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PERINATAL REGULATION OF CORTISOL IN THE PRIMATE

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SUMMARY

These studies were designed to assess regulation of steroidogenic function of the human and rhesus monkey fetal adrenal gland. Studies were performed *in vitro* using superfusion and, in the monkey, *in vivo*, using fetal monkeys bearing catheters chronically implanted *in utero*. *In vitro*, there is functional specialization of the fetal and definitive zones of the human and monkey fetal adrenal glands. The definitive zone produces cortisol stimulable by ACTH and the fetal zone produces principally dehydroepiandrosterone sulfate which also can be stimulated by ACTH. *In vivo*, an increase in cortisol was seen in the fetal circulation toward the end of gestation. Administration of dexamethasone caused a prompt reduction in fetal cortisol and ACTH, indicating integrity of the fetal pituitary–adrenal axis. When fetal monkeys were challenged with 0.5 i.u. of ACTH only three of the nine studied had a cortisol response. In contrast, newborns delivered by hysterotomy and those delivered vaginally following labor all responded to ACTH stimulation. The responses of the newborns delivered vaginally after labor were greater than in the group delivered by hysterotomy prior to labor. The fetal metabolic clearance rate (MCR), production rate (PR), secretion rate (SR) and placental transfer of cortisol were determined *in utero*. The MCR of cortisol in the fetus was markedly greater than in the newborn which, in turn, was greater than in the adult. The PR of cortisol was higher in the fetus than in the mother when expressed on the basis of body weight. The difference between PR and SR in the fetus is due to transplacental passage of cortisol from the mother. Expressed on the basis of body weight, SR's in fetus and mother were not significantly different. There is an increase in fetal cortisol SR toward the end of pregnancy and immediately prior to or during labor which may play a role in parturition.

In a variety of mammalian species, including humans and subhuman primates, glucocorticoids secreted by the fetal adrenal gland play an important role in effecting homeostatic adjustments necessary for extrauterine life. These secretions affect the maturation of a variety of organ systems, including the liver, pancreas, adrenal medulla and gastrointestinal tract [1]. In the lung, glucocorticoids induce the development of surfactant, important in the prevention of hyaline membrane disease in humans. In addition, in certain species, the fetal adrenal gland is involved in the initiation of parturition. In humans, the adrenals grow rapidly in early fetal life and, by the end of the first trimester, have attained a size equal to or greater than that of the fetal kidneys. The bulk of the fetal adrenal mass is attributable to a central "fetal zone" which comprises approximately 80% of the volume of the gland. This is surrounded by the outer, definitive, or adult zone. At the end of gestation, the fetal adrenal gland undergoes a significant increase in rate of growth. This increase in size is particularly striking in the primate. The growth spurt seen at the end of gestation involves growth of both the fetal and definitive zones of the gland. As can be seen in Fig. 1, there is functional specialization of the fetal and definitive zones in the human fetal adrenal gland. As shown in these *in vitro* superfusion studies, the definitive zone produces primarily cortisol which is stimulable by ACTH [2]. In contrast, as shown in Fig. 2, the fetal zone elaborates principally dehydroepiandrosterone sulfate (DHAS), the production of which also can be stimulated by ACTH [2].

Our studies of the human fetal adrenal gland were limited to midgestation because of ethical considerations. The rhesus monkey fetus has a pattern of adrenal development, steroid production and morphology similar to that of the human fetus. Because of these similarities, and because, as shown in Fig. 3, we demonstrated similar patterns of steroid production in the rhesus monkey fetus [3], we have chosen this animal as an experimental model to investigate the regulation of fetal cortisol in late gestation.

To study endocrine regulation in the fetal rhesus monkey in a relatively physiologic manner, we have utilized the chronically catheterized fetus *in utero* shown schematically in Fig. 4. Studies were performed beginning at about 130 days of gestation. A detailed description of the technique is published elsewhere [4]. In brief, the technique involves catheterization of the fetal carotid artery and jugular vein at the time of abdominal hysterotomy of the mother. Electrodes, for fetal electrocardiographic recording, are implanted in the fetal thoracic skin, the fetus is returned to the uterus and the arterial and venous catheters and fetal electrodes, together with an amniotic catheter, are exteriorized either through the vagina or flank of the mother. Fetal status is monitored by fetal blood pH

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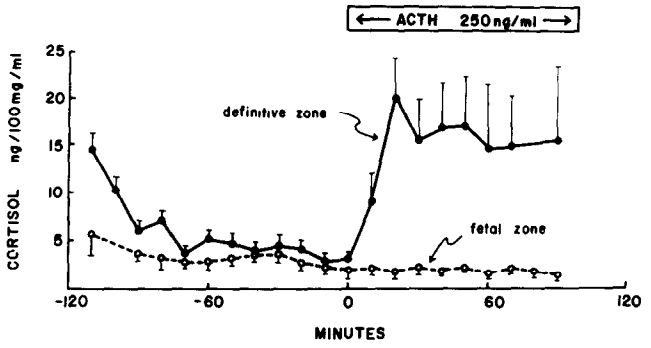

Fig. 1. Corticoid secretion (mean ± s.e.) by minces of fetal and definitive zone of human fetal adrenal gland from seven fetuses. The data were normalized, considering time of addition of ACTH as time zero. Corticoid secretion by the definitive zone after ACTH treatment is significantly greater ($p < 0.05$) when compared to time zero at all time intervals after 10 min. From Serón-Ferré M., Lawrence C. C., Siiteri P. K. and Jaffe R. B.; *J. Clin. Endocrinol. Metab.* **47** (1978) 603.

measurements, PO_2 and PCO_2 determinations and electrocardiographic recordings. Judged by these criteria, the preparations studied were stable. All experiments were completed prior to evidence of alteration in fetal status or initiation of maternal labor. The mother was maintained unanesthetized in a primate chair. The longest preparation has been maintained for 23 days, and the mean is approx. 5 days [4].

In the human fetus, cortisol concentrations have been shown to increase with advancing gestation in cord blood and amniotic fluid [5]. As shown in Fig. 5, a parallel increase in radioimmunoassayable cortisol was seen in the circulation of the chronically catheterized monkey fetus toward the end of gestation. Values rose from a mean of 75.6 ng/ml at 130–134 days to a mean of 134.9 ng/ml at 150–154 days [4]. As also can be seen, mean cortisol levels were higher in fetal samples obtained during maternal labor.

To assess the integrity of the pituitary–adrenal axis in the fetus at this stage of gestation, dexamethasone was administered to the mother and fetus [3, 4]. As can be seen in Fig. 6, a prompt reduction in both cortisol and ACTH was seen in the fetal circulation. Following discontinuance of dexamethasone suppression, there was a return to normal values. In other studies, we and others also have observed suppression of dehydroepiandrosterone sulfate and oestradiol in the maternal circulation following dexamethasone suppression [6].

Having demonstrated that cortisol could be stimulated *in vitro* with ACTH, and that the monkey fetus *in utero* has a functional pituitary–adrenal axis, we next assessed the response to ACTH in the chronically catheterized monkey fetus. As shown in Fig. 7, we challenged 9 fetal monkeys with 0.5 i.u. of ACTH. This is a supraphysiologic dose which produces a regular

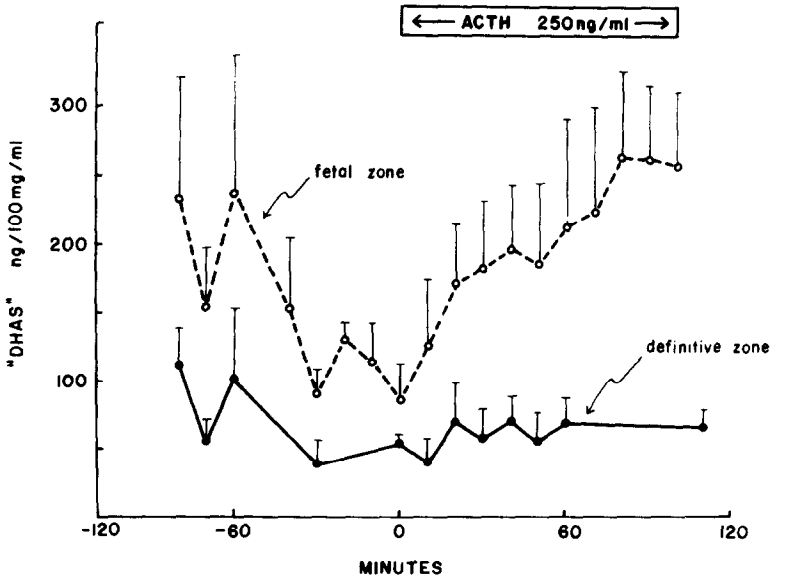

Fig. 2. Dehydroepiandrosterone sulfate (DHAS) secretion (mean ± s.e.) by minces of fetal and definitive zones of the same glands shown in Fig. 1. DHAS secretion by the fetal zone was significantly ($P < 0.05$) higher at 30 and 50 min after addition of ACTH than at time zero. From Serón-Ferré M., Lawrence C. C., Siiteri P. K. and Jaffe R. B.; *J. clin. Endocr. Met.* **47** (1978) 603.

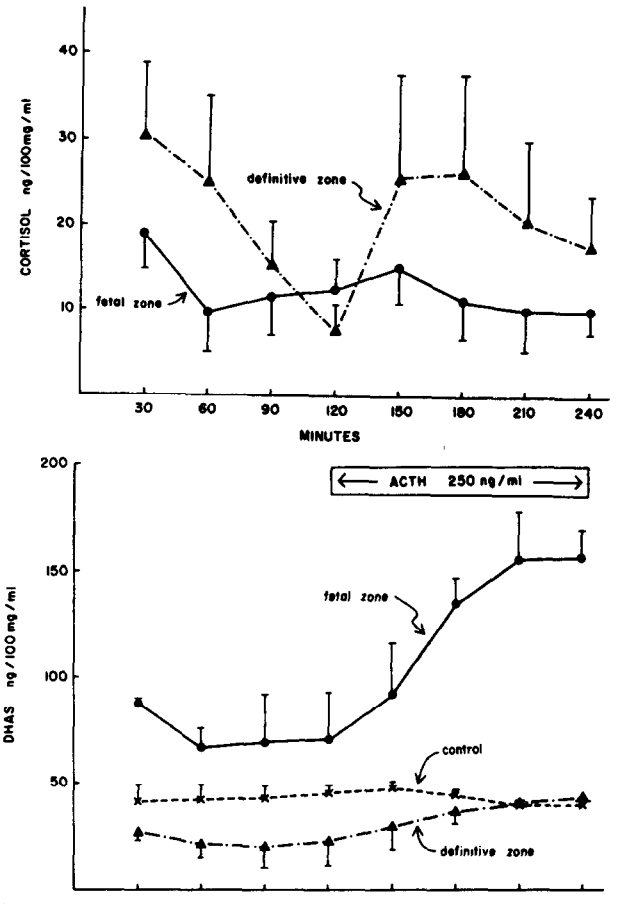

Fig. 3. Cortisol and DHAS responses to ACTH in the separated fetal and definitive zones of the rhesus monkey fetal adrenal gland studied in a superfusion system. In figure of DHAS data, curve from fetal adrenal minced preparations to which no ACTH was added is shown for comparison. From Jaffe R. B., Serón-Ferré M., Parer J. T. and Lawrence C. C.; *Am. J. Obstet. Gynecol.* **131** (1978) 164.

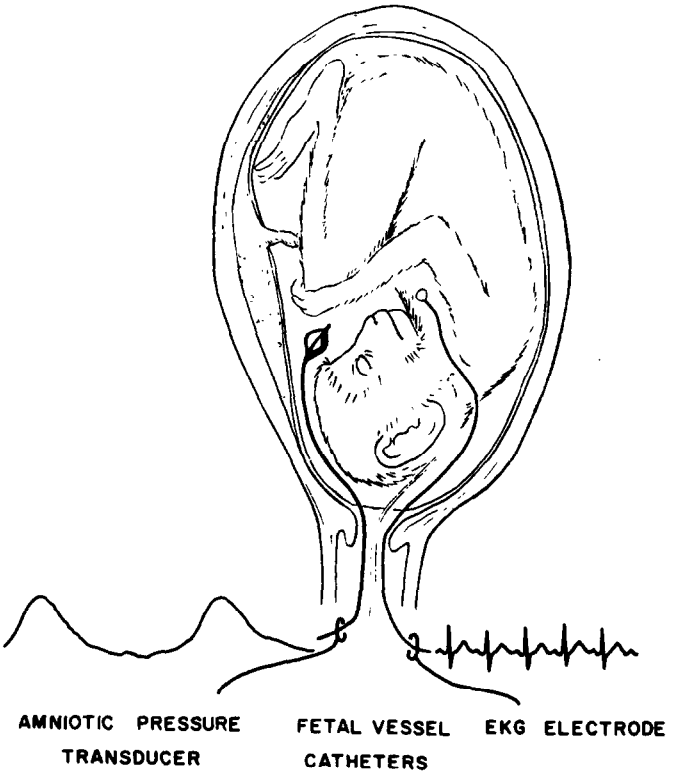

Fig. 4. Schematic illustration of preparation of fetal monkey chronically catheterized *in utero*.

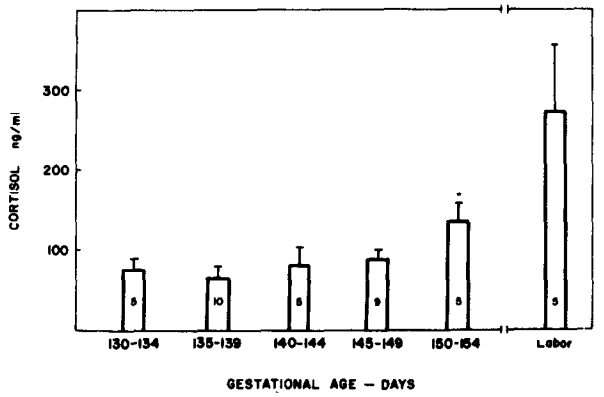

Fig. 5. Mean circulating cortisol concentrations in rhesus monkey fetuses from 130 to 154 days' gestational age. Data were obtained from 20 fetuses with long-term catheterization *in utero*. A daily morning sample was obtained from each fetus. When a fetus was sampled over several consecutive days in a single time interval, the mean cortisol value for that fetus was used in calculating the group mean. From Jaffe R. B., Serón-Ferré M., Parer J. T. and Lawrence C. C., *Am. J. Obstet. Gynecol.* **131** (1974) 164.

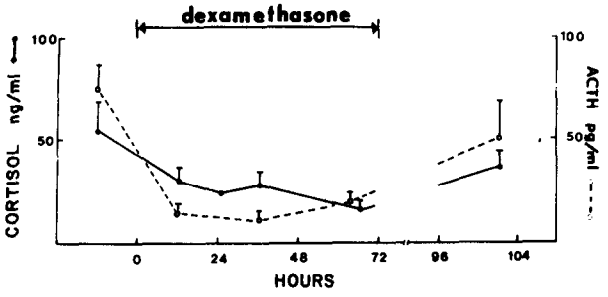

Fig. 6. Radioimmunoassayable ACTH and cortisol in the circulation of four chronically catheterized monkey fetuses *in utero* before, during and after dexamethasone administration. Each point represents mean (±S.E.) of four experiments. Mean ACTH and cortisol levels decreases significantly ($P < 0.05$) at 12 h after dexamethasone. From Serón-Ferré M., Rose J. C., Parer J. T., Foster D. B. and Jaffe R. B.; *Endocrinology* **103** (1978) 368.

rise in cortisol in the adult. Only 3 of the 9 animals showed a response to ACTH. It is of interest that these three animals all delivered within 24 h of the ACTH challenge. This finding in the group of fetuses challenged *in utero* is in contrast to newborns delivered either by hysterotomy prior to the initiation of labor or newborns delivered vaginally following labor (Fig. 7). It should be noted that the approximate age of delivery was similar in both of these latter groups.

There are several possible reasons for the differences between the *in vitro* and *in vivo* fetal adrenal responses to ACTH, as well as the difference in responsiveness of the three groups depicted in Fig. 7. First, there might be a relative insensitivity of the adrenal gland to ACTH *in utero*, as other investiga-

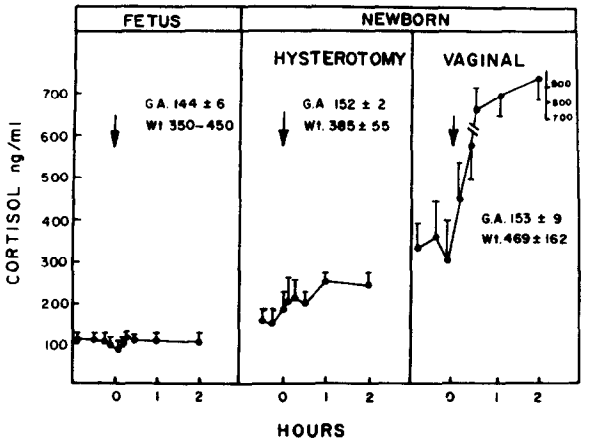

Fig. 7. Fetal and newborn rhesus monkey responses to bolus i.v. injections of 0.5 i.u. ACTH.

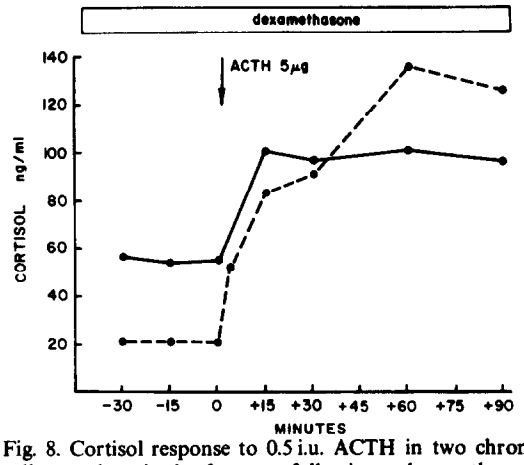

Fig. 8. Cortisol response to 0.5 i.u. ACTH in two chronically catheterized fetuses following dexamethasone suppression.

tors have demonstrated increased cortisol levels after treating with ACTH in doses considerably higher than those used in the present experiments. Second, there is a possibility that the response *in utero* to ACTH is blocked by fetal or placental factors. Third, there may be factors other than ACTH that could be trophic for the adrenal gland *in utero*. It is known that there are other corticotropin related peptides present in the fetal pituitary. Fourth, it is possible that *in utero* clearance of cortisol by the fetus and placenta is so rapid that an increase in cortisol secretion would not be detected in a peripheral sample. Finally, there could be a relative lack of capacity of the fetal adrenal gland *in utero* to produce enough corticosteroids to affect circulating cortisol levels, perhaps due to the relatively small size of the definitive zone of the gland.

We have attempted to address the first two possibilities, namely the relative insensitivity to ACTH and the possibility of an inhibited response to ACTH, by experiments in which the pituitary–adrenal axis was first suppressed with dexamethasone and then challenged *in utero* with ACTH. These data are shown in Fig. 8. A dose of 8 mg of dexamethasone per day was administered as a continuous infusion to the mother. As can be seen, when this was done, the response of the two fetuses shown here to a subsequent bolus of the same dose of ACTH used in the previous experiments in the fetus and newborns elicited a rise in fetal cortisol. Baseline cortisol levels were partially suppressed with this regimen. Novy and his colleagues also have demonstrated this response in dexamethasone suppressed fetal monkeys [7]. Thus, it is unlikely that there is absolute fetal adrenal insensitivity to ACTH or an inhibition of responsivity to ACTH, at least under these experimental conditions. However this may contribute, in part, to our inability to observe a response in the unsuppressed fetus.

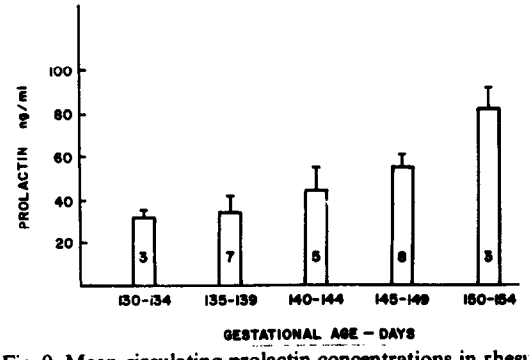

Fig. 9. Mean circulating prolactin concentrations in rhesus monkey fetuses with long-term catheterization *in utero* from 130 to 154 days' gestational ages. Numbers in bars indicate numbers of fetuses in each age group.

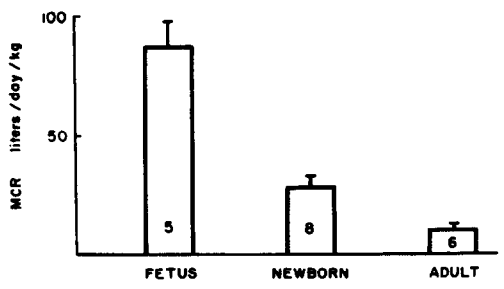

Fig. 10. Metabolic clearance rates of cortisol in the fetus, newborn and adult.

In regard to the third possibility, that of other trophic factors influencing adrenal corticoid response, it has been suggested that prolactin, αMSH and other trophic hormones may play a role in fetal adrenal regulation. Indeed, as shown in Fig. 9, we have demonstrated that prolactin concentrations increase with advancing gestation in a parallel manner to that observed for cortisol [8]. Currently, we are attempting to assess whether hyperprolactinemia in the fetus alters adrenal steroid production. In addition, we have preliminary data that αMSH also may increase cortisol production *in vitro*. Whether this situation obtains *in vivo* currently is under study. Thus, it is possible that other trophic factors also regulate fetal adrenal function.

We next addressed the possibility that the clearance of cortisol in the fetus might be different than that of the newborn and adult. We have performed two groups of experiments to address this possibility. The first group involved the bolus injection of labeled cortisol into the fetus, infant and adult which allowed the calculation of the metabolic clearance rate and production rate of cortisol [9]. The second employed a constant infusion of cortisol into the fetus and mother with different isotopic labels (^3H and ^{14}C) permitting the calculation of secretion rate and pla-

Table 1. Metabolic clearance rates, production rates and secretion rates in fetal and maternal rhesus monkeys

	Fetus	Mother
MCR (l/day/kg)	76.7 ± 13.9	20.4 ± 5.4
PR (mg/day/kg)	8.3 ± 1.6	3.4 ± 0.6
SR (mg/day/kg)	5.3 ± 1.9	3.2 ± 0.6

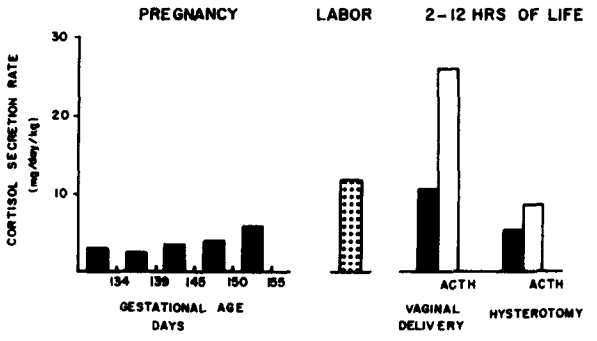

Fig. 11. Calculated cortisol secretion rates in fetal and newborn rhesus monkeys. In newborns, values before and after ACTH stimulation in infants delivered by the vaginal route and hysterotomy are shown.

cental transfer in addition to the metabolic clearance rate and production rate [10].

Figure 10 depicts the metabolic clearance rates, expressed as liters per kilogram per day, in the fetus, newborn and adult. As can be seen, the clearance rate of cortisol in the fetus is markedly greater than that in the newborn or adult. In addition, the clearance in the newborn is higher than the adult. This observation could contribute to our inability to observe an increase in cortisol following ACTH administration to the non-dexamethasone treated fetus.

Finally, attention was directed to actual measurements of the capacity of the fetal adrenal *in utero* to secrete cortisol. Table 1 depicts these results. Utilizing the constant infusion technique, data on metabolic clearance rates were obtained that were similar to those seen with the bolus infusion. Again, an increase in metabolic clearance rate in the fetus relative to the mother was observed. Further, the production rate of cortisol was higher in the fetus than in the mother when expressed on the basis of body weight. It is particularly noteworthy that the secretion rates in the fetus and the mother are not significantly different when expressed on the basis of body weight. Therefore, the fetal adrenal gland has the capacity at this late stage in gestation of making at least as much cortisol as the mother for their respective weights. We have found that the difference between production rate and secretion rate in the fetus is due to the transplacental passage of cortisol from the mother [11]. Thus, this is another factor which may mask the response to ACTH in the fetus.

Having demonstrated that the fetal adrenal does have the capacity to secrete significant amounts of cortisol, the remaining question is whether, indeed, the secretion of cortisol actually does increase at the end of gestation. As shown in Fig. 11, we have addressed this question indirectly by utilizing the data which have been presented previously to estimate the cortisol secretion rates in fetal and newborn monkeys. To make these calculations, we have assumed a constant transfer of cortisol during late gestation and labor and a constant fetal metabolic clearance rate at these times. Using these assumptions, it can be seen that there is an increase in secretion rate toward the end of pregnancy and immediately prior to or during labor.

It should be recalled that, in the neonate, the production rate is similar to the adrenal secretion rate since there is no other source of cortisol. It can be seen that the fetal cortisol secretion increases during late gestation and increases further either prior to or during labor. It is noteworthy that the secretion rate of the neonates delivered by hysterotomy prior to labor is greater than that of the late gestation fetus. We interpret the data to suggest that this increase in secretion occurs shortly prior to or during labor since the infants delivered by hysterotomy have cortisol secretion rates greater than those of the 155 day fetuses. The newborn secretion rate may be equal to that of the last two weeks of gestation, which we have not quantitated as yet. When given a large dose of ACTH, the cortisol secretion rate in these hysterotomy-delivered neonates increases, but is still less than the secretion rate of the fetuses in labor. It also is possible that labor further augments the adrenal capacity to secrete cortisol, since the vaginally delivered fetuses have higher basal and ACTH stimulated secretion rates than those delivered by hysterotomy despite similar gestational ages in these two groups.

In summary, we have reviewed some of the factors regulating fetal cortisol production. We have demonstrated that the late gestational fetal monkey adrenal has the capacity to secrete as much cortisol *in utero* as an adult on the basis of body weight. We also have demonstrated that ACTH is a trophic factor for the fetal adrenal gland. Further, we have shown that the cortisol secretion rate of the newborn monkey that has been through labor is significantly higher than that of the late gestation fetus or the mother and have interpreted these data to indicate that the increase in adrenal secretion of cortisol takes place prior to or during labor and may play a role in this process. The factor or factors responsible for this supersensitivity thus may have important physiologic implications.

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