rate was 10-15 ml/hr and 1-ml eluate fractions were collected.

(From Chatelain and Dupouy, 1985).

the lower ACTH<sub>1-39</sub> (« little » form) content of the pars neurointermedia as compared to the pars distalis.

## B. Plasma ACTH

1) *Origin of the ACTH present in fetal circulation.* — Several observations extensively discussed by Dupouy and Chatelain (1981) suggest that the fetal pituitary has both trophic and steroidogenic action on fetal adrenals. Indeed, fetal hypophysectomy performed *in utero* induced a sharp drop in plasma ACTH levels, adrenal atrophy and a decrease in the synthesis and release of corticosteroids ; ACTH given to the fetus suppressed the effects of hypophysectomy on growth as well as on adrenal activity. The ACTH found in the fetal circulation originated mainly or exclusively from the fetus. This hypothesis is also supported by a comparison of fetal and maternal plasma ACTH levels. In man, immunoreactive ACTH concentrations are reduced in anencephalic infants compared with the levels found in full-term babies or mothers (Allen *et al.*, 1973, 1974 ; Miyakawa *et al.*, 1974 ; Winters *et al.*, 1974). Similarly, plasma ACTH levels are reduced in encephalotomized rats (Dupouy and Chatelain, 1981 ; Chatelain and Dupouy, 1981a). At birth, ACTH concentrations are higher in the arterial than in the venous plasma of the human umbilical cord (Arai *et al.*, 1976 ; Puolakka *et al.*, 1982). Also, ACTH levels are higher in the arterial plasma of fetal sheep than in that of the mothers during the second half of gestation as well as during parturition (Jones *et al.*, 1977).

Immunoreactive ACTH concentrations are reduced in infants from mothers with Nelson's syndrome (Allen *et al.*, 1973) or from mothers infused with ACTH during labour (Miyakawa *et al.*, 1974). Moreover, no correlation has been reported between maternal and fetal ACTH levels in man (Allen *et al.*, 1973) or in sheep (Alexander *et al.*, 1971). No transplacental passage of ACTH was observed when labelled hormone was given to the mother or the fetus in several species including sheep (Jones *et al.*, 1975), rabbits (Genazzani *et al.*, 1975) and rats (Dupouy *et al.*, 1980). The perfusion of ACTH to rhesus monkey fetuses was unable to induce a rise in the maternal plasma ACTH level (Walsh *et al.*, 1979).

These experimental data suggest or demonstrate that ACTH is not transported across the placenta in several species of mammals, including man, and that the ACTH present in the fetal circulation is not of maternal origin. Although some factors other than ACTH could partly control the development of fetal adrenals in mammals and man (see review in Dupouy and Chatelain, 1981), the corticostimulatory activity of the fetal pituitary is mainly due to ACTH. Therefore, quantitative and qualitative changes in plasma ACTH levels during gestation are reliable tests of the corticostimulatory action of the pituitary gland.

2) *Polymorphism of circulating ACTH in the fetus and newborn.* — Many studies have been done on the polymorphism of immunoreactive ACTH in adult plasma ; in contrast, there are few observations concerning the plasma of the fetus and newborn.

Three molecular forms of immunoreactive ACTH are found in the fetal circulation of sheep between 115 and 125 days (Jones *et al.*, 1978 ; Jones and

Roebuck, 1979) and in that of rat between 17 and 21 days (Chatelain and Cheong, 1985a, b) of pregnancy. In the latter species, these forms are similar to those observed in the fetal pituitary (Chatelain and Dupouy, 1985b). High molecular forms of ACTH are also present in the plasma of fetal monkeys and guinea-pigs (Jones and Roebuck, 1979).

The proportions of the different immunoreactive forms of ACTH vary during gestation in rats. The « big » ACTH/total ACTH ratio gradually decreases, whereas the « little » ACTH/total ACTH ratio gradually increases (fig. 2), as also shown in the anterior lobe of the pituitary gland (Chatelain and Dupouy, 1985b). A similar evolution of these forms is reported for the plasma of fetal monkeys, sheep and guinea-pigs (Jones, 1976b ; Jones and Roebuck, 1979). In the plasma of the rat fetus, the « intermediate » ACTH/total ACTH ratio is higher on days 17 and 19 of gestation than at term on day 21. The rise in the low molecular form of ACTH in the fetal plasma has been correlated with the progressive disappearance of the higher forms (Chatelain and Cheong, 1985a, b). The plasma ACTH levels of newborn rats are so low (Dupouy and Chatelain, 1981 ; Chatelain and Cheong, 1985b) that the different molecular forms of ACTH cannot be identified by gel filtration. However, the « little » form is quite abundant in the plasma of 1 or 2-month old male and female rats subjected to ether inhalation (unpublished data).

## II. Released and circulating molecular forms of ACTH

A. *Release of ACTH by fetal rat pituitary glands in vitro.* — Is the evolution of plasma ACTH heterogeneity during gestation the result of both a selective

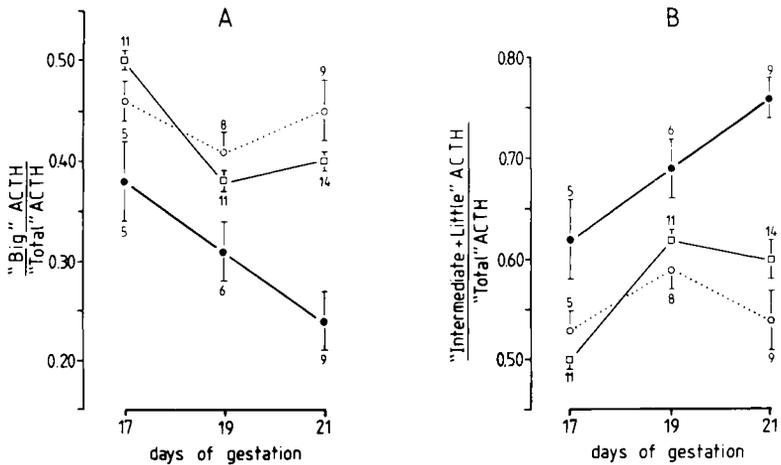


FIG. 2. — Variations of the « big » ACTH/« total » ACTH ratio (A) and the « intermediate » + « little » ACTH/« total » ACTH ratio (B) in the plasma (●—●), in the perfusate of pituitary gland anterior lobe (○- - -○) and in the anterior lobe (□—□) of rat fetuses collected on days 17, 19 and 21 of gestation. Each point represents the mean  $\pm$  SEM ; the number of chromatograms is indicated.

(From Chatelain and Cheong, 1985b)

secretion of the different molecular forms of pituitary ACTH and/or a further protease-induced conversion in the fetal circulation? As *in vivo* study of the ACTH released by both lobes of the fetal pituitary is impossible, Chatelain and Cheong (1985a, b) investigated *in vitro* ACTH release by the pars distalis of the fetal gland collected at different stages of gestation (days 17, 19 and 21) and stimulated with a crude extract of adult hypothalamus. The effluent perfusate was collected in 1-ml fractions which were subjected to gel filtration on Sephadex G50 fine, according to the procedure used previously for plasma studies.

Three immunoreactive forms of ACTH, isolated by chromatography of anterior lobe perfusate, were similar to those previously observed in the fetal plasma and pituitary gland (Chatelain and Cheong, 1985a). Moreover, on days 17, 19 and 21 of gestation, the fetal pituitary *in vitro* released the same proportions of all the molecular forms of ACTH it contained (fig. 2). If the same type of release occurs *in vivo*, this does not explain why the proportions between the three molecular forms of ACTH are so different in the fetal circulation and mainly on day 21 of gestation. Indeed, at term the ratio of « intermediate » + « little » ACTH/total ACTH is significantly greater in the fetal plasma than in the pars distalis and the lobe perfusate (fig. 2). *In vivo*, plasma ACTH could arise from the pars distalis as well as from the pars neurointermedia; however, as the « big » form of ACTH is found in the latter and represents less than 10 % of the pituitary ACTH content, the pars distalis of the pituitary gland is the main source of circulating ACTH.

The different forms of ACTH identified in fetal sheep plasma are also found in isolated anterior pituitary cells as well as in their incubation medium (Jones, 1976a). The predominant forms of immunoreactive ACTH released in both basal and [Arg-]-vasopressin (AVP)-stimulated cultures are the same as the major forms contained in the pituitary cells of mice (Paquette *et al.*, 1979) or rats (Eipper and Mains, 1978; Zimmerman and Kraicer, 1978; Itoh *et al.*, 1981). These data suggest that the evolution of ACTH polymorphism in the fetal plasma might not reflect a selective release of the molecular-weight forms of ACTH by the pituitary but some proteolytic modification after secretion into the fetal circulation.

*B. Conversion of released forms of ACTH in the circulation.* — Several findings strengthen the hypothesis that high molecular-weight forms of ACTH are converted by the action of plasmin or trypsin into « little » forms. Indeed, fetal sheep blood or plasma when incubated at 37 °C induces a sharp rise in bioactive ACTH concentration (600 %) associated with the conversion of the high molecular-weight form of ACTH into « little » ACTH of higher biological activity (Jones, 1976b). This process is slower with incubation at 4 °C and inhibited by lima bean trypsin inhibitor or by pre-heating the plasma at 80 °C for 60 min.

Controlled tryptic digestion *in vitro* of fetal rat « big » ACTH which has low biological activity (Chatelain and Dupouy, 1985) leads to the appearance of « intermediate » and « little » forms of ACTH (Chatelain and Cheong, 1985a, b) of high steroidogenic potency (Chatelain and Dupouy, 1985). However, when « intermediate » ACTH is submitted to tryptic digestion in the same conditions,

there is continuous loss of immunoreactivity but no change of hormonal form (fig. 3).

Yalow and Berson (1973) studying the pituitary gland and plasma and Gewirtz *et al.*, (1974) ectopic tumors showed the conversion of human ACTH into its « little » form. Trypsin-induced conversion of « big » ACTH into the « intermediate » form was observed in mice (Coslovsky *et al.*, 1975). In contrast, « intermediate » ACTH was not a precursor of the « small » form in rabbits and cattle (Coslovsky and Yalow, 1974) or mice (Coslovsky *et al.*, 1975).

These data suggest that the « big » form of ACTH in both adult and fetal plasma is converted into « intermediate » and « little » forms by the action of proteases. Such conversion might be higher in term fetuses than in younger ones.

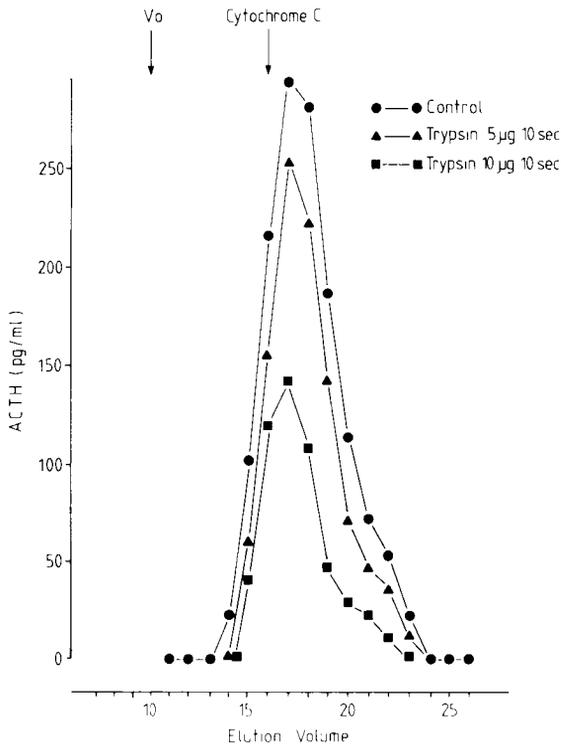


FIG. 3. — *Sephadex G50* fine filtration of the « intermediate » immunoreactive form of ACTH isolated from an acid extract (0.1 N HCl) of twenty anterior lobes of 21 day-old fetuses, before (●—●) and 10 sec after controlled digestion with 5 µg (▲—▲) or 10 µg (■—■) of trypsin.

Chromatography was performed at 4 °C on *Sephadex G50* fine columns (Pharmacia K9/60, 60 × 0.9 cm) previously equilibrated and eluted with 1 % formic acid containing 100 mg of polyep/100 ml. The flow rate was 10-14 ml/hr and 1 ml eluate fractions were collected in polyethylene tubes. Before ACTH radioimmunoassay the fractions were evaporated and reconstituted with 0.02 M veronal buffer ; pH was adjusted to 8.2 with 5 N NaOH.

(Original data)

### III. Biological activity of different molecular forms of ACTH

A. *In vitro* and *in vivo* corticosteroidogenic activity. — Low biological activity is reported to be associated with the « big » ACTH form isolated from the pituitary of adult rats (Zimmerman and Kraicer, 1978) and mice (Mains and Eipper, 1975) as well as from mouse pituitary tumors (Mains and Eipper, 1975) or human ectopic carcinoma (Gewirtz *et al.*, 1974). Gasson (1979) studied the biological activity of three high molecular-weight forms of ACTH in isolated rat adrenal cortex cells; the three forms (pro-ACTH/endorphin or « big » ACTH, biosynthetic « intermediate » or 23 000 MW ACTH, and glycosylated ACTH<sub>1-39</sub> or 13 000 MW ACTH) could stimulate corticosterone release; however, pro-ACTH/endorphin and glycosylated ACTH<sub>1-39</sub> were 100 and 300-fold less potent, respectively, than ACTH<sub>1-39</sub>. These observations disagree with those of Estivariz and Iturriza (1975) who detected high biological activity associated with « big » ACTH in the anterior and neurointermediate lobes of adult rats.

Fetal material has hardly been investigated. Roebuck *et al.*, (1980) studying fetal adrenal cells of 130 to 135-day old lambs showed that three high molecular-weight forms of ACTH (50 000, 30 000 and 20 000) were each biologically active but blocked ACTH corticosteroidogenic activity.

Macromolecular peptides in the fetal circulation might explain why fetal sheep adrenals are apparently less responsive to exogenous ACTH *in vivo* than *in vitro*, and they could explain the paradoxical effect of short-term dexamethasone infusion into fetal sheep which increases response to ACTH<sub>1-24</sub> (Liggins *et al.*, 1977). Dexamethasone could reduce the secretion of large inhibitory peptides as well as that of ACTH<sub>1-39</sub>. Similarly, dexamethasone increases adrenal response to exogenous ACTH in fetal rhesus monkeys (Novy and Walsh, 1981) while, without dexamethasone, this response is irregularly observed *in vivo* (Kittinger and Beamer, 1971; Jaffe *et al.*, 1977). However, isolated adrenal cells *in vitro* are responsive to physiological concentrations of ACTH (Roebuck *et al.*, 1984). The inhibition of ACTH<sub>1-39</sub> bioactivity by macromolecular forms could be related to the presence in their molecule of the 15-18 ACTH sequence which, by occupying corticotropin membrane receptors might prevent ACTH<sub>1-39</sub> from exercising its corticosteroidogenic activity (Hofmann, 1974 in Roebuck *et al.*, 1980). According to Gasson (1979), the low steroidogenic potency of biosynthetic « intermediate » ACTH and pro-ACTH/endorphin would be due to the addition of the 16 000-fragment extension on the amino terminal side of the ACTH<sub>1-39</sub> sequence. However, according to Gewirtz *et al.*, (1974), when present in 5-fold excess (on the basis of immunological equivalence) « big » ACTH extracted from human non-pituitary tumors had no inhibitory effect on the biological potency of standard ACTH.

The perfusion of 21-day old rat fetus adrenals with the same immunological quantities (2 ng) of « big », « intermediate » or « little » ACTH isolated from fetal pituitary showed that « big », ACTH has weak corticosteroidogenic activity, whereas « intermediate » and « little » ACTH highly stimulate corticosterone release (Chatelain and Dupouy, 1985). Moreover, with adrenals from 17, 19 and

21-day old fetuses, the production of corticosterone induced by « little » and « intermediate » ACTH is log-dose-dependent (fig. 4).

An immunologically equivalent amount (2 ng) of « intermediate » and « little » ACTH, which does not induce maximal response shows similar corticosteroidogenic activity on adrenals of 17 and 19-day old fetuses (fig. 5), whereas « little » ACTH has greater biological potency than the « intermediate » form on fetal adrenals at term (fig. 5).

To interpret differences in potency obtained with these ACTH forms on fetal adrenals, it is necessary to understand their fate in the assay system and to determine changes in the number, specificity and affinity of the related adrenal receptors during gestation.

**B. The physiological meaning of ACTH polymorphism.** — The physiological meaning of ACTH heterogeneity and its evolution during perinatal development is not well known.

According to the hypothesis of Coslovsky and Yalow (1974), the hormonal form of ACTH would be an important factor regulating the cortisol/corticosterone ratio in mammalian adrenal corticoid secretion. « Little » ACTH is reported to predominate in mammals primarily producing cortisol (humans, monkeys, sheep, dogs, cats, guinea-pigs), whereas « intermediate » ACTH is more important in mammals in which corticosterone predominates (rabbits, rats, mice). As the main hormones released by fetal and newborn adrenals are reported to change during

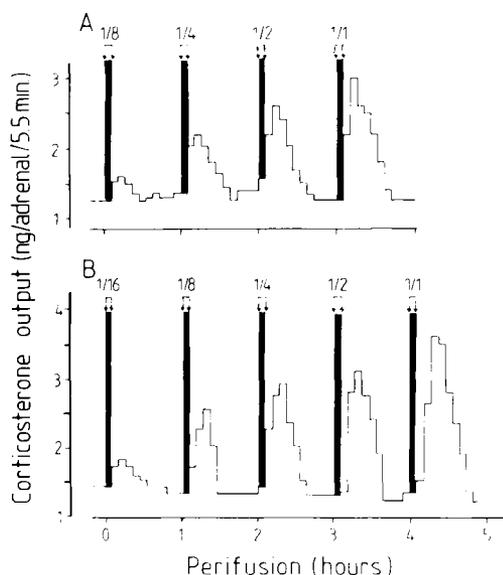


FIG. 4. — *Biological activity of « intermediate » (A) and « little » (B) ACTH forms on perfused adrenals of 19-day old rat fetuses.* Corticosterone content of 1 ml eluate fractions collected during 5.5 min was determined by radiocompetition. The production of corticosterone induced by the « intermediate » and « little » ACTH forms was log-dose-dependent (A :  $y = 7.36 \log x - 7.30$  ;  $r = 0.974$  ; B :  $y = 6.33 \log x - 3.85$  ;  $r = 0.995$ ).

(Original data)

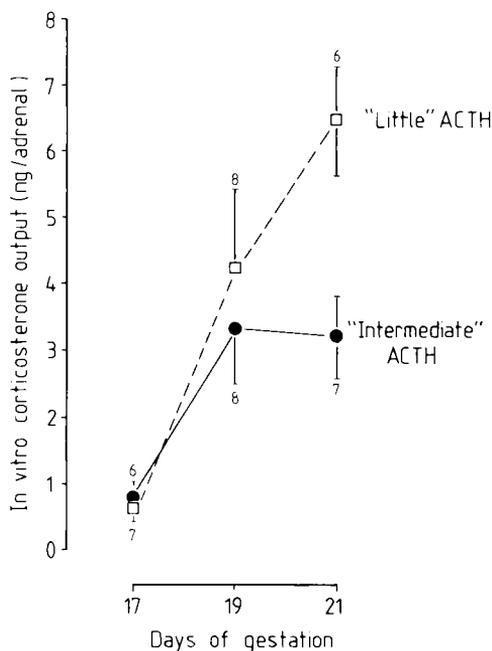


FIG. 5. — Biological activity of equivalent amounts (2 ng) of the « intermediate » and « little » ACTH forms on perfused adrenals of 17, 19 and 21-day old rat fetuses. This dose did not induce maximal response.

The corticosterone content of 1-ml eluate fractions was determined by radiocompetition.

(Original data)

the development of species such as rats, sheep and rabbits (see review in Chatelain and Dupouy, 1980b), it is possible that these drastic changes in adrenal activity are related to the evolution of the different molecular forms of ACTH during the perinatal period. However, Coslovsky and Yalow (1974) as well as Yalow and Berson (1971) performed gel filtration with a non-dissociative basic buffer containing plasma protein so that some ACTH molecules might have bound to albumin and other proteins, modifying the elution profiles (Ratter *et al.*, 1980). For example, adult rat pituitary extracts chromatographed under acid conditions contain the « little » form of ACTH (Lang *et al.*, 1973 ; Estivariz and Iturriza, 1975 ; Itoh *et al.*, 1981) associated with either the « intermediate » form (Lang *et al.*, 1973) or the « big » one (Estivariz and Iturriza, 1975) or both these forms (Itoh *et al.*, 1981). In contrast, under basal conditions, Coslovsky and Yalow (1974) identified only the « intermediate » form of ACTH. Similarly, besides a minor « big » molecular form, pituitary extracts of adult mice were reported to contain two equivalent major forms related to the « little » and « intermediate » forms when chromatographed under acid conditions (Mains and Eipper, 1975) or subjected to electrophoresis on SDS-polyacrylamide gel (Paquette *et al.*, 1979). In contrast, Coslovsky and Yalow (1974) identified only « intermediate » ACTH when gel filtration was performed with basic buffer.

Such discrepancies make it difficult to assert some link between ACTH polymorphism in the fetus and the newborn and the functional differentiation of the adrenals during the perinatal period.

**C. Quantitative and qualitative modulation of the corticostimulatory activity of the fetal and neonatal pituitary.** — Adrenal growth and adrenal and plasma corticosterone concentrations, which are used extensively as indirect but reliable tests of corticostimulatory activity in rat fetuses, suggest that this pituitary function increases between days 17 and 19 of gestation and then decreases until term (see review in Dupouy and Chatelain, 1981). During the last 3 days of gestation, pituitary corticotropic activity depends on both hypothalamic control and negative steroid feedback (see review in Dupouy and Chatelain, 1981). Immunoreactive ACTH levels reach a peak in the plasma of 18 to 19-day old fetuses (Chatelain *et al.*, 1980 ; Boudouresque *et al.*, 1984 ; Chatelain and Cheong, 1985b) in correlation with the activity of the fetal adrenals (fig. 6). The determination of plasma ACTH contents by either immunological or biological assay cannot account for the heterogeneity of the circulating forms of ACTH which differ in their biological and immunological activity as well as in their apparent molecular weight. The corticostimulatory activity of the fetal pituitary rises between days 17 and 19 of gestation due to the quantitative increase in plasma ACTH and to the qualitative change in circulating ACTH. Indeed, the proportion of bioactive forms (« little » and « intermediate ») increases over the « big » form which shows low biological activity.

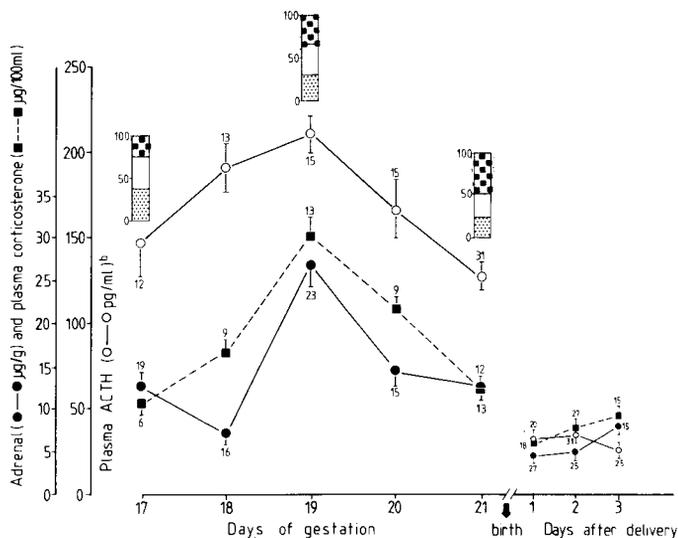


FIG. 6. — Immunoreactive plasma ACTH levels in rat fetuses (a) and newborns (b) in relation to adrenal and plasma corticosterone concentrations in fetuses (c) and newborns (b). Vertical columns: percentage of the different molecular forms of ACTH; « big », « intermediate », « little ».

(Data adapted from (a) Chatelain and Cheong, 1985 ; (b) Dupouy and Chatelain (original data), (c) Dupouy, Coffigny and Magre, 1975.)

Beyond day 19, the decrease in pituitary gland corticostimulatory activity is mainly due to a drop in ACTH secretion.

In early postnatal life, the plasma free corticosterone concentration rises significantly (Koch *et al.*, 1967) owing to the precipitous drop in corticosteroid-binding globulin (CBG) activity (Koch *et al.*, 1967 ; Nuñez *et al.*, 1971 ; Martin *et al.*, 1977 ; Van Baelen *et al.*, 1977 ; Raymoure and Kuhn, 1983 ; Dupouy and Chatelain, 1985) ; this results in a sharp decrease in pituitary ACTH secretion under the negative feedback effect of plasma corticosterone. The fall in plasma ACTH levels could be related to a reduction in both the adrenal and plasma corticosterone concentrations in newborn rats.

No study has been done on ACTH polymorphism in the plasma of newborns. However, it is possible that the evolution observed during fetal life simply continues after birth.

## Conclusion.

A drastic change in ACTH polymorphism occurs in both the pituitary and the plasma of mammals during perinatal development. This change is probably linked to functional maturation of the enzyme systems involved in the conversion of the high molecular-weight (« big ») form of ACTH with low biological activity into lower molecular-weight (« intermediate » and « little ») forms with higher corticosteroidogenic activity. Nevertheless, the physiological meaning of ACTH heterogeneity during the perinatal period is not well understood. The action mechanism of these different forms of ACTH on fetal and neonatal adrenals has yet to be defined.

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**Résumé.** *Hétérogénéité de l'ACTH hypophysaire et plasmatique dans la période périnatale.*

1) La filtration sur gel de Sephadex G50 fine d'extraits acides de lobes antérieurs (LA) et neurointermédiaires (LNI) d'hypophyses de fœtus et de nouveau-nés de rat met en évidence trois formes immunoréactives de l'ACTH caractérisées par leur poids moléculaire apparent (P. M.) et leur activité biologique.

La forme « big », dont le P. M. est voisin de 44 000, stimule peu la production de corticostérone par des surrénales fœtales périfusées.

Les formes « intermédiaire » (P. M.  $\leq$  13 000) et « little » (P. M.  $\leq$  4 500) ont par contre *in vitro*, une forte activité biologique log-dose dépendante.

La proportion entre ces formes varie au cours de la vie intrautérine et postnatale.

Dans les LNI de fœtus, prédomine la forme « big » ; après la naissance, le rapport « big » ACTH/ACTH totale décline régulièrement pour atteindre chez le nouveau-né de 4 semaines, une valeur comparable à celle de ♀ adultes gestantes.

Dans les LA coexistent les trois formes de l'ACTH mais, au cours de la période périnatale, la proportion de la forme « big » diminue tandis que celle des formes « little » et « intermédiaire » augmente.

2) *In vitro*, des LA d'hypophyses de fœtus de 17, 19 et 21 jours stimulées par un extrait d'hypothalamus d'adulte sécrètent les trois formes immunoréactives de l'ACTH dans les mêmes proportions que celles déterminées dans les adénohypophyses.

3) Dans le plasma de fœtus âgés de 17, 19 et 21 jours, l'ACTH se présente sous trois formes immunoréactives analogues à celles observées dans l'hypophyse. Au cours de la gestation, le rapport de concentration ACTH « big »/ACTH totale diminue alors que le rapport ACTH « little »/ACTH totale augmente. A terme, la proportion de « little » dans le plasma est plus grande que dans l'hypophyse.

4) La digestion trypsique ménagée des formes « big » et « intermédiaire » montre que la forme « big » doit être un précurseur des formes « little » et « intermédiaire » mais que cette dernière n'est pas un précurseur de la « little ».

*In vivo*, une transformation de ces formes moléculaires de l'ACTH est suggérée dans la circulation du fœtus à terme.

5) Une bonne corrélation paraît exister entre l'ACTHémie du fœtus qui présente un maximum à 19 jours et l'activité de la corticosurrénale fœtale. Dans les jours qui suivent la naissance, l'ACTHémie s'effondre et la surrénale du nouveau-né est faiblement active.

Au cours de la vie fœtale, l'activité corticostimulante de l'hypophyse paraît être modulée quantitativement par le taux de sécrétion de l'ACTH et qualitativement par l'activité biologique des différentes formes moléculaires de l'ACTH sécrétée.

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