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Developmental and Functional Biology of the Primate Fetal Adrenal Cortex*

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I. Introduction

STEROID hormones produced by the fetal adrenal cortex regulate intrauterine homeostasis, the maturation of fetal organ systems necessary for extrauterine life, and, in some species, the timing of parturition (Refs. 1–3 for review). Appropriate development and function of the fetal adrenal cortex therefore are critical for fetal maturation and perinatal survival. Moreover, the fetal adrenal cortex must itself undergo maturational changes in preparation for its essential role postnatally, *i.e.* production of glucocorticoids and mineralocorticoids, and to ensure adrenal cortical autonomy once the placenta has separated.

Development and function of the primate fetal adrenal cortex are distinct from those in other species. During the latter two thirds of gestation in humans and higher primates, the fetal adrenal glands are disproportionately enlarged and exhibit extraordinary growth and steroidogenic activity in a specialized cortical compartment known as the fetal zone (4–7). As its name implies, the fetal zone exists only during fetal life; it atrophies soon after birth and has no counterpart postnatally. During midgestation, the fetal zone occupies 80–90% of the cortical volume and produces 100–200 mg/day of the androgenic C_{19} steroid, dehydroepiandrosterone sulfate (DHEA-S), which is quantitatively the principal steroid product of the primate fetal adrenal gland throughout gestation. The primate fetal adrenal cortex also produces cortisol, which promotes the maturation of fetal organ systems, including the lungs, liver, thyroid, and gut, needed for extrauterine life (1). In some species (*e.g.* sheep, goats, and rabbits), cortisol produced by the fetal adrenals also regulates the timing of parturition (2, 8); however, a similar role in primates is not apparent.

Experiments of nature in humans (*e.g.* anencephaly) (9–11) and studies in pregnant rhesus monkeys (12, 13) indicate that ACTH secreted from the fetal pituitary is the principal trophic regulator of the fetal adrenal cortex. However, several observations indicate that ACTH may not be acting directly. During the latter two thirds of gestation, the fetal zone grows rapidly and produces large amounts of steroid even though circulating ACTH concentrations may be decreasing (14). Furthermore, soon after birth the fetal zone rapidly involutes although exposure to ACTH continues. Thus, other factors, possibly specific to the intrauterine environment, appear to play a role in the regulation of fetal adrenal cortical growth and function. Substances produced by the placenta (*e.g.* CG) have been implicated (10, 15), and peptide growth factors produced locally within the fetal adrenal (16) are thought to influence fetal adrenal cortical growth and function by mediating and/or modulating the tropic actions of ACTH. Specific nuclear receptor transcription factors also appear to be important regulators of adrenal cortical development by influencing early embryonic differentiation of adrenal cortical progenitors and the maintenance of steroidogenic function (17, 18). Thus, regulation of the primate fetal adrenal cortex

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is a complex process involving the net effect of a cohort of factors that modulate and/or mediate the trophic actions of ACTH.

Our purpose is to review the literature and synthesize the current understanding of the developmental and functional biology of the primate fetal adrenal cortex. In particular, we will discuss the recent literature concerning the role of growth factors and nuclear receptors and factors emanating from the placenta in the regulation of fetal adrenal cortical growth and function. We will also address recent advances in understanding the function of the fetal adrenal cortex in the regulation of fetal development and parturition.

II. Development

In their extensive study of the development of zonal patterns in the human adrenal gland, Sucheston and Cannon (19) described five landmark phases: 1) condensation of the celomic epithelium (3–4 weeks of gestation); 2) proliferation and migration of celomic epithelial cells (4–6 weeks of gestation); 3) morphological differentiation of fetal adrenal cortical cells into two distinct zones (8–10 weeks of gestation); 4) decline and disappearance of the fetal zone (first 3 postnatal months); and 5) establishment and stabilization of the adult zonal pattern (10–20 yr of age). Thus, human adrenal development can be thought of as a continuum beginning at around the fourth week of gestation and continuing into adult life.

Development of the primate fetal adrenal cortex differs qualitatively and quantitatively from that of other species and is characterized by extraordinarily rapid growth, high steroidogenic activity, and a unique morphological appearance. For much of gestation, the human fetal adrenal cortex is composed of two morphologically distinct zones: the fetal zone and the definitive zone (Fig. 1). The fetal zone accounts for the bulk (80–90%) of the cortex and is the primary site of growth and steroidogenesis. The definitive zone (also referred to as the adult cortex, neocortex, or permanent zone), occupies the remainder of the cortex and comprises a narrow band of tightly packed cells surrounding the fetal zone. Excellent studies of the early embryonic and fetal development of the human adrenal cortex have been reported (4, 20–25). The following description is synthesized from these works.

A. Embryonic adrenal development

The anlage of the human adrenal cortex is first identified at about the fourth week of gestation as a thickening of the celomic epithelium in the notch between the primitive urogenital ridge and the dorsal mesentery. By the fifth week, these primitive cells begin to migrate, forming cords that stream medially and cranially, eventually accumulating at the cranial end of the mesonephros where they condense to form what Jirasek (4) referred to as the "adrenal blastema," the earliest recognizable manifestation of the adrenal gland. Interestingly, cells destined to become the steroidogenic cells of the adrenal and gonad appear to be derived from neighboring areas of the celomic epithelium and are morphologically identical (21). In general, the portion of celomic epithelium medial to the mesonephros produces cells destined for the adrenal cortex, whereas the portion ventral to the mesonephros produces cells destined for the gonad.

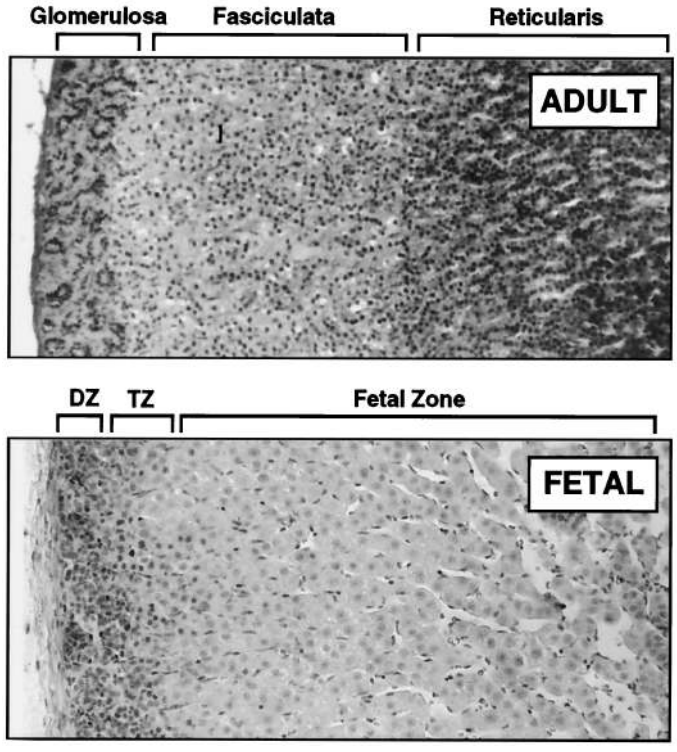

FIG. 1. Morphology of the human fetal (midgestation) and adult adrenal cortex. The adult cortex is composed of three distinct zones: the glomerulosa, fasciculata, and reticularis (medulla not shown). In contrast, the fetal adrenal cortex is comprised of two distinct zones: the outer definitive zone (DZ) and the large inner fetal zone. The transitional zone (TZ) comprises the outer edge of the fetal zone and forms a functionally distinct compartment between the fetal and definitive zones.

By the eighth week of gestation, the mass of cells migrating into the adrenal blastema organize into anastomosing cords and exhibit ultrastructural characteristics consistent with steroidogenic capability. These cells eventually differentiate into large polyhedral cells and become the primordium of the fetal zone. Uotila (22) reported that the definitive zone is derived about a week later when a separate population of cells in the same area of celomic epithelium migrates to the adrenal blastema and surrounds the primordial fetal zone. Although Crowder (23) also proposed that the fetal and definitive zone are derived from separate populations of cells in the celomic epithelium, that study concluded that the anlage of both zones develops simultaneously in the celomic epithelium and enters the adrenal primordium at the same time but in discrete juxtaposed columns. Conversely, Jirasek (4), concluded that both zones are derived from a single progenitor population and that the entire adrenal blastema is initially made up of small primitive epithelial cells. The cells in the center of the adrenal blastema then differentiate into fetal zone cells, whereas the peripheral cells maintain their small blastematous morphology and eventually form the definitive zone. Despite these differences in the dynamics of fetal and definitive zone formation, it is clear that by the eighth week of human pregnancy the fetus acquires a rudi-

mentary but distinct adrenal cortex made up of two zonal compartments.

At around the ninth week of gestation, the adrenal blastema is completely enclosed by the adrenal capsule, which is composed of specialized mesenchymal cells migrating from the area of Bowman's capsule. At the same time, an extensive network of sinusoidal capillaries develops between the cords of the fetal zone. This vasculature predominates in the central portion of the fetal zone and persists throughout fetal life (26). Consequently, the adrenal cortex is one of the most highly vascularized organs in the primate fetus. Abundant vascularization is likely required to facilitate access of hormonal products to the circulation.

The medulla is absent from the primate fetal adrenal as a discrete structure throughout most of gestation except for small islands of chromaffin cells scattered through the body of the cortex. Only after the involution of the fetal zone during the first postnatal week do the chromaffin elements coalesce around the central vein and begin to form a rudimentary medulla. By the fourth postnatal week, essentially all of the chromaffin cells have clustered in the center of the gland. However, it is not until 12 to 18 months that the medulla becomes adult-like in appearance (23).

B. Fetal adrenal development

After 10–12 weeks of gestation, the morphology of the adrenal cortex remains relatively constant. By midgestation (16–20 weeks), the fetal zone clearly dominates and is composed of large (20–50 μm) eosinophilic cells that exhibit ultrastructural characteristics typical of steroid-secreting cells (*i.e.* large amounts of tubular smooth endoplasmic reticulum, mitochondria with tubulovesicular cristae, large Golgi complexes, abundant lipid, and numerous dense bodies). In the outer regions of the fetal zone, the cells are arranged in tightly packed cords. However, the cells in the central portion are more widely spaced into a reticular pattern and separated by many vascular sinusoids. Clusters of immature neuroblasts that will aggregate eventually into a functional medulla are also present between the innermost fetal zone cells (26).

The definitive zone is composed of a narrow band of small (10–20 μm) tightly packed basophilic cells that exhibit structural characteristics typical of cells in a proliferative state (*i.e.* small cytoplasmic volume containing free ribosomes; small, dense mitochondria with lamelliform cristae and scant lipid). Its inner layers form arched cords that send finger-like columns of cells into the outer rim of the fetal zone. Although definitive zone cells are lipid-poor during midgestation, they accumulate some cytoplasmic lipid and begin to resemble steroidogenically active cells with increasing age. By late gestation, the definitive zone cells may be likened to cells of the adult zona glomerulosa (26).

Ultrastructural studies have also demonstrated a third zone between the fetal and definitive zones, the cells of which have intermediate characteristics (27). We have referred to this cortical area as the 'transitional zone' (28) (Fig. 1). Studies in our laboratory (discussed below) indicate that after midgestation, transitional zone cells may have the capacity to synthesize cortisol and thus be analogous to cells of the zona fasciculata of the adult adrenal. By the 30th week of gestation, the definitive zone and transitional zone begin to take on the appearance of the zona glomerulosa and the zona fasciculata, respectively (19). Thus, by late gestation the fetal adrenal cortex resembles a rudimentary form of the adult adrenal cortex.

C. Neonatal adrenal development

Soon after birth, the primate adrenal cortex dramatically remodels. Keene and Hewer (20) in 1927 reported that during the first 6 weeks of postnatal life "... *The whole gland shrinks owing to the rapid disappearance of the foetal cortex*... " The demise of the fetal zone was thought to occur by necrosis and hemorrhage. However, more recent studies have suggested that the necrosis and hemorrhage were probably artifacts related to the cause of death and time elapsed before tissue fixation. Detailed studies by Sucheston and Cannon (19) in humans and by McNulty (29) in rhesus monkeys, demonstrated that the postnatal remodeling of the primate adrenal cortex involves a complex wave of differentiation such that the fetal zone atrophies and the zonae glomerulosa and fasciculata develop. Recent studies by Spencer *et al*. (30) indicate that fetal zone remodeling in the human is an apoptotic process.

It has generally been thought that the adult cortical zones develop from the persistent definitive zone. However, there is no evidence of adrenal cortical insufficiency during the perinatal period and the postnatal remodeling process. Thus, it is likely that the nascent adult cortical zones are present and functional before birth. Indeed, morphological studies have identified rudimentary zonae glomerulosa and fasciculata during late gestation (19). This lends support to the notion that the postnatal remodeling of the primate adrenal cortex involves apoptosis of the fetal zone and the simultaneous expansion of preexisting zonae glomerulosa and fasciculata.

D. Growth

Rapid growth of the human fetal adrenal cortex begins at approximately the 10th week of gestation and continues to term (Fig. 2). The growth is almost entirely due to enlargement of the fetal zone and, as a consequence, the gland becomes as large as the fetal kidney by 20 weeks. Between 20 and 30 weeks, the size and weight of the fetal adrenal gland doubles, achieving a relative size 10- to 20-fold that of the adult adrenal. A further doubling in fetal adrenal weight occurs after 30 weeks of gestation such that by term the gland weighs approximately 3–4 g (4, 20).

The dynamics of primate fetal adrenal cortical growth involve cellular hyperplasia, hypertrophy, migration, and senescence. Growth of the embryonic (4–5 weeks of gestation) adrenal cortex probably occurs by hyperplasia, as mitotic activity can be observed throughout the adrenal blastema (4). However, after 8 weeks, when the definitive and fetal zones can be delineated, mitotic activity is limited to the definitive zone (31). By 10–12 weeks of gestation, the definitive zone exhibits numerous mitotic figures, whereas mitotic figures in the fetal zone are scant. The cells of the fetal zone

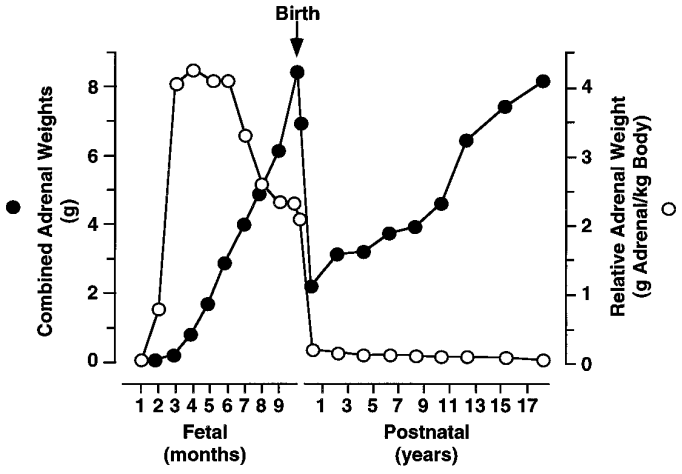

FIG. 2. Mean weight of the human adrenal glands (*closed symbols*) and the ratio of adrenal weight to body weight (*open symbols*) during fetal and postnatal life. After the second month of gestation, the fetal adrenals begin to grow rapidly due to hypertrophy of the fetal zone, and their size becomes disproportionate relative to body weight. Soon after birth, the fetal zone involutes and the weight of the glands rapidly decreases. The relative weight of the adrenals after birth markedly decreases and remains constant for the remainder of life. [Adapted from A. M. Neville and M.J. O'Hare: *The Human Adrenal Cortex*. Springer-Verlag, Berlin, 1982 (252).]

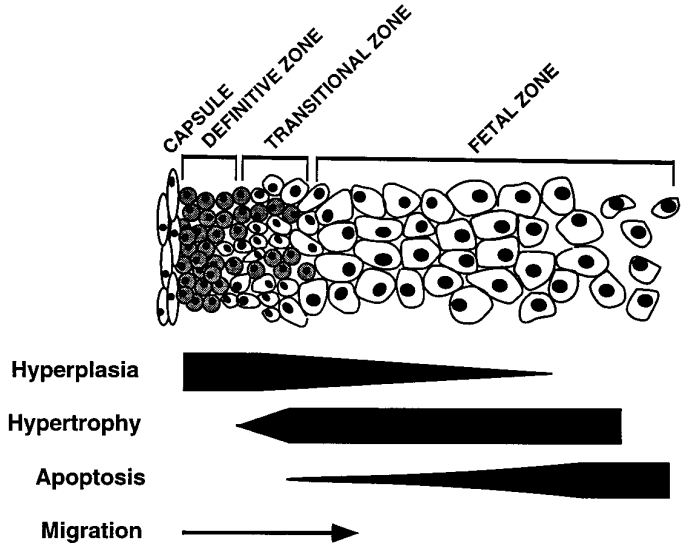

FIG. 3. Schematic structure of the midgestation human fetal adrenal gland and proposed primary modes of growth in each cortical zone and cell migration. Hyperplasia occurs mainly in the definitive zone; hypertrophy occurs mainly in the fetal zone; apoptosis occurs mainly in the central areas of the fetal zone; and cells migrate from the periphery to the center of the gland.

are not necessarily more numerous but are much larger than those of the definitive zone. Coulter *et al*. (32) have shown that in the fetal rhesus monkey, growth of the fetal zone in response to increased endogenous ACTH secretion occurs primarily by hypertrophy. Taken together, these data suggest that the fetal zone grows by hypertrophy and limited proliferation whereas definitive zone growth occurs mainly by hyperplasia.

Centripetal migration of lipid-containing cells from the definitive to the fetal zone was first reported by Keene and Hewer (20) and later confirmed by Crowder (23). Jirasek (4) described the daughter cells resulting from mitoses in the definitive zone forming cords that invade into the outer layers of the fetal zone. The disparate level of proliferation between the definitive and fetal zones and evidence of centripetal migration lend support to the migration theory of adrenal cortical cytogenesis and suggest that the definitive zone is the germinal/stem-cell compartment from which the inner cortical zones are derived. Thus, cells proliferate in the definitive zone and then migrate inward to form the fetal zone. This concept is supported by studies in the adult rat and mouse showing that mitotic pressure in the periphery of the adrenal cortex causes centripetal migration of cells from the glomerulosa to the inner cortical compartments (33–38). The adrenal cortical zones therefore appear to be interdependent and derived from a common pool of cells in the periphery. Thus, growth of the fetal zone not only involves limited proliferation of existing fetal zone cells but also the differentiation and hypertrophy of inwardly migrating cells from the definitive zone.

Apoptosis also may occur in the developing human fetal adrenal cortex. By morphological criteria, Jirasek (4) detected evidence of cellular apoptosis primarily in the central portions of the fetal zone and similarly, Spencer *et al.* (30) using a technique that identifies apoptotic cell *in situ* based on the detection of increased accumulation of cleaved DNA found that the labeling index of apoptotic nuclei was greater in the central areas of the fetal zone than in the definitive zone. In contrast, Albrecht *et al*. (39) could not detect evidence of apoptosis during early-, mid-, and late-gestation in the baboon fetal adrenal. However, in that study apotosis was assessed by measuring that extent to which genomic DNA extracted from whole baboon fetal adrenals was cleaved into specific oligonucleosomes (the DNA-laddering method). The low number of apoptotic cells detected by Spencer *et al*. (30) in the central areas of the cortex probably would not contribute enough cleaved DNA for detection in the laddering assay. Thus, the primate (and most likely other species') fetal adrenal cortex is a dynamic organ in which cells proliferate in the periphery, migrate centripetally, differentiate to form the specialized cortical compartments (and possibly continue to proliferate within the compartments), and then undergo senescence when they reach the center of the cortex (Fig. 3). The size of the fetal adrenal cortex and its constituent zones represents the net effect of forces that modulate these dynamic parameters of growth.

E. Functional development

The following discussion will address several key issues regarding the functional development of the primate fetal adrenal cortex. First, we will discuss data concerning the ontogeny of steroidogenic activity and the time in gestation when the gland begins producing steroids. Second, we will review the literature concerning the functional differentiation of the cortical zones and the ontogeny of steroidogenic enzyme expression. Finally, we will discuss how changes in responsiveness to ACTH may influence fetal adrenal development.

1. Ontogeny of steroidogenic activity. Morphological analyses indicate that the human fetal adrenal cortex has steroidogenic capabilities early in gestation. This is first seen at 6–8 weeks when the cells in the adrenal blastema differentiate and acquire steroidogenic characteristics (23, 27). In cord blood from human fetuses between 10 and 20 weeks of gestation, concentrations of cortisol (40–42), corticosterone, and aldosterone (40) are greater in the umbilical artery than in the umbilical vein, indicating that these steroids are produced by the fetus. Perfusion of previable human fetuses with radiolabeled progesterone showed that the fetus has the capacity to produce cortisol from progesterone as early as the 16th week of gestation (43–45). Small amounts of aldosterone have been detected in human amniotic fluid at 9 weeks of gestation (46); however, it is not certain whether it originates from the maternal or fetal adrenals at this stage of gestation. At 20 weeks, low levels of aldosterone can be produced by the previable human fetus perfused with radiolabeled corticosterone (47, 48).

Estrogens, particularly estriol, in the maternal circulation are indicative of fetal adrenal steroidogenic activity. The placenta, which is the principal source of estrogens during pregnancy, utilizes exogenous androgens supplied to an increasing extent by the fetal adrenal cortex and to a decreasing extent by the maternal adrenal cortex as gestation proceeds (49), as precursors for estrogen synthesis (see *Section IV*). Low levels of estriol can first be detected in the maternal circulation at the eighth week of gestation, indicating that DHEA-S is being produced by the fetus at this stage. At around the 12th week of gestation, estriol concentrations in the maternal circulation rapidly increase approximately 100-fold (50). This increase coincides with the initiation of fetal zone hypertrophy and ACTH secretion by the fetal pituitary gland (51). In contrast, estriol levels are markedly decreased and sometimes undetectable in women bearing anencephalic fetuses (49, 52), if fetal death occurs (49), or in placental sulfatase deficiency (53, 54). These observations indicate that the human fetal adrenal cortex produces DHEA-S beginning at around 8–10 weeks of gestation in sufficient quantities to effect increases in maternal estrogen levels. Production of DHEA-S by the fetal adrenal cortex continues for the remainder of pregnancy and during the second and third trimesters increases considerably such that by term the human fetal adrenal produces around 200 mg/day (49).

A major unanswered question regarding function of the primate fetal adrenal cortex is the stage of gestation at which the fetal adrenal cortex begins to produce cortisol. Observations of infants with congenital adrenal hyperplasia (CAH) suggest that the fetal adrenal cortex produces cortisol early in gestation. The most common form of CAH is caused by a deficiency of the 21-hydroxylase enzyme (P450c21) (55) and, as a result, the fetal adrenal cortex cannot synthesize adequate amounts of cortisol. The reduced glucocorticoid negative feedback to the hypothalamus and pituitary leads to a compensatory increase in ACTH secretion, the trophic actions of which cause hyperplasia of the fetal adrenal cortex. The elevated ACTH also increases production of DHEA-S, as biosynthesis of this C_{19} steroid is not affected by P450c21 deficiency. Consequently, the primary manifestations of P450c21 deficiency are those of androgen excess, which are first expressed *in utero*, resulting in the masculinization of the external genitalia in female fetuses. Inasmuch as differentiation of external genitalia in both sexes begins at week 7 of gestation and is complete by week 10 (Ref. 56 for review), these effects of androgen excess in P450c21 deficiency most likely occur before week 10 of gestation. These observations imply that, under normal circumstances, the fetal adrenal produces cortisol early in gestation which exerts negative feedback control on fetal pituitary ACTH secretion. Moreover, these observations indicate that the human fetal pituitary produces ACTH before 10 weeks of gestation, which regulates fetal adrenal steroidogenesis. Immunohistochemical studies demonstrate the presence of corticotropes in the human fetal pituitary at about this time (51). It is conceivable that cortisol is produced by the fetal adrenal early in gestation to suppress ACTH secretion and prevent overproduction of adrenal androgens during the androgen-sensitive phase of sexual differentiation (particularly in females).

Studies of human fetal adrenal tissue *in vitro* have provided more direct evidence of its steroidogenic capability early in gestation. Incubations with labeled acetate (57) or progesterone (58, 59) and superfusion of human fetal adrenal tissue *in vitro* with media containing ACTH (60) indicate that the human fetal adrenal cortex is responsive to ACTH and produces corticoids and DHEA-S as early as the tenth week of gestation. The pattern of glucocorticoid production by the primate fetal adrenal cortex during the rest of pregnancy is not clear. Expression of key steroid-metabolizing enzymes suggests that the human fetal adrenal cortex does not produce cortisol *de novo* from cholesterol until around week 30 of gestation (see below). However, this does not preclude the possibility that cortisol is produced utilizing progesterone as precursor early in gestation. MacNaughton *et al.* (45) infused radiolabeled progesterone into previable human fetuses between 16 and 18 weeks of gestation and found that it was metabolized to cortisol and that the rate of metabolism was increased by ACTH. The abundant amount of progesterone produced by the placenta provides the fetal adrenal cortex with a potential source of Δ^4-C_{21} steroid substrate for cortisol and aldosterone production even though the adrenal may not be sufficiently mature to produce these steroids *de novo*.

Mineralocorticoid production by the primate fetal adrenal cortex is very low early in gestation but increases during the third trimester. At term, 80% of the aldosterone in human and rhesus monkey fetal blood appears to originate from the fetal adrenal (47, 61). In 18- to 21-week human fetal adrenals, the mineralocorticoid metabolic pathway is localized to the definitive zone, although its activity is very low and unresponsive to secretagogues (62). The angiotensin-II receptors, AT_1 and AT_2, are present on human fetal adrenal cortical cells after 16 weeks of gestation (63). The AT_2 receptor is localized mainly on definitive zone cells, whereas the AT_1 receptor is detectable to a lesser extent in cells from both fetal and definitive zones. Thus, during the first and second trimesters, the ability of the human fetal adrenal cortex to synthesize mineralocorticoids is minimal even though the cells express angiotensin-II receptors.

2. Functional zonation and ontogeny of steroidogenic enzyme expression. The contributions of the fetal and definitive zones to

human fetal adrenal steroidogenic potential have been studied *in vitro* using tissue perfusion and cell culture techniques (60, 64, 65). In response to ACTH, fetal zone cells produce mainly DHEA-S and little cortisol, whereas definitive zone cells produce mainly corticoids and little DHEA-S. It was concluded that, during midgestation, the fetal zone is the site of DHEA-S synthesis and that the definitive zone is the site of cortisol synthesis (60).

Another approach to assessing the steroidogenic potential of adrenal cortical zones has been to determine which steroidogenic enzymes are expressed by the cells of each zone. As the fate of pregnenolone metabolism is determined by the branch-point enzymes cytochrome P450 17α hydroxylase/17,20 lyase (P450c17) and 3β-hydroxysteroid dehydrogenase/Δ^{4-5} isomerase (3βHSD), the steroidogenic potential of cells may be inferred by the pattern of expression of these two enzymes. Thus, if both enzymes are expressed, the cells have the potential for cortisol production, whereas if 3βHSD is expressed and P450c17 is lacking, steroidogenesis would be directed toward mineralocorticoid synthesis, and conversely, if P450c17 is expressed and 3βHSD is lacking, steroidogenesis would be limited to the Δ^5 pathway and be directed toward DHEA-S synthesis. Thus, expression of 3βHSD is particularly relevant for the *de novo* synthesis of mineralocorticoids and glucocorticoids, and expression of P450c17 is essential for C_{19} steroid (*e.g.*, DHEA) biosynthesis.

The metabolism of radiolabeled progesterone to cortisol by previable human fetuses indicates that the fetal adrenal cortex expresses the enzymes downstream from 3βHSD [*i.e.* P450c17, 21-hydroxylase (P450c21), and 11β-hydroxylase (P450c11)] needed for cortisol synthesis early in gestation. However, studies in the late gestation fetal rhesus monkey (66) and baboon (67) show that the conversion of placental progesterone to cortisol by the fetus is minimal. Expression of 3βHSD by the primate fetal adrenal cortex is therefore the critical step in the metabolism of pregnenolone, as it confers on cells the ability to convert Δ^5-3β hydroxysteroids to Δ^{4-3}-ketosteroids essential for mineralocorticoid and glucocorticoid production. Data regarding the expression of 3βHSD by the human fetal adrenal cortex early in gestation are conflicting. Goldman *et al*. (68) first indicated that 3βHSD activity is present in the human fetal adrenal cortex as early as 12 weeks of gestation and that it is localized to the definitive zone. However, the specificity of their assay is uncertain. Voutilainen *et al*. (69) examined 3βHSD expression using the highly sensitive technique of RT-PCR and could not detect mRNA encoding 3βHSD (either type I or type II) in human fetal adrenals between 12 and 22 weeks of gestation but could detect its expression after 22 weeks. In contrast, Parker *et al*. (70), using immunohistochemistry, detected 3βHSD staining exclusively in the definitive zone between 11 and 15 weeks of gestation. However, between 15 and 24 weeks, 3βHSD expression in the human fetal adrenal cortex decreased to undetectable levels and after 24 weeks was again detectable in the definitive zone. Thus, although masculinization of the female fetus with P450c21 deficiency indicates glucocorticoid production by the fetal adrenal cortex before 10 weeks of gestation, evidence of its *de novo* synthesis (based on 3βHSD expression) is uncertain.

The steroidogenic potential of each cortical zone in the primate fetal adrenal also has been examined by determining the *in situ* pattern of cholesterol side chain cleavage (P450 scc) and P450c17 and 3βHSD expression during midgestation (16–24 weeks) in the human and late-gestation rhesus monkey fetal adrenal by *in situ* hybridization and immunocytochemistry (28). In those studies, the late-gestation fetal rhesus monkey was used as a model for the late-gestation human fetus, as the growth, structure, and function of its adrenals closely resemble those of the human (26). Moderate levels of P450scc expression were detected in all cortical zones of the human and rhesus monkey fetal adrenals at all gestational ages. Consistent with other studies (70–72), 3βHSD was not expressed in any cortical zone in midgestation, *i.e.* 16–22 weeks. At 22–24 weeks, 3βHSD staining was detected in the outermost layer of definitive zone cells. Dupont *et al*. (72) and Parker *et al*. (70) showed that by 28 weeks, this staining extended inward to encompass all of the definitive and transitional zone cells (cells at the interface between the fetal and definitive zones). At no time in gestation was 3βHSD expression detected in the fetal zone. This ontogenetic pattern of 3βHSD expression suggests that the human fetal adrenal cortex cannot synthesize cortisol *de novo* between 16 and 22 weeks because it cannot convert pregnenolone to progesterone due to the lack of 3βHSD.

Interestingly, expression of P450c17, although highly abundant in the transitional and fetal zones, was lacking in the definitive zone at all gestational ages (28, 63, 71). The lack of P450c17 in the definitive zone was unexpected and implies that these cells cannot synthesize cortisol *in vivo* either *de novo* or from progesterone. Expression of P450c17 was highest in the transitional zone cells which, late in gestation, also expressed 3βHSD. Therefore, the transitional zone may be the site of *de novo* glucocorticoid production by the human fetal adrenal cortex and may acquire this capability late in gestation when its cells begin to express 3βHSD.

Recently, Coulter and Jaffe (C. L. Coulter and R. B. Jaffe, unpublished data) examined the ontogenetic localization of P450c21 and P450c11 expression in the human (13–24 weeks) and rhesus monkey (109 days to term) fetal adrenal cortex using immunohistochemistry. In the human and rhesus fetal adrenals at all gestational ages, P450c21 immunoreactive staining was detected in all of the cortical zones. Staining for P450c21 was less intense in the fetal zone than in the definitive and transitional zones and was increased in adrenals from rhesus monkey fetuses treated with metyrapone, which was administered to increase endogenous ACTH secretion. P450c11 peptide was detected with a polyclonal antibody that also detects P450c11AS (aldosterone synthase). Staining for P450c11 was detected early in gestation only in the transitional zone. Later in gestation, P450c11 staining was found in the definitive and transitional zones and was increased by metyrapone treatment. These data suggest differential regulation of P450c21 and P450c11 expression in the primate fetal adrenal cortex. The presence of P450c21 throughout gestation in all cortical zones suggests that this is not a rate-limiting step in steroidogenesis. The colocalization of P450c21 and P450c11 in the transitional zone further implicates this cortical compartment as the site of *de novo* glucocorticoid synthesis. The onset of P450c11 in the definitive zone late in gestation indicates that this zone does not have

the capacity to synthesize aldosterone until near term. The onset of 3βHSD expression in definitive and transitional zone cells late in gestation (73), coupled with the presence of P450c21 and P450c11, suggests that these cells acquire the capacity to synthesize mineralocorticoids and glucocorticoids, respectively. Thus, the ontogeny of 3βHSD expression in specific zones allows the functional differentiation of the primate fetal adrenal cortex. Studies in the fetal rhesus monkey *in vivo* suggest that the ontogeny of 3βHSD in the fetal adrenal cortical zones is regulated by ACTH (73) (Fig. 4). Future studies of the ontogeny and localization of P450c21 and P450c11 in the human fetal adrenal cortex will provide

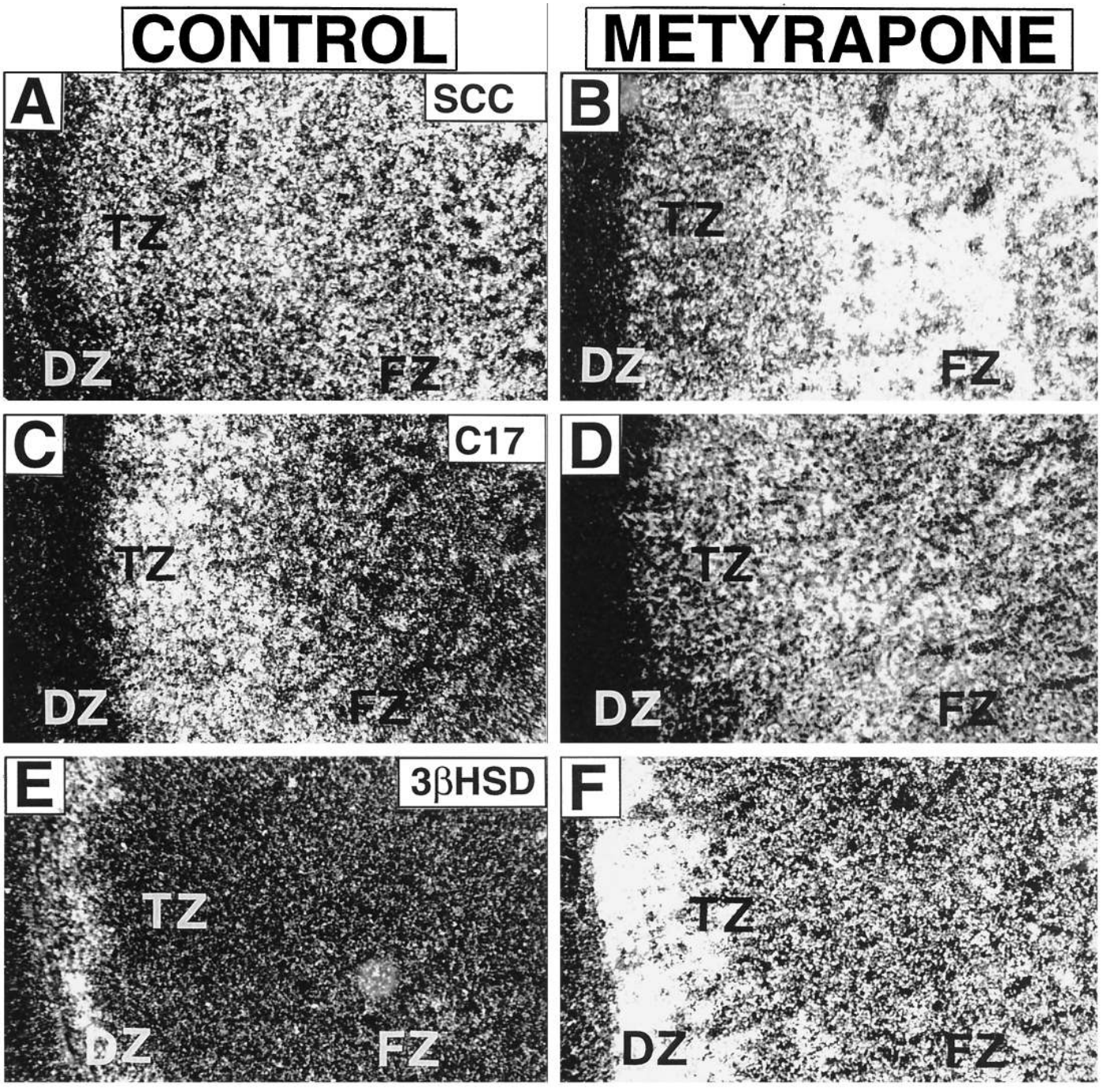

FIG. 4. Photomicrographs of sections of adrenal glands from 140-day gestation control (A, C, and E) and 137-day gestation metyrapone-treated (B, D, and F) fetal rhesus monkey. Localization of mRNAs encoding P450scc (A and B), P450c17 (C and D), and 3βHSD (E and F) was determined by *in situ* hybridization. White grains visualized by darkfield optics indicate positive hybridization. The location of each cortical zone is indicated by the following abbreviations: DZ, definitive zone; TZ, transitional zone, and FZ, fetal zone (see Fig. 1 for lightfield micrograph). Note the lack of P450c17 expression in the definitive zone and the increase in 3βHSD expression in the definitive and transitional zones in response to metyrapone treatment. Magnification, ×109. [From C.L. Coulter *et al.*: *Endocrinology* 137:4953–4959, 1996 (73). © The Endocrine Society.]

valuable information and should help resolve the issue of whether cortisol is produced *de novo* or derived from progesterone early in gestation.

Taken together, these data indicate that each fetal adrenal cortical zone has a different rate of functional maturation depending on the ontogeny of expression of specific steroidogenic enzymes. Furthermore, the human fetal adrenal cortex appears to be composed of three functionally distinct zones: 1) the definitive zone, which late in gestation is the likely site of mineralocorticoid synthesis, 2) the transitional zone, which appears to be the site of glucocorticoid synthesis as it expresses all the necessary biosynthetic enzymes, and 3) the fetal zone, which is the site of Δ^5-steroid production, particularly DHEA-S (Fig. 5).

An intriguing characteristic of the fetal zone is that its cells do not express 3βHSD *in vivo* but will readily express this enzyme *in vitro* if they are stimulated by ACTH. More recently, we have found that the *in vivo* phenotype of fetal zone cells is maintained *in vitro* when cells are exposed to relatively low concentrations of ACTH (74). In most studies, cultured fetal zone cells are exposed to an ACTH concentration of 0.1–10 nM. However, based upon the data of Winters *et al*. (14), circulating concentrations of ACTH in the human fetus at midgestation are around 50 pM. We found that exposure of fetal zone cells to this concentration of ACTH *in vitro* increased DHEA-S production but had no effect on cortisol synthesis (Fig. 6). Fetal zone cells can be induced to synthesize cortisol *de novo* if they are exposed to a sufficiently high concentration of ACTH. Thus, it is possible that, although fetal zone cells exhibit some differentiation when placed under culture conditions, their expression of 3βHSD *in vitro* may be due to exposure to supraphysiological concentrations of ACTH.

3. Responsiveness to ACTH. The rapid growth and abundant steroid production by the human fetal adrenal cortex are not paralleled by increases in plasma ACTH (14). Instead, Winters *et al*. (14) found that mean circulating ACTH levels in the human fetus decrease by almost 50% during this period of maximum fetal zone enlargement. An obvious explanation for this paradox, and one which is supported by studies in fetal sheep *in vivo* (75–78), is that responsiveness of the fetal adrenals to ACTH increases during the second and third trimesters. Studies of cultured human fetal adrenal cortical cells have shown that responsiveness to ACTH is augmented by ACTH itself (79–81) and other factors, particularly the insulin-like growth factors I and II (74). Exposure of fetal zone and definitive zone cells to ACTH increases the subsequent acute response to further ACTH stimulation (79, 80). This increased responsiveness is due to increased ACTH-binding capacity (80) as a result of increased expression of the

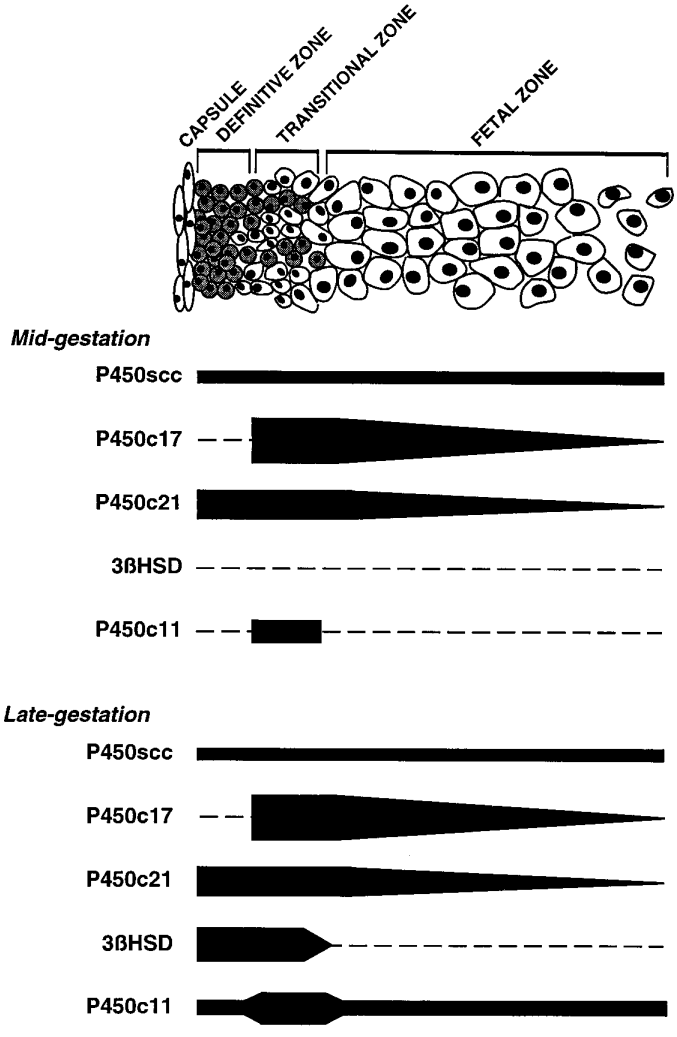

FIG. 5. Schematic representation of the localization of expression of P450 scc, P450c17, P450c21, 3βHSD, and P450c11 in the primate fetal adrenal cortex during mid- and late gestation. Thickness of the line indicates relative abundance of expression. *Dashed line* indicates lack of expression. Note the lack of P450c17 expression in the definitive zone at all stages of gestation and the ontogenetic expression of 3βHSD only in the definitive and transitional zones late in gestation. [Derived from Refs. 28, 65–70.]

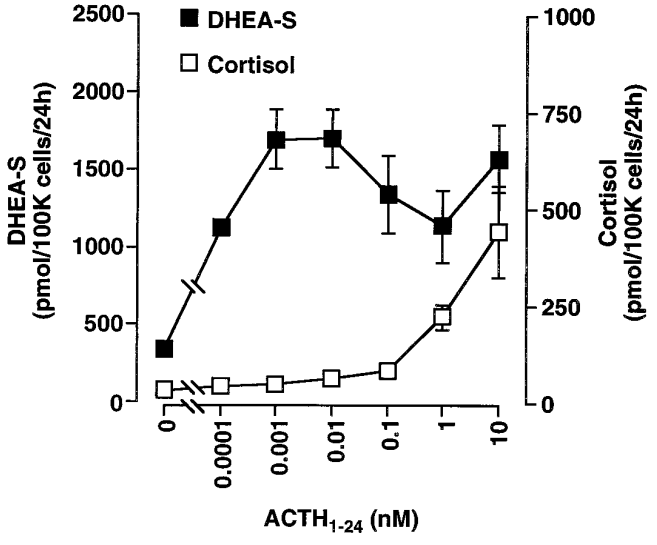

FIG. 6. Dose-responsive effect of ACTH on DHEA-S and cortisol production by cultured human fetal zone cells derived from midgestation human fetal adrenals. Cells were exposed to ACTH (0–10 nM) for 24 h. Data are mean ± SE of triplicate samples. [Adapted from S. Mesiano *et al.*: *J Clin Endocrinol Metab* 82:1390–1396, 1997 (74). © The Endocrine Society.]

ACTH receptor (81). Interestingly, similar effects of ACTH have been observed in adult humans *in vivo* (82) and in experimental animals (76, 83, 84), indicating that, under normal circumstances, the adrenals are not maximally sensitive to ACTH but instead have the capacity for increased responsiveness. Although others have shown that ACTH production by human fetal pituitary incubates (85) and abundance of mRNA encoding ACTH in the baboon fetal pituitary (86) increase with advancing gestation, the report by Winters *et al*. (14) is the only study to date which directly examined circulating ACTH concentrations in the human fetus. However, in that study blood samples from fetuses were not collected until 30 min after the uterine arteries were clamped for hysterectomy, raising the possibility that endogenous fetal ACTH may have degraded before assay. The extent to which the fetal adrenal cortex is exposed to ACTH in the primate throughout gestation therefore remains uncertain.

Ligand-induced up-regulation of ACTH receptor expression may be an important adaptive process directed toward optimizing adrenal responsiveness to ACTH in concert with physiological requirements for hypothalamic-pituitary-adrenal activity. This is particularly important for the physiological response to stress and the maintenance of metabolic homeostasis in which the adrenals play a pivotal role. Inhibition, by ligand-induced receptor down-regulation, of mechanisms involved in the response to stress would be detrimental, whereas enhancement by ligand-induced up-regulation would be advantageous by permitting a more efficient and rapid response. Whether such a process is advantageous for fetal development is not clear. It is possible that this up-regulation is part of the process by which the fetus prepares for responding to the stresses of delivery and the perinatal period. Clearly, an efficient and functionally responsive fetal adrenal cortex is essential for survival during the perinatal period.

In summary, functional development of the primate fetal adrenal cortex is a complex process involving the temporal and spatial expression of specific steroid-metabolizing enzymes and changes in responsiveness to ACTH. Early in gestation (8–12 weeks), the glands appear to be capable of cortisol and DHEA-S synthesis. However, it is not clear whether cortisol is produced from progesterone or whether the full complement of enzymes necessary for *de novo* cortisol synthesis are expressed. Later in gestation, the ontogenetic expression of 3βHSD, first in the definitive zone and subsequently in the transitional zone, appears to reflect the functional maturation of these zones as analogs of the zonae glomerulosa and fasciculata, respectively. Throughout gestation, the fetal zone expresses high levels of P450c17 but lacks 3βHSD, consistent with its continued production of DHEA-S (Fig. 4).

III. Regulation

Literature before 1990 concerning the regulation of primate fetal adrenal cortical growth and function has been elegantly reviewed by Pepe and Albrecht (87). To avoid repetition, the following discussion will be limited to more recent findings, particularly those concerning the role of peptide growth factors, orphan nuclear receptors, and substances produced by the placenta.

A. The fetal pituitary and ACTH

It is not unexpected that the extraordinary growth and steroidogenic activity of the primate fetal adrenal cortex are dependent on an intact fetal pituitary gland as it produces ACTH, the primary tropic regulator of the adrenal cortex, postnatally. Several observations firmly establish the pivotal role of ACTH secreted by the fetal pituitary in primate adrenal cortical regulation: 1) Disruption of hypothalamic-pituitary function in the human fetus (*e.g*. in anencephalics or associated with maternal glucocorticoid treatment) results in failure of the fetal zone to develop beyond the size attained at approximately the 15th week of gestation (9, 10, 88, 89) and is associated with dramatically reduced estrogen concentrations in the maternal circulation (49, 90). 2) Administration of dexamethasone to normal human fetuses *in utero* reduces maternal estrogen levels (91), and dexamethasone treatment of pregnant rhesus monkeys during late gestation causes atrophy of the fetal zone and a marked decrease in maternal plasma estradiol and estrone levels and fetal plasma cortisol levels (12, 13). Similarly, experimental anencephaly (produced by fetal decapitation at around day 80 of gestation; term = 165 $\pm$ 5 days) in the fetal rhesus monkey also caused marked atrophy of the fetal adrenal cortex and decreased maternal estrogen levels (12, 92); 3) *In utero* administration of ACTH to normal human fetuses (91) increases maternal estrogen levels and in anencephalics (89), partially restores adrenal size. 4) In congenital adrenal hyperplasia, the fetal zone is markedly enlarged, and adrenal androgen concentrations are elevated to virilizing levels (93). 5) In fetal rhesus monkeys, administration of ACTH increases fetal cortisol and maternal estrone concentrations (12), and increased endogenous ACTH secretion (induced by metyrapone treatment) increases fetal adrenal cortical growth and advances functional maturation, as assessed by 3βHSD expression (Refs 32 and 73 and see Fig. 4).

The mechanism by which ACTH regulates fetal adrenal cortical growth and function is not clearly understood. The actions of ACTH are mediated via its interaction with specific receptors on the cell surface of adrenal cortical cells. A human ACTH receptor has been cloned and characterized (94). This receptor is coupled to heterotrimeric guanine nucleotide-binding proteins that activate adenylate cyclase leading to an increase in intracellular cAMP that activates protein kinase A and initiates the cascade of intracellular signaling events. Consequently, the actions of ACTH can be mimicked by cAMP analogs (*e.g*. 8-bromo-cAMP) or substances that increase adenylate cyclase activity (*e.g*. forskolin). The signaling events downstream from the activation of protein kinase A have not been characterized. The contribution of other signaling pathways in adrenal cortical steroidogenic activity has been reviewed by Pepe and Albrecht (87).

Although the principal regulator of fetal adrenal cortical development appears to be ACTH, several observations support the concept that human fetal adrenal growth and function also are influenced by factors that act independently from, or in conjunction with, ACTH. These lines of evidence

are as follows: 1) human fetal adrenal cortical growth and steroidogenic activity are maximal during mid- to late gestation even though circulating ACTH concentrations in the fetus appear to be decreasing (14); 2) before 10–15 weeks of gestation, adrenal development in anencephalic fetuses (presumably with markedly reduced ACTH) is normal, but thereafter the fetal zone fails to develop and does not exhibit its characteristic growth and steroidogenic activity (9, 10), indicating that early in gestation fetal zone growth and function are independent of ACTH; 3) in contrast, the definitive zone appears normal in anencephalic fetuses despite the absence of ACTH stimulation (9, 10, 89), suggesting that its growth is not dependent on ACTH at any stage in gestation, although its functional maturation appears to be regulated by ACTH (73); 4) the fetal zone rapidly involutes once the newborn is delivered and separated from the placenta despite relatively unchanged exposure to ACTH (29), indicating that fetal zone growth and function are maintained by a factor(s) specific to intrauterine life, and 5) ACTH is not a growth factor *per se* and is not a mitogen for adrenal cortical cells *in vitro* (95, 96). However, it stimulates proliferation of adrenal cortical cells *in vivo*, suggesting that its proliferative actions may be mediated by growth factor(s).

B. Growth factors

Specific growth factors, acting in an autocrine and/or paracrine fashion, are likely candidates as mediators and/or modulators of the trophic actions of ACTH on the primate fetal adrenal cortex. This notion is not without precedent, as several classic growth-promoting hormones are known to act through the stimulation of local autocrine/paracrine growth factors. Studies of growth factor involvement in the regulation of fetal adrenal development have addressed: 1) the effects of growth factors on proliferation and function of cultured fetal adrenal cortical cells; 2) growth factor expression by the fetal adrenals, and 3) the regulation of this growth factor expression by the fetal adrenals.

1. Basic fibroblast growth factor (bFGF). Basic FGF is a peptide mitogen that stimulates the proliferation of mesodermal- and neurectodermal-derived cells and also is a potent angiogenic and neurotrophic agent. It is a member of a family of related peptides that include acidic FGF, FGF-6, FGF-7, keratinocyte growth factor, *hst*, and *int* (97). Four different FGF receptors have been identified, each with intrinsic tyrosine kinase activity. Differential mRNA splicing results in multiple isoforms of each receptor (98).

The mitogenic effects of bFGF on adrenal cortical cells was first observed in the Y-1 mouse adrenal cortical cell line (99). Gospodarowicz *et al*. (100) and Hornsby and Gill (101) subsequently reported that bFGF is a potent mitogen for primary cultures of bovine adult adrenal cortical cells, and both groups suggested that it mediates the growth-stimulatory actions of ACTH *in vivo*. Basic FGF was later purified from adult bovine adrenals (102) and shown to be synthesized by cultured bovine adrenal cortical cells (103). More recently, bFGF has been shown to be involved in the compensatory adrenal growth response to unilateral adrenalectomy in the rat (104).

Crickard *et al*. (105) examined the effect of bFGF on the proliferation of fetal and definitive zone cells from midgestation human adrenals. Proliferation of both cell types was stimulated by bFGF, a finding that was later confirmed by Hornsby *et al*. (106). Interestingly, bFGF elicited a greater proliferative response (relative to control) in definitive zone cells (4-fold increase) than in fetal zone cells (2-fold increase). This suggests that the definitive zone may be more sensitive than the fetal zone to the mitogenic actions of bFGF and that bFGF may preferentially influence definitive zone development *in vivo*.

In light of its potent mitogenic actions on human fetal adrenal cortical cells, we hypothesized that bFGF is a local mediator of the stimulatory effects of ACTH (16). Therefore, we determined whether the human fetal adrenals express bFGF and, if so, whether this expression is regulated by ACTH. Basic FGF bioactivity and mRNA were detected in protein and total RNA extracts, respectively, from midgestation human fetal adrenals. Messenger RNA encoding bFGF also was detected in cultured human fetal adrenal cortical cells and interestingly, its abundance was increased 2- to 3-fold by ACTH. Thus, bFGF, a potent mitogen for human fetal adrenal cortical cells, is expressed by these cells and regulated by ACTH. Therefore, bFGF may be an important mediator of ACTH action in human fetal adrenal development. It is of interest that bFGF also is a potent angiogenic factor (107) and that the adrenal cortex, particularly the fetal zone, is one of the most highly vascularized organs in the human fetus (26, 27). It is possible that bFGF not only affects the growth of the fetal adrenal cortex but also its vascularization and may be an important local mediator of these events in response to ACTH. In this regard, our preliminary data also indicate that vascular endothelial growth factor, a potent stimulator of angiogenesis, may promote vascularization of the developing adrenal cortex (108).

2. Epidermal growth factor (EGF). The effects of EGF on primary adrenal cortical cell cultures was first examined by Gospodarowicz *et al*. (100) who reasoned that because EGF is a potent mitogen for granulosa cells (109), and granulosa and adrenal cortical cells are derived from the same embryonic germ layer, *i.e.* the celomic epithelium, it also may be mitogenic for adrenal cortical cells. Their studies of cultured adult bovine adrenal cortical cells, however, revealed that EGF was not mitogenic, a finding that was later confirmed by Hornsby *et al*. (106). In contrast to these negative results in bovine adrenal cortical cells, Crickard *et al*. (105) found EGF to be a potent mitogen for cultured fetal and definitive zone cells from midgestation human fetal adrenals, a finding that was also confirmed by Hornsby *et al*. (106). Interestingly, as with their response to bFGF, definitive zone cells were more responsive to the proliferative action of EGF than were fetal zone cells, suggesting that EGF (like bFGF) preferentially regulates definitive zone growth. High-affinity surface-binding sites, characteristic of EGF receptors, were detected in both cell types (105). These findings imply that the human fetal adrenal cortex is a target for EGF (or other EGF receptor ligands).

The effect of EGF treatment on fetal adrenal development in the late gestation rhesus monkey *in vivo* has been exam-

ined (110). Treatment with EGF significantly increased adrenal weight and the width of the definitive zone. Interestingly, Coulter *et al*. (110) found that cell density in the definitive zone of EGF-treated animals was less than that of controls, indicating that definitive zone enlargement in EGF-treated animals was due to cellular hypertrophy rather than hyperplasia. This finding indicates that EGF stimulated hypertrophy and not hyperplasia of definitive zone cells, an unexpected effect given the potent mitogenic action of EGF on definitive zone cells *in vitro*. Thus, *in vivo* EGF may not be a direct mitogen for definitive zone cells but instead may affect its growth by modulating the hypothalamic-pituitary axis and possibly increasing ACTH secretion and/or ACTH responsiveness. EGF has been shown to affect the fetal hypothalamic-pituitary-adrenal axis in other species: in fetal sheep, EGF stimulates secretion of cortisol from the fetal adrenals, corticotropin releasing hormone (CRH) from the hypothalamus, and ACTH from the pituitary (111, 112). Therefore, EGF could affect fetal adrenal development indirectly by modulating the fetal hypothalamic-pituitary axis. This also is suggested by the finding of Coulter *et al*. (110) that EGF treatment *in vivo* increased the amount of 3βHSD protein in definitive and transitional zone cells of fetal rhesus monkeys. Similar effects were reported in fetal rhesus monkeys in which endogenous ACTH secretion was increased by administration of metyrapone (32). Thus, in addition to its potential direct effect on adrenal cortical cell proliferation, EGF also may modulate adrenal growth and functional maturation by affecting the hypothalamic-pituitary-adrenal axis.

EGF is a member of a large family of peptide growth factors that includes transforming growth factor-α (TGFα), vaccinia virus growth factor, amphiregulin, heparin-binding EGF-like factor, and betacellulin (113). Each of these peptides shares considerable sequence homology with EGF and, because they all bind to the EGF receptor, have similar biological activities. Therefore, studies of EGF action on adrenal development must take into account the multiple ligands for the EGF receptor and that, although EGF may have effects on cultured cells, it may not be a biologically significant ligand *in vivo*. Sasano *et al*. (114), aware of this issue, examined the expression of EGF, TGFα, and the EGF receptor in human adult adrenals. They found that TGFα and the EGF receptor were expressed, whereas EGF was not, and concluded that TGFα is the significant locally produced ligand for the EGF receptor in adult human adrenals. Similar experiments in human fetal adrenal glands, in which the expression of EGF, TGFα, and the EGF receptor were examined, yielded similar findings (115). Immunostaining for TGFα was greatest in the fetal and transitional zones and less intense in the definitive zone. Immunostaining for the EGF receptor was detected in each of the cortical zones with equal intensity. These data indicate that TGFα may be the predominant locally produced ligand for the EGF receptor in the developing human fetal adrenal cortex. These observations indicate that activation of the EGF receptor on human fetal adrenal cortical cells may be an important component in the regulation of fetal adrenal development.

3. Insulin-like growth factors I and II (IGF-I and IGF-II). IGF-I and IGF-II affect growth and function in a wide variety of cell types and can act as autocrine, paracrine, or endocrine factors (116). IGF-I (formerly known as somatomedin-C) mediates many of the somatotropic actions of GH (117). Although the role of IGF-II is less defined, it is thought to be involved in the regulation of fetal development because its circulating and tissue levels are highest during fetal life and decrease postnatally (118). Two IGF receptors, designated type I and type II, have been identified (119). The type I receptor is structurally related to the insulin receptor and binds both IGF-I and IGF-II with high affinity and insulin with lower affinity. The type II receptor, also known as the mannose-6-phosphate receptor, binds IGF-II with high affinity but will not bind IGF-I or insulin. Most of the known actions of IGF-I and -II appear to be mediated via activation of the type I receptor. Effects mediated via the type II receptor are not clearly understood, although this receptor is known to be involved in targeting lysosomal enzymes. The IGF system is made more complex by the presence of six high-affinity IGF-binding proteins that associate with the IGF peptides and modulate their biological activity (120).

The IGF peptides and their receptors have been identified in normal and neoplastic adrenals (121). Growth and differentiated function in several steroidogenic cell types, including granulosa (122, 123), Leydig (124, 125), and adrenal cortical cells (126), are modulated by IGFs. In adult bovine (127) and fetal ovine (128) adrenal cortical cells, IGF-I increases proliferation and enhances the steroidogenic responsiveness to ACTH. IGF-I augments responsiveness of adult bovine adrenal cortical cells to ACTH by increasing ACTH receptors (127). Similar findings were reported by Pham-Huu-Trung *et al*. (129), who showed that IGF-I modulates steroidogenesis in cultured human adult adrenal cortical cells by enhancing responsiveness to ACTH and the activity of key steroidogenic enzymes, including P450c17. These effects of IGF-I on adrenal cortical cells were most likely mediated via the type I receptor, which has been identified in bovine (126) and human (121, 130, 131) adrenal cortical cells. Penhoat *et al*. (132) showed that IGF-I is synthesized by adult bovine adrenal cortical cells and that its secretion is enhanced by ACTH, suggesting that it may be a local paracrine/autocrine mediator of ACTH action.

The role of the IGFs in human fetal adrenal development has also been examined. Han *et al*. (133, 134) studied IGF-I and IGF-II expression in a variety of midgestation human fetal tissues and showed that, consistent with other species, IGF-II is quantitatively the predominant IGF expressed during fetal life. In the fetal adrenals, abundance of mRNA encoding IGF-II was high (second only to the liver), whereas mRNA encoding IGF-I was low. Ilvesmäki *et al*. (135), using RT-PCR analysis of total RNA extracted from whole adrenals, demonstrated that all of the components of the IGF system (*i.e*. IGFs, receptors, and binding proteins) are expressed by human fetal adrenals. Interestingly, Han *et al*. (133), using *in situ* hybridization analysis, found that mRNAs encoding the IGFs were generally restricted to mesenchymal cells, and in the adrenals were detected only in the capsule. These findings led Han *et al*. to hypothesize that IGFs produced by the mesenchymal component regulates the growth of associated epithelial elements.

Expression of IGF-I and IGF-II in cultured cortical cells

from midgestation human fetal adrenals was first examined by Voutilainen and Miller (136) who found that the high level of IGF-II expression reported by Han *et al*. (133) *in vivo* also was present *in vitro* and could be stimulated by ACTH and factors that increase intracellular cAMP, a finding that we later confirmed (137). This effect of ACTH is inconsistent with IGF-II expression by capsular fibroblasts as suggested by Han *et al*. (133), insasmuch as fibroblasts are not known to be responsive to ACTH, and their presence in the cell culture preparations was minimal. Instead, the data suggest that IGF-II was expressed by the ACTH-responsive cortical cells. In light of this, we reexamined the localization and regulation of IGF-I and IGF-II expression in midgestation human fetal adrenals (137).

In situ hybridization analysis revealed that IGF-II is expressed by all cortical cells in relatively high abundance, whereas IGF-I is only detectable in the adrenal capsule. Similarly, in cultured human fetal adrenal cortical cells, mRNA encoding IGF-II is highly abundant in the cortical cells and not present in the contaminating fibroblasts, and its abundance in cortical cells is markedly up-regulated by ACTH. In contrast, IGF-I mRNA was not detected in cultured fetal adrenal cortical cells and could not be stimulated with ACTH. These findings recently have been confirmed in the fetal rhesus monkey *in vivo* in which endogenous ACTH secretion was increased by administration of metyrapone (32). Adrenals of metyrapone-treated fetuses were larger than controls and expressed higher levels of IGF-II but not IGF-I. Taken together, these data strongly implicate IGF-II as an important local regulator of fetal adrenal development and a possible mediator of at least some of the trophic actions of ACTH. Interestingly, cultured adrenal cortical cells from a 6-week human neonate responded to ACTH with increased cortisol production but failed to express IGF-II (138), suggesting that expression of IGF-II by the human adrenal cortex and its regulation by ACTH are unique to fetal life.

The role of the IGFs in human fetal adrenal development was further characterized in studies of their effects on the growth and function of cultured human fetal adrenal cortical cells (137). Both IGF-I and IGF-II are specific mitogens for human fetal adrenal cortical cells. Interestingly, the IGFs act cooperatively with bFGF and EGF, other known mitogens for human fetal adrenal cortical cells (see above), resulting in an additive effect on cell proliferation. That both IGF-I and IGF-II stimulated proliferation in an almost equipotent fashion suggests that their mitogenic actions are mediated through a common receptor, most likely the type-I IGF receptor. In other cell types for which IGF-II is a mitogen, its actions have been found to be mediated via the type-I IGF receptor (139, 140).

In conjunction with its mitogenic activity, IGF-II also affects the differentiated function of human fetal adrenal cortical cells. In cultured fetal zone cells, IGF-II augments ACTH-stimulated cortisol and DHEA-S production (Fig. 7) and ACTH-stimulated expression of the steroidogenic enzymes P450scc, P450c17, and 3βHSD (74, 141) (Fig. 8). As with its mitogenic actions, the effects of IGF-II on steroid production were mimicked by IGF-I, again suggesting that the actions of both peptides were mediated by the type-I IGF receptor. Both IGF-I and IGF-II directly up-regulated basal

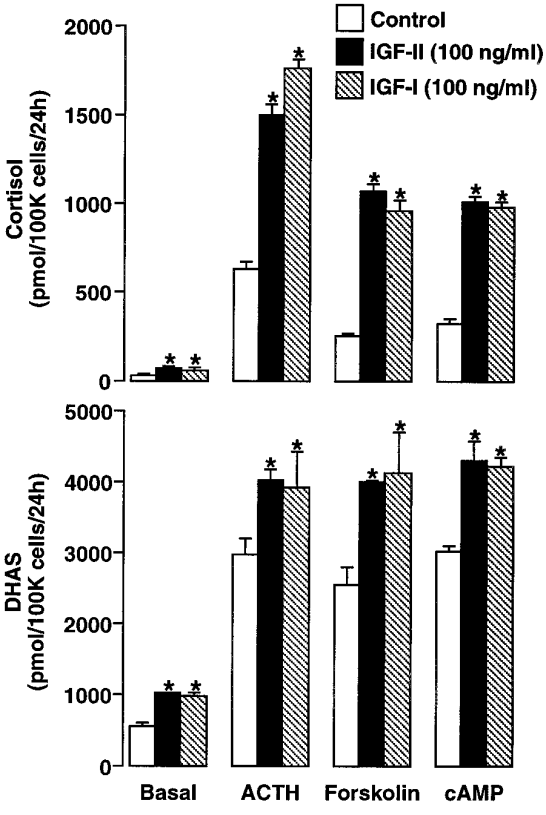

FIG. 7. Effects of IGF-I and IGF-II on basal and agonist-stimulated [ACTH (1 nM), forskolin (1 μM), and cAMP (1 mM)] production of cortisol and DHEA-S by primary cultures of fetal zone cells. Cells were exposed to IGF-I or IGF-II for 24 h, media were changed, the IGFs were replenished, and some wells were exposed to agonist for a further 24 h. Data are mean ± SE of triplicate samples and are representative of three separate experiments. (* $P < 0.05$). [Reproduced with permission from S. Mesiano *et al.*: *J Clin Endocrinol Metab* 82:1390–1396, 1997 (74). © The Endocrine Society.]

expression of P450c17 as assessed by mRNA abundance, but did not affect basal expression of P450scc or 3βHSD (Fig. 8). A similar effect of IGFs on P450c17 activity and expression has been reported in human adult adrenal cortical cells (129, 142). The increased P450c17 activity suggests that IGFs may be important regulators of adrenal androgen production. Activation of the type-I IGF receptor by either IGF-I (postnatally) or IGF-II (prenatally) may directly augment adrenal androgen synthetic capacity by augmenting P450c17 expression and activity. This may be an important mechanism by which adrenal androgen production is regulated during fetal and postnatal life. Interestingly, the onset of adrenarche coincides with an increase in circulating IGF-I (143).

Responsiveness to ACTH appears to be an important issue in human and nonhuman fetal adrenal development. Similarly, at adrenarche, adrenal androgen production increases even though circulating concentrations of ACTH do not change (144), suggesting that changes in adrenal responsiveness to ACTH are involved. The IGFs increase the responsiveness of fetal and adult adrenal cortical cells to ACTH (74, 129, 141, 142). A similar effect has been reported in adult bovine (127) and fetal ovine (128) adrenal cortical cells. In adult human (142) and bovine (127) adrenal cortical cells, the

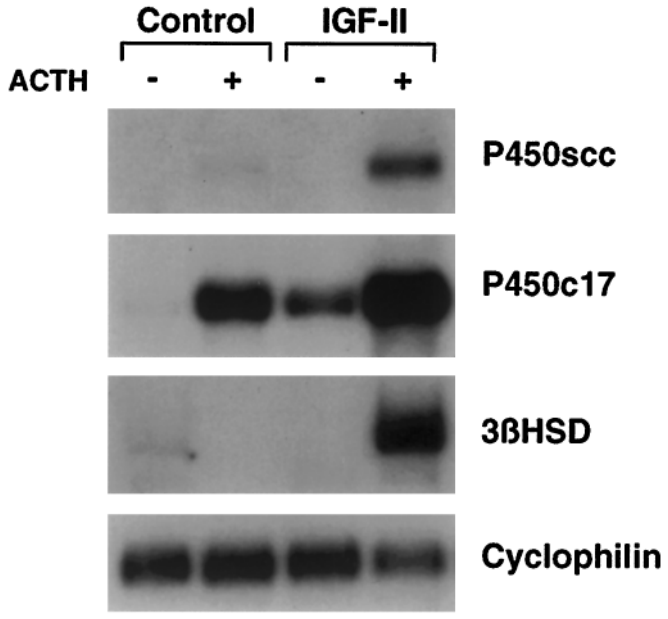

FIG. 8. Effects of IGF-II (100ng/ml) and ACTH1–24 (1 nM) on steady-state abundance of mRNAs encoding P450scc (overnight exposure), P450c17 (2 h exposure), 3βHSD (2 day exposure) and cyclophilin (4 h exposure) in fetal zone cells. Northern blot analysis of total RNA (10 μg) from cultured midgestation fetal zone cells exposed to IGF-II, ACTH, or both for 24 h. [Adapted from S. Mesiano and R. B. Jaffe: *J Clin Endocrinol Metab* 77:754–758, 1993 (141). © The Endocrine Society.]

increase in ACTH responsiveness induced by the IGFs is associated with increased expression of the ACTH receptor. However, in human fetal adrenal cortical cells, neither IGF-I nor IGF-II affect ACTH receptor expression even though responsiveness to ACTH is increased (74). Furthermore, the IGFs also augmented responsiveness to forskolin and cAMP (Fig. 7), indicating that their stimulatory effects on ACTH responsiveness were exerted at some point distal to ACTH receptor binding and activation.

4. Activin/inhibins. Activin and inhibin are homodimeric (βA-βA, βB-βB, or βA-βB) and heterodimeric (α-βA or α-βB) glycoproteins, respectively, which are structurally related to other members of the TGFβ family of peptides. Both inhibin and activin originally were isolated from follicular fluid based on their ability to modulate FSH secretion from the pituitary (145). Inhibin suppresses, while activin stimulates, FSH secretion. It is now apparent that activin's actions extend beyond the pituitary-gonadal axis to affect many key biological functions. The amino acid sequence of activin is strongly conserved between species and is closely related to that of TGFβ, mullerian inhibiting substance, fly decapentaplegic complex, and bone morphogenesis proteins, all of which are regulators of development and differentiation of a variety of tissues. Activin may subserve different functions in the organism depending on the stage of development. For example, activin induces mesoderm development in *Xenopus* during early embryogenesis (146), whereas later in life it appears to be a significant regulator of ovarian folliculogenesis (147). A family of activin receptors has been characterized according to subunit structure. These include the type-I activin receptor and two homologous type-II receptors (IIA and IIB) (148).

Activin appears to play a role in the regulation of adrenal cortical development and function. This is not unexpected as activin has profound effects on the growth and function of granulosa cells (149), which originate from the same germ layer as adrenal cortical cells. The subunits of activin and inhibin (α, βA and βB) have been identified in the adrenal glands of the adult rat (150) and the fetal and adult sheep (151). Interestingly, inhibin knockout mice develop adrenal tumors, suggesting that inhibin acts as a tumor suppressor gene in adrenal cortical cells (152). Activin/inhibin subunit localization, as well as the mitogenic and steroidogenic actions of activin and inhibin in human fetal and adult adrenals, has been examined (153, 154). Each of the activin/inhibin subunit proteins and their mRNAs were detected in fetal and adult adrenals by immunohistochemistry. In midgestation fetal adrenals, specific immunostaining for the subunits was localized in a scattered pattern in both the fetal and definitive zones, whereas specific staining for intact activin-A (βA-βA homodimer) was detected predominantly in the definitive and transitional zones. In cultured fetal adrenal cortical cells, ACTH stimulated secretion of immunoreactive α-subunit. This suggests that ACTH stimulates inhibin production by fetal adrenal cortical cells, as the α-subunit is only present in the inhibin molecule. ACTH enhances the abundance of mRNAs encoding the α- and βA-subunits, but not the βB-subunit, in cultured fetal adrenal cortical cells. Thus, during midgestation, the human fetal adrenals express each of the activin/inhibin subunits and appear to produce immunoreactive activin-A in the definitive and transitional zones. ACTH stimulates expression of the α- and βA-subunits, suggesting that fetal adrenal production of activin and inhibin is under trophic hormone regulation.

In cultured human luteinizing granulosa cells activin stimulates proliferation and steroidogenesis, whereas inhibin has no effect (149). In contrast to its mitogenic affects on granulosa cells, recombinant human activin-A inhibits proliferation of fetal zone cells and, at the same time, increases ACTH-stimulated cortisol production (153, 154). Activin has no effect on DHEA-S production by fetal zone cells or growth and steroidogenesis in definitive zone or adult adrenal cortical cells. Recombinant human inhibin has no effect on proliferation or function of any of the adrenal cortical cell types. Thus, activin acts directly and specifically on fetal zone cells to inhibit their rate of growth and enhance their capacity for cortisol production in response to ACTH. Activin may inhibit fetal zone cell growth by stimulating cellular apoptosis and therefore may be involved in the postnatal demise of the fetal zone, a process involving apoptosis (30). In addition, activin may coordinately stimulate the differentiation of other fetal zone cells into a cortisol-producing phenotype.

5. TGFβ. TGFβ is the prototypical peptide of a large family of growth factor proteins including activin, inhibin, mullerian inhibiting substance, bone morphogenic protein, and several closely related proteins designated TGFβ2, TGFβ3, TGFβ4, and TGFβ5 (155). Specific receptors for TGFβ have been identified on almost all mammalian cells. The effect of TGFβ is variable and appears to be dependent on the cell

type. In general, TGFβ stimulates proliferation of cells of mesenchymal origin and inhibits proliferation of cells of epithelial or neurectodermal origin (156). TGFβ also modulates the differentiated function of cells and, in particular, has marked effects on the function of steroid-producing cells. In adult bovine and ovine adrenal cortical cells, TGFβ inhibits basal and ACTH-stimulated cortisol and agonist-stimulated aldosterone production (157–160) and reduces ACTH receptor binding (161). TGFβ is produced by, and interacts with, specific receptors on adult bovine adrenal cortical cells (162), suggesting that it acts as an intraadrenal autocrine/paracrine factor.

Several studies have indicated that TGFβ is involved in the regulation of human fetal adrenal development. Riopel *et al*. (163) investigated the effects of TGFβ on growth and function of cultured fetal zone cells and found that it significantly inhibits fetal zone cell proliferation but had no effect on steroidogenesis. Subsequently, Spencer *et al*. (154) confirmed these inhibitory effects of TGFβ on fetal zone cell proliferation. Parker *et al.* (164) later reported that TGFβ also inhibits proliferation of definitive zone cells and, in a more detailed study of the effects of TGFβ on fetal adrenal steroid production, Stankovic *et al*. (165) found that both basal and ACTH-, forskolin-, and cAMP-stimulated DHEA-S and cortisol production and expression of P450c17 by fetal and definitive zone cells were inhibited by TGFβ. They also showed that TGFβ binds to specific sites on human fetal adrenal cortical cells and that these binding sites are regulated by ACTH (166). Lebrethon *et al*. (167) reported similar findings and, in addition, showed that TGFβ has no effects on ACTH receptor and P450scc expression but enhances ACTH-stimulated expression of 3βHSD, an intriguing finding in light of its inhibitory effects on steroid production. Thus, TGFβ inhibits proliferation and steroid production by fetal and definitive zone cells, likely via interaction with specific cell surface-binding sites. Whether TGFβ is expressed by human fetal adrenal cortical cells is uncertain. Taken together, these data indicate that TGFβ-related peptides (particularly TGFβ and activin) are significant negative regulators of human fetal adrenal growth and may play an important role in balancing the positive effects of other growth factors during adrenal development.

C. Nuclear receptors/transcription factors

Nuclear receptors are essential elements in cellular regulation as they mediate the link between an extracellular signal and the transcriptional response (Ref. 168 for review). Examples of well characterized nuclear receptors for which the ligand is known include the steroid hormone receptors, thyroid hormone receptor, retinoic acid receptor, and vitamin D receptor. Each of these proteins, when bound to its cognate ligand, acts on specific sequences of DNA (response elements) to either promote or inhibit transcription of target genes. Many transcription factors that share sequence homology (especially in the DNA- and ligand-binding domains) with classical nuclear receptors have been identified, but their ligands have not been identified. These are referred to as "orphan" nuclear receptors. Two of these, steroidogenic factor-1 (SF-1) and DAX-I, appear to play major roles in adrenal development and function.

SF-1 is a transcription factor that regulates the expression of genes encoding steroidogenic enzymes [Refs. 169 and 170 and see review by Parker and Schimmer (170a) in this issue]. Consensus-binding sites for SF-1 have been identified in the promoter regions of genes for most steroidogenic enzymes. In the mouse, SF-1 is expressed in all steroidogenic tissues, including the adrenal gland. Interestingly, SF-1 also is expressed in the embryonic anlage of steroidogenic cells before their acquisition of a steroidogenic phenotype, suggesting that it is involved in the early embryonic development of steroidogenic tissues. To examine the role of SF-1, Luo *et al*. (18) performed targeted disruption of the *Ftz-F1* gene, which encodes SF-1, in intact mice. SF-1 null mice had normal survival *in utero* but died by postnatal day 8 due to severe adrenal insufficiency. These animals lacked adrenal glands and gonads, and all animals (male and female) had female internal reproductive organs. These findings demonstrate the essential role of SF-1 in the embryonic differentiation of steroidogenic tissues, in particular the embryonic development of the adrenal cortex. Thus, the role of SF-1 in mice extends beyond the regulation of steroidogenic enzyme expression and includes the regulation of fundamental events in adrenal and gonadal differentiation. A similar role of SF-1 in the regulation of adrenal development in humans and higher primates is likely but presently is unproven. Several studies have demonstrated SF-1 expression in human steroidogenic tissues, and a growing body of literature demonstrates a role for SF-1 in the regulation of the genes for human steroidogenic enzymes [see review by Parker and Schimmer (170a) in this issue].

Another putative transcription factor (based on its structural homology to other transcription factors) that appears to be an important regulator of adrenal development is DAX-1. Mutations in the DAX-1 gene are responsible for X-linked adrenal hypoplasia congenita (AHC), an inherited disorder in humans that is characterized by hypoplasia of the fetal adrenal glands with absence of the definitive zone and the structural disorganization of the fetal zone (Refs. 171–173 and Ref. 17 for review). The DAX-1 gene was identified by positional cloning and derives its name from its proximity to the dosage-sensitive sex reversal locus and the AHC locus on the X chromosome (173). Like SF-1, DAX-1 also is a member of the nuclear receptor superfamily and as its ligand is not yet known, is considered an orphan nuclear receptor (17). DAX-1 is expressed in the human adrenal gland and testis and, to a lesser extent, in the ovary. Abundance of mRNA encoding DAX-1 is much lower in the adult adrenal than in the fetal adrenal (173), possibly reflecting its more important role in fetal adrenal development. The tissue distribution of DAX-1 expression is similar to that for SF-1 (18, 169, 174), suggesting that these two factors may be coregulators of steroidogenic tissue development and function. Interestingly, putative SF-1 response elements have been identified in the 5′-flanking region of the human DAX-1 (175, 176) gene, suggesting that SF-1 is involved in the regulation of DAX-1 expression and that SF-1 is proximal to DAX-1 in the regulatory cascade. The complete absence of adrenals and gonads associated with SF-1 deficiency, but not with DAX-1 defi-

ciency, suggests that SF-1 is absolutely required for adrenal and gonadal development, whereas the requirement for DAX-1 is partial; DAX-1 appears to be required for the development of the definitive zone but not the fetal zone. The molecular mechanism underlying the actions of DAX-1 are not yet fully characterized.

D. Placental factors

The rapid disappearance of the fetal zone when the newborn and placenta separate at birth suggests that substances produced by the placenta play a role in fetal zone development and/or maintenance. The human placenta produces a large variety of hormones and growth factors, including EGF (177), bFGF (178), and IGF-I and -II (179), which may influence fetal adrenal growth and function. However, the roles of these factors *in vivo* as endocrine regulators of adrenal development are uncertain.

1. Human CG (hCG). Placental production of CG appears to be unique to primate species and is thought to act primarily as a luteotropin to ensure maintenance of the corpus luteum and its production of progesterone during the early stages of pregnancy before the onset of placental progesterone synthesis. In humans, hCG production peaks at around the 10th week of gestation and gradually declines thereafter, reaching a nadir of around 20 IU/ml in the maternal plasma at 17–20 weeks (180). The involvement of hCG in the regulation of human fetal adrenal development was first proposed by Lanman (181). Studies by Lauritzen and Lehmann (182) showed that administration of hCG to human infants during the first week of postnatal life (when fetal zone remnants persist) significantly increases urinary excretion of DHEA, suggesting that it may regulate steroid production by the fetal zone. Interestingly, there was no concomitant rise in 17-hydroxycorticosteroids in response to hCG, whereas in response to ACTH both DHEA and corticosteroid levels increased. Moreover, Lauritzen and Lehmann found that the excretion of DHEA in response to hCG was greater in premature infants than in infants born at term. Based on those data, they proposed that hCG is an adrenocorticotrophic hormone in the human fetus and that it regulates the supply of fetal adrenal DHEA-S as precursor for placental estrogen production. Similar results were obtained by Jaffe *et al*. (6) who found that hCG sustained DHEA-S production by the rhesus monkey adrenal gland when administered during the first postpartum month. Consistent with these *in vivo* findings, Pabon *et al*. (183) recently demonstrated that the zona reticularis of the human adult adrenal cortex (which may be analogous to the fetal zone) expresses the hCG receptor. In contrast, hCG had no effect on fetal adrenal steroid production when administered to human anencephalic (89) or rhesus monkey fetuses during midgestation (12) probably because the fetal adrenal was already maximally stimulated. Several studies have examined the effects of hCG on cultured fetal adrenal cortical cells. Serón-Ferré *et al*. (15), using superfusion techniques on fetal zone tissue derived from human fetuses between 12 and 17 weeks of gestation, found that DHEA-S production increased significantly when hCG was added to the perfusing medium. Lehmann and Lauritzen (184) also found that hCG increased the production of DHEA by slices of human fetal adrenal tissue obtained from 14- to 22-week abortuses. Interestingly, Abu-Hakima *et al*. (185) found that hCG obtained from a commercial source stimulated DHEA-S production by cultured fetal zone cells, whereas hCG obtained from the NIH was without effect. They suggested that the effect seen with the less pure commercial hCG was due to contaminants in the preparation. However, the robust stimulation of fetal zone DHEA-S production by hCG reported by Serón-Ferré *et al*. (15) was obtained using NIH hCG (preparation CR-21). These inconsistent and conflicting data have not yet been resolved, and consequently the role of hCG in primate fetal adrenal development remains uncertain.

2. Placental CRH and ACTH. The human placenta produces CRH, which has identical immunoreactivity and bioactivity to that produced by the hypothalamus (186–194). Interestingly, immunoassayable and bioassayable ACTH activity also has been detected in human placental tissue and dispersed trophoblasts (195), and cultured human placental trophoblastic cells synthesize a high molecular weight protein with physicochemical similarities to POMC (196). Placental CRH can stimulate production of POMC and some of its derivatives, including ACTH, αMSH, and β-endorphin in syncytiotrophoblast cells (194). Thus, it is possible that CRH produced by syncytio- and cytotrophoblast (189) stimulates the production of POMC-derived peptides, including ACTH from the syncytiotrophoblast, which then can influence the fetal adrenal cortex. Although the extent to which placental ACTH contributes to the regulation of the fetal adrenal cortex is not known, it would appear that it is not sufficient to maintain fetal adrenal growth and function in anencephalics, suggesting that its role in the regulation of fetal adrenal development is minor. It is more likely that placental CRH influences the fetal adrenal cortex by modulating the fetal pituitary-adrenal axis. Placental CRH is secreted into the fetal circulation resulting in elevated CRH levels in the fetus throughout gestation (197, 198). Thus, CRH produced by the placenta may modulate fetal adrenal growth and function. Other physiological roles of placental CRH are discussed below.

3. Indirect effect of placental glucocorticoid metabolism. Based on a series of studies in the baboon, Pepe and Albrecht (Refs. 87 and 199 for review) proposed that the placenta, via its capacity to metabolize glucocorticoids of maternal origin, influences ACTH secretion by the fetal pituitary and therefore indirectly affects fetal adrenal development and function. Studies in late gestation humans and rhesus monkeys showed that although maternal cortisol freely traverses the placenta, it is efficiently oxidized to cortisone, a less active glucocorticoid, by the placenta before it can gain access to the fetal circulation (200–204). Thus, the placenta can protect the fetus from the relatively high maternal cortisol concentrations. Pepe and Albrecht (205) examined the metabolism of maternal cortisol by the baboon placenta *in vivo* at various times in gestation and found that, as with the human near term, cortisol is preferentially oxidized to cortisone. However, during midgestation they found that the reduction of

cortisone to cortisol was substantial and exceeded the oxidation of cortisol to cortisone. They proposed that during midgestation, the baboon placenta permits maternal cortisol to enter the fetal circulation. The maternal cortisol then can act on the fetal hypothalamus and pituitary to suppresses ACTH secretion. These investigators proposed that factors other than ACTH (possibly CG) maintain fetal adrenal cortical growth and function during the period when maternal glucocorticoids suppress ACTH production by the fetal pituitary. As pregnancy proceeds, the placental metabolism of maternal cortisol becomes oxidative, leading to decreased cortisol concentrations in the fetal circulation and a concomitant rise in ACTH secretion by the fetal pituitary. The increased ACTH then can stimulate growth of, and DHEA-S production by, the fetal adrenal cortex (Ref. 199 for review).

4. Placental estrogens. Interestingly, in the baboon, oxidation of cortisol to cortisone by the placenta is induced by estrogen (206, 207). As placental estrogens are derived from DHEA-S, this represents a positive feedback loop whereby further increases in placental estrogens lead to increased conversion of cortisol to cortisone, decreasing circulating cortisol in the fetus and leading to increased secretion of ACTH by the fetal pituitary. This, in turn, could stimulate increased DHEA-S production by the fetal adrenal cortex, providing more substrate for placental estrogen formation. The increased ACTH eventually could promote functional maturation of definitive and transitional zones and the ability to synthesize cortisol *de novo*. The production of cortisol by the fetal adrenal suppresses ACTH secretion by the fetal pituitary and effectively interrupts the loop. This intriguing hypothesis is supported by an extensive amount of data, and Pepe and Albrecht (Refs. 87 and 199 for review) have proposed that this mechanism may underlie the rapid growth and high level of DHEA-S production by the primate fetal adrenal cortex during midgestation.

Estrogens also directly influence steroid production by primate fetal adrenal cortical cells. Several studies have shown that estradiol suppresses ACTH-stimulated cortisol and augments ACTH-stimulated DHEA-S production by human fetal adrenal cortical cells (141, 208–210). Fujieda *et al*. (210) postulated that placental estrogens influence fetal zone function by inhibiting 3βHSD expression and proposed that ACTH and estradiol interact to cause fetal zone cells to exhibit their characteristic steroidogenic phenotype. However, although estradiol inhibited cortisol production, it did not inhibit the expression of P450scc, P450c17, or 3βHSD (141). Hirst *et al*. (211) showed that the fetal zone of midgestation rhesus monkeys does not express estrogen receptors, suggesting that any effects of estrogens on fetal zone function are not mediated via classic estrogen receptor interactions. These data indicate that effects of estradiol on fetal zone steroidogenesis are exerted at the level of the activity of steroidogenic enzymes rather than their gene transcription, and that although estradiol restores fetal zone phenotype with respect to cortisol synthesis, it does not inhibit expression of 3βHSD.

In contrast to the stimulatory effects of estrogen on DHEA-S production by cultured midgestation human fetal adrenal cortical cells in response to ACTH, Pepe and Albrecht and colleagues (212–214) have demonstrated a negative feedback loop in the baboon whereby placental estrogens inhibit DHEA production by the fetal adrenal cortex. Interestingly, this attenuation of DHEA production by estrogen occurred at midgestation but not near term *in vitro* (213) and *in vivo* (214). These investigators proposed that estrogen down-regulates DHEA-S production by the fetal adrenal cortex to maintain a physiologically normal balance of estrogen production during primate pregnancy (Ref. 199 for review).

IV. Physiology

After birth, the major physiological role of the adrenal cortex is to provide glucocorticoids for the maintenance of metabolic homeostasis and response to stress and mineralocorticoids for maintenance of fluid and electrolyte balance. Although these functions are performed mainly by the placenta during fetal life, the primate fetal hypothalamic-pituitary-adrenal axis is able to respond to stress with increased cortisol production as it does postnatally (215), and it is capable of aldosterone secretion late in gestation (47, 61). The fetal adrenal cortex also is involved in the maintenance of intrauterine homeostasis and in the preparation of the fetus for extrauterine life by regulating the maturation of essential organ systems including the lungs, liver, and gut. In some mammalian species, the fetal hypothalamic-pituitary-adrenal axis also plays a pivotal role in regulating the timing of parturition. A unique feature of the primate fetal adrenal cortex is the fetal zone and its abundant production of DHEA-S. One physiological role of DHEA-S is as a source of C_{19} steroids for placental estrogen production. Thus, the fetal adrenal cortex is essential in establishing the estrogenic milieu of primate pregnancy. Cortisol also is produced by the primate fetal adrenal cortex, and one of its physiological roles is to promote the maturation of organ systems needed for survival in the extrauterine environment. Unlike in other species, fetal adrenal cortisol does not appear to be involved in the regulation of primate parturition.

A. Placental estrogen formation

The primate placenta has a high level of aromatase activity and produces large amounts of estrogens (216). Studies in the 1950s showed that, although radiolabeled cholesterol administered to pregnant women is converted to estrogens by the placenta, human placental tissue *in vitro* does not produce estrogens *de novo* from acetate or cholesterol and cannot convert pregnenolone or progesterone into C_{19} steroids because it lacks the P450c17 enzyme (Refs. 217–219 for review). Thus, the estrogen synthetic pathway in the primate placenta is incomplete. The involvement of the fetus in placental estrogen production was first demonstrated by Cassmer (220), who found that maternal estrogens decreased sharply when the umbilical cord was cut, whereas progesterone, the other major steroid produced by the placenta, was unchanged until the placenta was delivered. Involvement of the fetal adrenal cortex in placental estrogen production was first suggested by Fransden and Stakemann (52) who found markedly reduced estrogen concentrations in women bearing anencephalic fetuses. Siiteri and MacDonald (49), Bolté *et al*. (221),

and Baulieu and Dray (222) subsequently demonstrated that the human placenta produces estrogens by the aromatization of C_{19} precursors, particularly DHEA-S and its 16-hydroxylated metabolite produced by the fetal liver and adrenal cortex. Thus, the primate placenta is capable of converting C_{19} steroids to estrogens but cannot produce C_{19} steroids from pregnenolone or progesterone because it lacks the P450c17 enzyme. In contrast, the fetal adrenal cortex expresses high levels of P450c17 and produces large amounts of the C_{19} steroid DHEA-S. Siiteri and MacDonald (49) estimated DHEA-S production by the fetal adrenal during the third trimester to be around 200 mg/day. The combination of these two incomplete steroidogenic pathways in these two disparate organs results in a complete estrogen-synthesizing system. Thus, one of the physiological functions of the primate fetal adrenal cortex is to provide C_{19} substrate to the placenta for the formation of estrogens. This strategy for estrogen formation in pregnancy is unique to primate species and is accomplished by the 'feto-placental unit' (223).

The principal placental estrogen of human pregnancy is estriol. Inasmuch as the placenta lacks the 16-hydroxylase enzyme, it can only produce estriol from 16-hydroxylated C_{19} steroid precursor (223). Most of the DHEA-S produced by the fetal zone is converted to 16α-hydroxy-DHEA-S by the fetal liver and to a lesser extent within the adrenal itself (224). In the placenta, the sulfatase enzyme removes the sulfate moiety from DHEA-S and 16α-hydroxy DHEA-S producing DHEA and 16α-hydroxy DHEA, respectively, which are then aromatized to estradiol, estrone, and estriol after further metabolism and 19-hydroxylation.

In other species, the fetal adrenal cortex also influences placental estrogen production. As with the human placenta, the sheep placenta lacks P450c17 for most of gestation and produces estrogens (mainly as sulfoconjugates) primarily from androstenedione supplied by the fetal adrenal cortex. Late in gestation, the prenatal rise in cortisol secretion by the sheep fetal adrenals induces increased expression of P450c17 in the placenta, which results in the conversion of progesterone to androstenedione and subsequently to estrone and estradiol. The consequence of this is that during the final days before parturition in sheep, placental production of progesterone declines as its conversion to androstenedione and estrogen increases (Refs. 2 and 3 for review). In most species, an increase in the estrogen/progesterone ratio occurs at the end of pregnancy (225). In general, progesterone maintains pregnancy by sustaining uterine quiescence, whereas estrogens stimulate events necessary for parturition, *e.g.* formation of myometrial gap junction, cervical effacement and dilatation, and uterine contractions (Ref. 199 for review). A rise in estrogens and a decrease in progesterone at the end of pregnancy therefore are requisite for parturition in most species. In primates, the placenta lacks P450c17 throughout gestation; therefore, progesterone production does not decline at the end of pregnancy as it does in sheep. However, toward the end of pregnancy, increased DHEA-S production by the fetal adrenal cortex results in increased substrate available to the placenta for estrogen production and a rise in maternal estrogen levels. Unlike the sheep, maternal estrogen concentrations in the pregnant woman (226), rhesus monkey (12), and baboon (227) rise gradually over several weeks prepartum. The physiological roles of estrogens in primate pregnancy are diverse (Ref. 199 for review) and beyond the scope of this review; however, it is clear that the fetal adrenal cortex is essential for the production of placental estrogens.

B. Timing of parturition and fetal maturation

The pioneering work of Liggins and colleagues (Refs. 2 and 3 for review) in sheep first demonstrated that increased activity of the fetal hypothalamic-pituitary-adrenal axis triggers the initiation of parturition and stimulates the maturation of the fetal organ systems essential for extrauterine life. In this species, increased secretion of cortisol from the fetal adrenal glands during the final week of pregnancy initiates a cascade of events that culminates in the birth of a viable neonate. In most mammalian species, including humans, cortisol also stimulates events associated with preparation for extrauterine life, *e.g.* surfactant production by the fetal lungs, activity of enzyme systems in the fetal gut, retina, pancreas, thyroid, and brain, and deposition of glycogen in the fetal liver (Refs. 1 and 228 for review). As in the sheep, the primate fetal adrenal cortex must produce cortisol *de novo* toward the end of gestation to ensure fetal maturation and neonatal competence. Clearly, perinatal survival is dependent on the timely initiation of labor when organ systems necessary for extrauterine life are sufficiently mature to allow the newborn to live outside of the uterus and independent of the placenta. Thus, in a number of species, regulation of fetal maturation and the timing of parturition are controlled by a single hormone, cortisol, produced by the fetal adrenals, which appears to coordinate these processes such that fetal maturation proceeds appropriately before parturition.

The discoveries by Liggins and colleagues in sheep caused excitement among clinicians seeking to understand the physiological basis for the timing of parturition and to develop strategies to deal with the problems of preterm labor in humans. However, it was soon realized that fundamental differences exist between sheep and humans with regard to the regulation of parturition and that, although fetal adrenal cortisol clearly orchestrates the initiation of parturition in sheep, a similar role for cortisol in primates was not apparent.

In anencephalics and infants with congenital abnormalities that prevent glucocorticoid synthesis, pregnancy is not significantly prolonged, on average, although labor occurs over a wider time interval (10, 89, 229). Similarly, adrenalectomy (230) or experimental anencephaly (92) of fetal rhesus monkeys does not prevent parturition but increases the window of time in gestation during which birth occurs. Treatment of rhesus monkey fetuses with dexamethasone does not lead to premature induction of parturition, as it does in sheep, but instead results in prolonged pregnancy (231). As glucocorticoid treatment inhibits ACTH production by the fetal pituitary leading to a decrease in DHEA-S production and suppression of the feto-placental unit, these findings implicate the feto-placental unit in the regulation of primate parturition. Interestingly, fetectomy of the rhesus monkey at midgestation results in delivery of the placenta postterm (232) and alters the rhythm of uterine activity (233). Recently, Nathanielsz and colleagues (234) infused androstenedione,

which is readily aromatized by the placenta to estrogen, into pregnant rhesus monkeys late in gestation to assess the effect of augmented placental estrogen synthesis on parturition. Androstenedione infusion increased maternal estrogen and nocturnal oxytocin concentrations and induced cervical dilation and normal parturition. These investigators proposed that, in primates, androgen produced by the fetal adrenals as a source of aromatizable substrate for estrogen synthesis by the placenta is the link between the fetus and mother in the initiation of parturition. However, studies in the baboon have provided conflicting data. Albrecht *et al*. (235) found that fetectomy in baboons does not prolong gestation of the placenta. Interestingly, administration of estradiol prevented placental delivery and prolonged gestation in fetectomized animals, indicating that in this species the feto-placental unit may actually inhibit parturition. Moreover, human aromatase deficiency does not appear to be associated with abnormal length of gestation (236), although as Mecenas *et al*. (234) point out, the patient with aromatase deficiency was treated frequently with tocolytic agents between 24 and 35 weeks, and it is unclear whether membrane rupture was spontaneous or induced. Based on this literature, the role of the feto-placental unit in the regulation of primate (especially human) parturition is unclear.

Studies of CRH production by the primate placenta have led to novel theories regarding the mechanism by which the feto-placental unit is involved in the regulation of parturition. A role for placental CRH in the regulation of the fetal hypothalamic-pituitary-adrenal axis and parturition was suspected when it was found that concentrations of CRH in the fetal (197, 237, 238) and maternal (197, 237, 239, 240) peripheral circulation and abundance of mRNA encoding CRH in the human placenta (189) increase sharply from 28 weeks of gestation until delivery. Interestingly, Majzoub and colleagues (241, 242) found that, unlike its effects on the hypothalamic CRH production, glucocorticoid increases CRH expression by the human placenta. The marked increase of CRH expression and maternal circulating concentrations at the end of gestation, and the capacity of glucocorticoids to enhance placental CRH expression, led these investigators to propose that the rise in placental CRH that precedes parturition could result from the rise in fetal glucocorticoids that occurs at this time. The increase in placental CRH may stimulate, via stimulation of fetal pituitary ACTH (243–245), a further rise in fetal glucocorticoids, completing a positive feedback loop that would be terminated by delivery. They also postulated that environmental stresses may stimulate fetal hypothalamic, as well as placental, CRH production, leading to increases in fetal ACTH production and activation of the positive feedback loop (Fig. 9). Subsequent studies showing that CRH receptors are present in the myometrium and fetal membranes (246, 247) and that CRH stimulates the release of prostaglandins from human decidua and amnion *in vitro* (248) and can potentiate the action of oxytocin and prostaglandin $F_{2\alpha}$ *in vitro* (249, 250) and *in vivo* (251) provide further circumstantial evidence that placental CRH may be directly involved in the regulation of human parturition by increasing myometrial contractility associated with labor.

More recently, Majzoub and colleagues (242) investigated the mechanism by which glucocorticoid increases CRH expression in the human placenta (242). Their data indicate that, late in gestation, cortisol may compete with progesterone for binding to the glucocorticoid receptor and that since progesterone, by interacting with this receptor, inhibits placental CRH expression, its displacement would result in increased CRH expression. In their theoretical model (*cf*. Fig. 9), concomitant stimulation of fetal cortisol and DHEA by placental CRH would couple the glucocorticoid effects on fetal organ maturation with the timing of parturition which, as they note, is of obvious benefit for postnatal survival.

McLean *et al* (240) recently proposed that placental CRH is associated with a 'placental clock,' which is active beginning at least by the 16th week of pregnancy, and which participates in the determination of the length of pregnancy and the timing of parturition. They found that concentrations of CRH in the maternal circulation, presumably secreted by the placenta, are predictive of the subsequent length of gestation. Maternal plasma CRH concentrations were predictive of those women who were destined to have normal term, preterm, or postterm delivery. The CRH curve in women who delivered preterm was shifted to the left by a magnitude of 6 weeks, which was equivalent to the degree of prematurity later observed at delivery in this group. Conversely, in women who delivered postterm, the CRH curve was shifted to the right by a magnitude of 2 weeks, which corresponded to the extent of postmaturity that was observed subsequently.

The exponential rise in maternal plasma CRH concentrations with advancing pregnancy is associated with a concomitant fall in the concentrations of the CRH-binding protein in late pregnancy. These reciprocal concentration curves suggest that there is a rapid increase in circulating levels of bioavailable CRH concurrent with the onset of parturition. The causal relationship between the increase in unbound CRH and the timing of parturition remains to be elucidated.

Clearly, the role of the fetal adrenal cortex in the regulation of parturition in primates is highly complex and different from that in other species. In addition, the primate may have several redundant mechanisms that may regulate parturition. This is not surprising given the critical nature of the timing and occurrence of this event.

V. Summary

The unique characteristics of the primate (particularly human) fetal adrenal were first realized in the early 1900s when its morphology was examined in detail and compared with that of other species. The unusual architecture of the human fetal adrenal cortex, with its unique and disproportionately enlarged fetal zone, its compact definitive zone, and its dramatic remodeling soon after birth captured the interest of developmental anatomists. Many detailed anatomical studies describing the morphology of the developing human fetal adrenal were reported between 1920 and 1960, and these morphological descriptions have not changed significantly. More recently, it has become clear that fetal adrenal cortical growth involves cellular hypertrophy, hyperplasia, apopto-

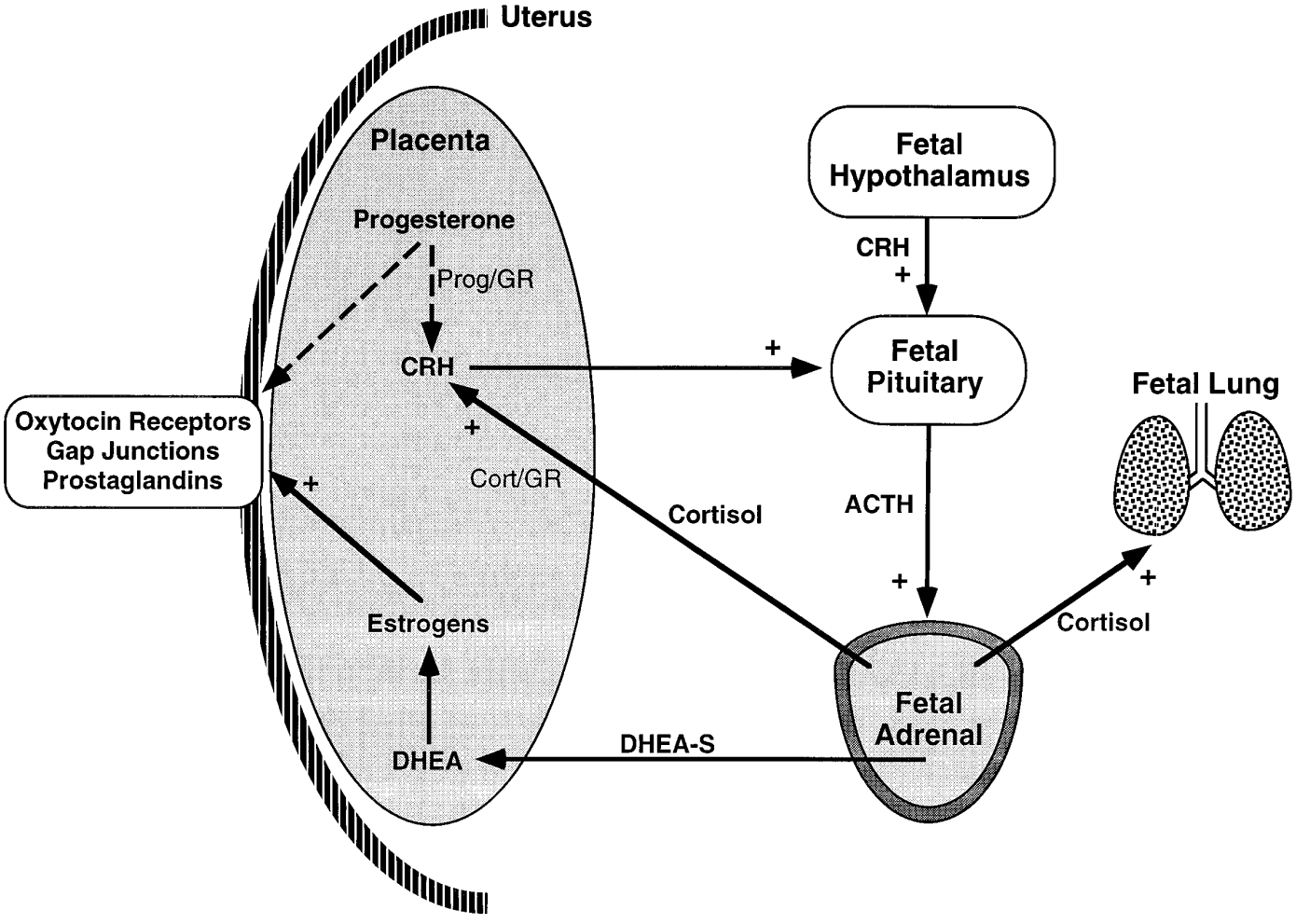

FIG. 9. Schematic depiction of the theoretical model proposed by Karalis *et al.* (242) describing the endocrine interaction between the fetal hypothalamic-pituitary-adrenal axis and the placenta in the regulation of human parturition. Late in gestation, cortisol produced by the fetal adrenal cortex blocks the inhibitory effects of progesterone on placental CRH production by competing with progesterone for the glucocorticoid receptor (GR). As a consequence, CRH secretion by the placenta into the fetal compartment increases, providing further stimulation to fetal pituitary ACTH production which in turn stimulates cortisol and DHEA-S production by the fetal adrenal cortex. Cortisol promotes maturation of fetal organ systems, such as the lungs, in preparation for extrauterine life and, by further enhancing placental CRH production, completes a positive feedback loop. DHEA-S is converted to estrogens by the placenta, which stimulates gap junction formation and oxytocin receptor expression by the myometrium and prostaglandin production by the aminon and decidua, events necessary to facilitate uterine contraction and labor. CRH also may directly enhance prostaglandin production and myometrial responsiveness to oxytocin. These events are inhibited by progesterone. [Adapted with permission from K. Karalis *et al.*: *Nat Med* 2:556–560, 1996 (242).]

sis, and migration and is best described by the migration theory, *i.e.* cells proliferate in the periphery, migrate centripetally, differentiate during their migration to form the functional cortical zones, and then likely undergo apoptosis in the center of the cortex. Consistent with this model, cells of intermediate phenotype, arranged in columnar cords typical of migration, have been identified between the definitive and fetal zones. This cortical area has been referred to as the transitional zone and, based on the expression of steroidogenic enzymes, we consider it to be a functionally distinct cortical zone.

Elegant experiments during the 1950s and 1960s demonstrated the central role of the primate fetal adrenal cortex in establishing the estrogenic milieu of pregnancy. Those findings were among the first indications of the function and physiological role of the human fetal adrenal cortex and led Diczfalusy and co-workers to propose the concept of the feto-placental unit, in which DHEA-S produced by the fetal adrenal cortex is used by the placenta for estrogen synthesis. Tissue and cell culture techniques, together with improved steroid assays, revealed that the fetal zone is the primary source of DHEA-S, and that its steroidogenic activity is regulated by ACTH.

In recent years, function of the human and rhesus monkey fetal adrenal cortical zones has been reexamined by assessing the localization and ontogeny of steroidogenic enzyme expression. The primate fetal adrenal cortex is composed of three functionally distinct zones: 1) the fetal zone, which throughout gestation does not express 3βHSD but does express P450scc and P450c17 required for DHEA-S synthesis; 2) the transitional zone, which early in gestation is functionally identical to the fetal zone but late in gestation (after 25–30

weeks) expresses 3βHSD, P450scc, and P450c17, and therefore is the likely site of glucocorticoid synthesis, and 3) the definitive zone, which lacks P450c17 throughout gestation but late in gestation (after 22–24 weeks) expresses 3βHSD and P450scc, and therefore is the likely site of mineralocorticoid synthesis. Indirect evidence, based on effects of P450c21 deficiency and maternal estriol concentrations, indicate that the fetal adrenal cortex produces cortisol and DHEA-S early in gestation (6–12 weeks). However, controversy exists as to whether cortisol is produced *de novo* or derived from the metabolism of progesterone, as data regarding the expression of 3βHSD in the fetal adrenal cortex early in gestation are conflicting.

During the 1960s, Liggins and colleagues demonstrated that in the sheep, cortisol secreted by the fetal adrenal cortex late in gestation regulates maturation of the fetus and initiates the cascade of events leading to parturition. Those pioneering discoveries provided insight into the mechanism underlying the timing of parturition and therefore were of particular interest to obstetricians and perinatologists confronted with the problems of preterm labor. However, although cortisol emanating from the fetal adrenal cortex promotes fetal maturation in primates as it does in sheep, its role in the regulation of primate parturition, unlike that in sheep, appears minimal. More recently, Nathanielsz and colleagues have proposed, based on studies in the rhesus monkey, that the feto-placental unit plays a role in regulating the timing of parturition in primates. These investigators provided strong evidence supporting the hypothesis that estrogens produced by the placenta from C19 precursor supplied by the fetal adrenal cortex influence the timing of parturition in the rhesus monkey. However, studies by Albrecht and colleagues indicated that estrogens are not involved in the regulation of parturition in the baboon. These conflicting data may be due to species differences, and further studies are required to resolve this intriguing issue and to elucidate the role of the fetal adrenal cortex in the regulation of primate parturition.

In all mammalian species studied to date, growth and function of the fetal adrenal cortex are primarily regulated by ACTH secreted from the fetal pituitary. Studies in humans and non-human primates have clearly demonstrated the dependence of the fetal adrenal cortex, particularly the fetal zone, on the fetal hypothalamic-pituitary axis and on ACTH. As ACTH is not a growth factor *per se*, we have proposed that at least some of its trophic actions are mediated by locally expressed growth factors. Several growth factors including bFGF, EGF, IGF-I and -II, TGFα and -β, and the activins/inhibins can modulate the growth and function of primate fetal adrenal cortical cells. Moreover, the expression of some of these growth factors, *e.g.* bFGF and IGF-II, by fetal adrenal cortical cells is up-regulated by ACTH, suggesting that they act as local mediators of ACTH action.

The placenta also may influence fetal adrenal development. In pregnant baboons, Pepe and Albrecht have proposed that the placenta modulates fetal adrenal cortical development indirectly by limiting the amount of maternal cortisol passing into the fetal circulation, which could inhibit ACTH secretion by the fetal pituitary. They also found that placental estrogens promote the conversion of maternal cortisol to cortisone and inhibit DHEA-S production by the fetal adrenal. More recently, the primate placenta was found to produce CRH and ACTH. It has been hypothesized that placental CRH influences fetal adrenal cortical growth and function by stimulating ACTH secretion from either the fetal pituitary or within the placenta itself. The human placenta produces very large quantities of CRH late in gestation that may stimulate ACTH secretion from the fetal pituitary. Interestingly, cortisol can stimulate CRH production by the placenta, suggesting that a positive feedback loop develops whereby placental CRH stimulates ACTH secretion from the fetal pituitary, which then augments cortisol production by the fetal adrenal, which stimulates further CRH production by the placenta. Some investigators have proposed that this regulatory feedback mechanism may be involved in the regulation of parturition. Although Serón-Ferré *et al*. showed that hCG increases DHEA-S production by human fetal zone cells, other workers have not detected an effect of hCG on fetal adrenal cortical function; therefore, its role in fetal adrenal cortical development remains uncertain.

In conclusion, development and function of the primate fetal adrenal cortex are unique among mammalian species. Much effort has been directed at elucidating the mechanism by which fetal adrenal growth and function are regulated, and significant progress has been made in understanding the mechanism by which ACTH exerts its trophic actions and the role of growth factors in this process. In addition, studies of adrenal cortical function based on the expression of steroidogenic enzymes have provided new insight into the functional zonation of the fetal adrenal cortex. However, despite these advances, we still do not fully understand the physiological role of the primate fetal adrenal cortex and its possible involvement in the regulation of parturition. This problem is made more intriguing by the recent discovery that the primate placenta produces CRH. This finding puts a new and exciting perspective on the concept of the feto-placental unit and broadens our understanding of the physiological interaction between the placenta and fetal adrenal. Future studies directed at this issue will likely contribute significantly to understanding the developmental and functional biology of the primate fetal adrenal cortex.

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