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Maturity of the Adrenal Cortex in Very Preterm Infants Is Related to Gestational Age

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ABSTRACT

To study the maturity of the adrenal cortex in preterms born before 33 wk of gestation, basal levels of cortisol and cortisone and the cortisol and 17-hydroxyprogesterone (17-OHP) response to 1 μg/kg adrenocorticotropic hormone stimulation were measured in 24 appropriate-for-gestational age preterm infants (26-33 wk; 690-1985 g). Gestational age influenced the response of cortisol, 17-OHP, and the ratio between cortisol/17-OHP in the studied infants. In preterms born <30 wk of gestation, levels of cortisol, and the ratio between cortisol/17-OHP were lower compared with preterms born between 30 and 33 wk. Levels of cortisone were higher in preterms born <30 wk, suggesting a lower activity of 11β-hydroxysteroid dehydrogenase that may be related to maturity as well. These findings indicate that the adrenal cortex function in preterm infants is closely related to the duration of gestation and may be important in neonatal morbidity. **(*Pediatr Res* 52: 405–410, 2002)**

Abbreviations
11β HSD, 11β-hydroxysteroid dehydrogenase
17 OHP, 17-hydroxyprogesterone
ACTH, adrenocorticotropic hormone
AGA, appropriate, for-gestational age
DHEAS, dehydroepiandrosterone sulfate
HPA axis, hypothalamic-pituitary-adrenal axis
SGA, small-for-gestational age

The adrenal gland plays an important role during gestation. Steroid hormones produced by the fetal adrenal cortex are involved in the maturation of organ systems necessary for intrauterine and extrauterine life. For example, secretion of cortisol in late gestation is essential for lung maturation in sheep, rats, monkeys, and possibly humans (1, 2). It has been suggested that the adrenal cortex function may be more closely related to gestational age rather than to birth (3, 4), which implicates that in very preterm infants the adrenal activity may still be immature. Immaturity of the hypothalamic-pituitary-adrenal (HPA)-axis in preterm infants has been suggested to be associated with the occurrence of respiratory distress syndrome, chronic lung disease of prematurity, or even cardiovascular instability (5–7). Therefore, the ability of the HPA-axis to regulate, synthesize, and secrete cortisol in response to stress may be critical for survival and pulmonary development in very preterm infants.

To evaluate the adrenal function the plasma cortisol response to i.v. administered ACTH is a common screening test. In general, 250 μg synthetic ACTH is given as a bolus dose to stimulate cortisol release from the adrenal cortex, in adults as well as children as described by Wood *et al*. in 1965 (8). This supra-physiologic dose is much higher than required to produce a maximal adrenal response and could elicit an appropriate response despite of adrenal insufficiency (9, 10). Therefore, a lower dose of ACTH has been proposed to study the adrenal function.

We hypothesize that differences in the maturity of the HPA-axis related to gestational age can be observed in the levels of hormones produced by the adrenal cortex and the adrenal response to ACTH in preterm infants of different gestational ages. The objective was to study basal levels of glucocorticoids and to establish the effect of stimulation of the adrenal cortex with ACTH in very preterm infants at the end of the 1st week of life.

PATIENTS AND METHODS

Patients. The study group consisted of 24 preterm infants with gestational ages ranging from 26 to 33 wk and birth weights ranging from 690 to 1985 g. Patients admitted to our

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neonatal intensive care unit were eligible to be enrolled if they were appropriate for gestational age (*i.e.* birth weight SD score adjusted for gestational age, parity, and sex between −2 SD and +2 SD according to a Dutch reference population) (11). In addition, all patients required respiratory support (high frequency ventilation, conventional mechanical ventilation, or continuous positive airway pressure) during the 1st week of life, and had no major congenital anomalies. All infants to be studied had an arterial and/or venous indwelling catheter, and had not received postnatal treatment with corticosteroids. Infants did not receive packed cell transfusions within 2 d of the test. Results were analyzed using gestational age as a continuous variable. However, based on clinical observations that preterm infants born before 30 wk of gestation have a higher risk for the development of chronic lung disease compared with infants born after 30 wk of gestation (6), patients were also subdivided into two groups. Group A consisted of 13 patients with gestational ages ranging from 26 to 29 wk and group B consisted of 11 patients with gestational ages ranging from 30 to 33 wk. Clinical data of both groups are summarized in Table 1. Most preterm infants (21 out of 24 infants) received antenatal glucocorticoid treatment (*i.e.* 2 doses of 11.4 mg betamethasone in 48 h at weekly intervals). Two infants were born before the second dose of antenatal steroids was administered, and one infants was born within 24 h after the second dose of antenatal steroids. Three infants received a second course and one infant a third course of antenatal steroids. None of the patients showed signs of birth asphyxia. Infants in group A required comparable levels of oxygen during the 1st week, but the duration of mechanical ventilation and hospital stay was significantly longer compared with infants in group B (Table 1). Written informed consent was obtained from all parents. The study protocol was approved by the Ethics Committee of the VU University Medical Center, Amsterdam, The Netherlands.

Testing protocol. To minimize the possible influences of birth associated stress and antenatal steroid treatment due to premature labor, ACTH tests were performed not sooner than the 5th to 10th day of life between 08:00 h and 09:00 h. Infants underwent the ACTH test only when they were clinically stable and did not show any clinical or laboratory signs of infection. After basal blood sampling, 1 μg per kilogram body weight (1 μg/kg) ACTH (tetracosactide, Synacthen, Ciba-Geigy, The Netherlands) was administered i.v. (without the use of additional plastic tubing connections and after flushing the heparinized indwelling i.v. cannula with NaCl 0.9%). The dose was obtained by dilution of ACTH in normal saline and prepared within 30 min before administration. Blood samples (each 0.3 milliliter) were withdrawn just before, as well as 30 and 60 min after i.v. ACTH administration for determination of cortisol and 17-hydroxyprogesterone (17-OHP). Because of limited blood sample volumes in preterm infants, we have carefully chosen only to determine cortisol and 17-OHP. The latter was chosen as a determinant of the capacity of the adrenal cortex to convert steroid precursors, while cortisol was chosen as the final product of glucocorticoid synthesis. In addition, the activity of 11β-hydroxysteroid dehydrogenase (11β-HSD) in the adrenal cortex of preterm infants was indirectly studied by the determination of cortisol and cortisone in a blood sample taken at 08:00 h 1 to 2 d before the ACTH test.

Table 1. *Clinical characteristics of the study population (mean ± SD)*[§]

	Group A°	Group B°
Gender	7 male/6 female	6 male/5 female
Gestational age (weeks)*	28.1 ± 1.2	30.9 ± 1.1
Birth weight (grams)*	996 ± 178	1471 ± 294
Birth length (cm)*	35.8 ± 1.6	40.1 ± 2.7
Head circumference (cm)*	26.1 ± 1.9	28.9 ± 1.6
Antenatal steroids	12/13	9/11
Apgar score at 5 min <5	0/13	0/11
Oxygen during first week (median (range) % of inspired air)	31 (21–57)	30 (22–43)
Days on mechanical ventilation* median (range)	19 (9–38)	9 (1–29)
Length of stay in hospital (days)*	76.9 ± 15.0	51.7 ± 22.1

[§] Results are presented as the mean ± SD.

° Group A: gestational age <30 weeks (n = 13); group B: gestational age 30–33 weeks (n = 11). Asterisks (*) indicate statistical difference between both groups ($p < 0.05$).

Hormone assays. Blood samples were allowed to clot for 15 min at room temperature. Serum was separated and stored at −20°C until analysis. Serum cortisol was determined in duplicate by RIA (Coat-A-Count, DPC, Los Angeles, U.S.A.). Serum 17-OHP was determined in duplicate by a competitive ELISA (DRG Instruments, Marburg, Germany) directly in serum without an extraction step because of limited blood sample volumes in preterm infants. Therefore, steroids originating from the fetal zone of the (immature) adrenal cortex, such as 17-hydroxypregnenolonesulfate, were not removed and could contribute to the 17-OHP values because of cross-reactivity (12). However, the levels of cross-reaction of other steroids, including DHEAS and cortisol, were less than 0.05%, with the exception of 11-desoxycortisol (1.4%) and progesterone (1.2%). The detection limit was 30 nmol/L for cortisol and 0.5 nmol/L for 17-OHP. The intraassay and interassay coefficients of variation were 5% and 7% at a cortisol level of 550 nmol/L, 4% and 7% at a cortisol level of 1000 nmol/L. For 17-OHP the intraassay and interassay coefficients of variation were 8% and 16% at a 17-OHP level of 3 nmol/L, and 4% and 11% at a 17-OHP level of 9 nmol/L. The intra-assay coefficient of variation was 5% at a 17-OHP level of 20 nmol/L. In addition, cortisol and cortisone levels were determined by RIA after extraction and paperchromatographic purification, as described elsewhere (13). The detection limit using this methods was 5 nmol/L for cortisol and 1.3 nmol/L for cortisone. Using this technique the intraassay and interassay coefficients of variation were 5% and 8% for cortisol at a level of 0.27 μmol/L, and 6% and 11% for cortisone at a level of 48 nmol/L.

Statistical analysis. Results are presented as the mean ± SEM unless indicated otherwise. The baseline level was defined as the concentration at 0 min. Group differences were analyzed with t tests, Mann-Whitney U tests, and χ^2 tests where appropriate. For related samples, results were analyzed using repeated measures analysis of covariance (ANCOVA) with gestational age as covariable. To study the hypothesis that the postnatal age (5–10 d of life) influences the adrenal response, the analysis was repeated with postnatal age as an

additional covariable. Within-group differences and groups differences were analyzed with repeated measures analysis of variance (ANOVA). Significance was assumed when the probability value exceeded 95% ($p < 0.05$).

RESULTS

Response of cortisol. The overall effect of gestational age on cortisol showed a trend toward higher cortisol responses with increasing gestational age (ANCOVA; $p = 0.08$). Results of individual cortisol increments after ACTH stimulation in the two groups are shown in Figs. 1*A* (group A) and 1*B* (group B). The variability of cortisol levels is significantly smaller in group A (difference from the mean at 0 min: 32 ± 11; 30 min: 64 ± 8; 60 min: 100 ± 17 nmol/L) compared with group B (0 min: 113 ± 21; 30 min: 155 ± 32; 60 min: 230 ± 51 nmol/L) at all time points studied.

Cortisol levels in group A increase significantly from 178 ± 15 nmol/L at baseline to 391 ± 20 nmol/L and 516 ± 33 nmol/L at 30 and 60 min, respectively (Fig. 2: $p < 0.001$). In group B cortisol levels increase significantly from 250 ± 42 nmol/L at baseline to 542 ± 59 nmol/L and 733 ± 89 nmol/L at 30 and 60 min, respectively (Fig. 2: $p < 0.001$). Levels of cortisol were significantly lower in group A compared with group B at 30 and 60 min after ACTH stimulation, but not at baseline. Postnatal age was not a significant factor that influenced the cortisol response (repeated measures ANCOVA; $p = 0.21$).

No significant correlation was found between baseline cortisol and the increase in cortisol concentration after stimulation ($r = 0.11$; $p = 0.62$).

Response of 17-OHP. The overall effect of gestational age on 17-OHP levels was significant (ANCOVA; $p = 0.02$), and lower gestational ages were associated with higher 17-OHP levels. The 17-OHP concentration increases significantly from 112 ± 21 nmol/L at baseline to 120 ± 22 nmol/L at 30 min and 131 ± 22 nmol/L at 60 min in group A (Fig. 3: $p < 0.05$). However, no significant increase in 17-OHP levels is found in group B (from 71 ± 10 nmol/L at baseline to 80 ± 10 nmol at 30 min and 89 ± 11 nmol/L at 60 min). No significant difference in the level of 17-OHP is found between group A and B at all time points studied. Postnatal age was not a significant factor that influenced the 17-OHP response (repeated measures ANCOVA; $p = 0.68$).

Response of the ratio cortisol/17-OHP. The overall effect of gestational age on the ratio cortisol/17-OHP in response to ACTH stimulation was significant (ANCOVA; $p = 0.01$). The cortisol/17-OHP ratio in group A increases from 2.1 ± 0.3 at baseline to 4.2 ± 0.5 and 5.1 ± 0.7 at 30 and 60 min, respectively (Fig. 4; $p < 0.001$). In group B, the cortisol/17-OHP ratio increases from 3.6 ± 0.4 before ACTH stimulation to 7.1 ± 0.8 and 8.6 ± 1.0 at 30 and 60 min, respectively (Fig. 4; $p < 0.005$). The cortisol/17-OHP ratio is significantly higher at all time points in group B compared with group A ($p < 0.05$). The increase in cortisol/17-OHP ratio from baseline to 60 min is significantly higher in group B (5.0 ± 0.8) compared with group A (3.0 ± 0.5). Postnatal age was not a significant factor that influenced the response of the ratio cortisol/17-OHP (repeated measures ANCOVA; $p = 0.45$).

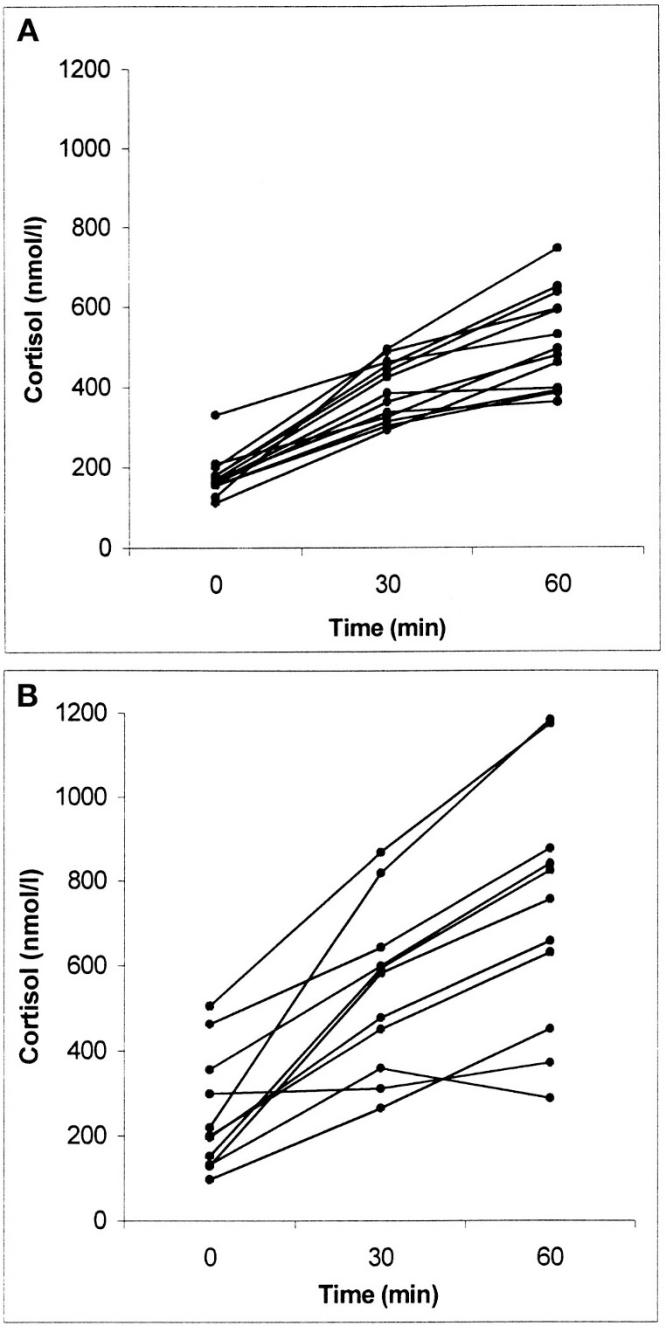

Figure 1. (*A*) Individual cortisol responses after ACTH stimulation in preterm infants with a gestational age <30 wk ($n = 13$). (*B*) Individual cortisol responses after ACTH stimulation in preterm infants with a gestational age between 30 and 33 wk ($n = 11$).

Levels of cortisol and cortisone. Cortisone levels were significantly higher in group A (99.7 ± 35.2 nmol/L) compared with group B (64.6 ± 40.1 nmol/L; $p < 0.05$). Cortisol levels determined in the same sample were comparable between group A (0.05 ± 0.03 μmol/L) and group B (0.08 ± 0.05 μmol/L). No significant correlation was found between cortisol and cortisone levels ($r = 0.23$; $p = 0.30$).

Clinical outcome and cortisol response. To study the relation between the maximal cortisol concentration after stimula-

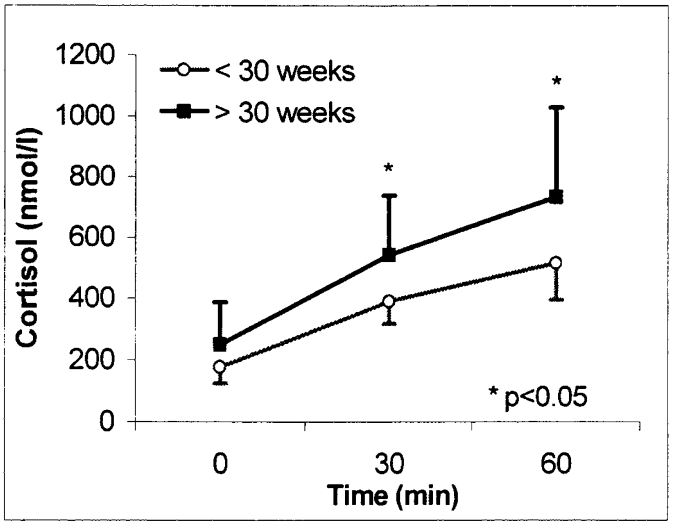

Figure 2. Cortisol concentrations (mean±SD) in response to ACTH stimulation in infants born <30 wk of gestation and infants born between 30–33 wk of gestation. Asterisks (*) indicate a significant difference between the groups ($p < 0.05$).

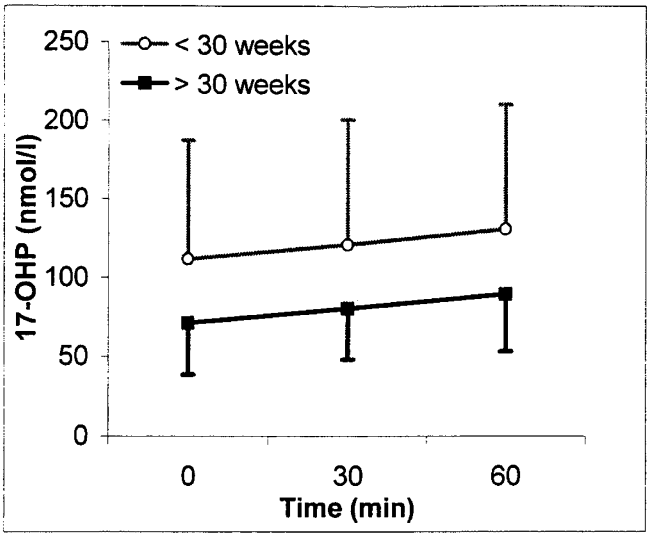

Figure 3. 17-Hydroxyprogesterone concentrations (mean±SD) in response to ACTH stimulation in infants born <30 wk of gestation and infants born between 30–33 wk of gestation.

tion and the clinical outcome, the maximal stimulated cortisol concentrations of the infants were divided into three equal groups based on the cortisol concentrations (first group: cortisol <460 nmol/L; second group: cortisol 460–650 nmol/L; third group: cortisol >650 nmol/L). The group of infants with the lowest stimulated cortisol did not require mechanical ventilation for a longer period of time (17 ± 21 versus 9 ± 13; $p = 0.35$) but were admitted to hospital longer (84.4 ± 18.7 versus 41.3 ± 23.9; $p < 0.05$) compared with the group of infants with the highest stimulated cortisol. Two infants (one with a gestational age >30 wk and one <30 wk) had stimulated cortisol concentrations below 360 nmol/L. Korte *et al.* suggested that a cortisol level below 360 nmol/L indicates an insufficient cortisol secretion in preterm infants (14). However, both these infants had good clinical outcomes and did not show signs of adrenal insufficiency.

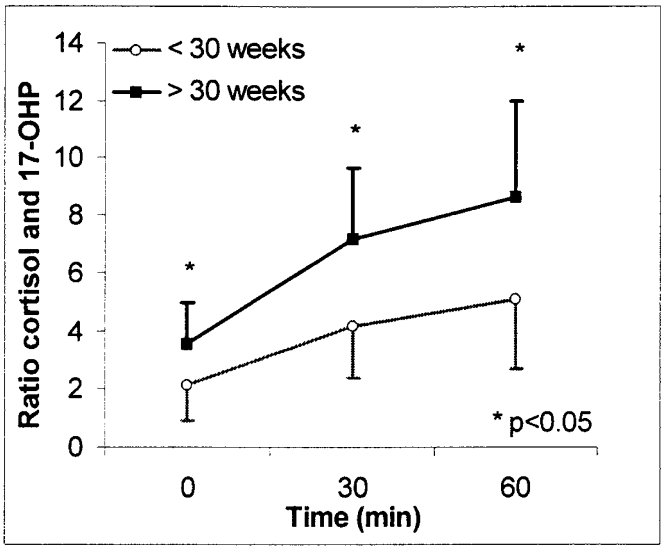

Figure 4. Ratio between cortisol and 17-hydroxyprogesterone (mean±SD) in response to ACTH stimulation in infants born <30 wk and infants born between 30–33 wk. Asterisks (*) indicate a significant difference between the groups ($p < 0.05$).

DISCUSSION

The present study shows that cortisol concentrations increase after stimulation with 1 μg/kg ACTH in preterm infants born before 33 wk of gestation and that this response is influenced by gestational age. A different response is found between preterm infants with a gestational age below 30 wk and preterms with a gestational age between 30 and 33 wk of gestation. The cortisol levels in infants born before 30 wk of gestation were lower after stimulation and showed less variation compared with more mature preterm infants. These findings suggest that the human adrenal function is not yet fully matured in preterm infants and indicates a decreased capacity for cortisol synthesis and/or a decreased responsiveness to ACTH (15). This may be related to a gradual maturation of the human fetal HPA axis during the third trimester of gestation and is in agreement with the work of Mesiano and Jaffe in primates (16, 17). No differences were found in levels of 17-OHP between infants born before 30 wk and infants born after 30 wk. The ratio between cortisol and 17-OHP, which represents the ratio between a corticosteroid precursor and cortisol, was significantly lower in infants born before 30 wk of gestation compared with "older" preterm infants. Lower cortisol levels after ACTH stimulation combined with lower cortisol/17-OHP ratios in the extreme preterm infants indicate an insufficient response of the adrenal cortex due to HPA-axis immaturity. The relatively high levels of 17-OHP combined with lower levels of cortisol in the more immature preterm infants suggest a lower activity of adrenal enzymes in their adrenal cortex, for instance 21-hydroxylase and/or 11β-hydroxylase. Other enzymes such as 11β-HSD type 1 and 2, that interconvert cortisone and cortisol, may also influence circulating levels of cortisol. To indirectly estimate the activity of both 11β-HSD type 1 and 2, we simultaneously measured cortisone and cortisol concentrations in serum. Preterm infants born before 30 wk of gestation had higher cortisone and similar

cortisol compared with those born between 30 and 33 wk, which suggests a change in activity of 11β-HSD type 1 and 2 with increasing gestational age. Others have already suggested differences in activity of 11β-HSD between term and preterm infants, that may be related to relative high levels of 11β-HSD type 2 in the persisting fetal zone of the adrenal cortex in preterm infants (18). However, since 11β-HSD type 1 is not only expressed in the adrenal, but also in other tissues including the liver, the amount of circulating cortisone is not solely determined by activity of 11β-HSD type 1 and 2 in the adrenal cortex. The exact regulation of cortisone and the activity of 11β-HSD type 1 and 2 remains uncertain in preterm infants and requires further study.

It has been suggested that an immature HPA-axis in preterm infants produces sufficient levels of corticosteroids in unstressed infants, but might be insufficient during stress in case of severe illness when higher levels of cortisol are required (19, 20). In preterm infants an insufficient adrenocortical function may be related to neonatal outcome due to its involvement in the development of chronic lung disease and cardiovascular instability in preterms (5, 6, 21–23). Previous studies using ACTH stimulation have shown that the HPA-axis in preterm infants functions inadequately compared with term infants (24–26). However, to our knowledge this is the first study showing that the adrenal function of extremely young preterm infants at higher risk for pulmonary morbidity *i.e.* chronic lung disease is different from "older" preterm infants. Infants born before 30 wk of gestation indeed showed signs of adrenal immaturity and required more mechanical ventilation and were hospitalized for a longer period of time (Table 1). The present study was not designed to establish cut-off values indicative of inadequate adrenal responses in preterm infants in relation to clinical outcome. However, preterm infants with a stimulated cortisol less than 460 nmol/L (lowest thirtile of cortisol secretion) were admitted to the hospital longer than infants with higher stimulated cortisol concentrations. In children and adults stimulated levels of cortisol below 500 nmol/L are commonly used to suggest adrenal insufficiency (14). However, a lower level of cortisol of 500 nmol/L may not be appropriate for very preterm infants and Korte *et al*. suggested a level of 360 nmol/L as cut-off value (15). In the present study only two infants had stimulated cortisol concentration less than 360 nmol/L. No data exists regarding the optimal lower limit of cortisol secretion after 1 μg/kg ACTH stimulation in very preterm infants.

Because of the possible relationship between adrenal development and neonatal morbidity in preterm infants, pediatricians, and neonatologists have shown considerable interest in the development of HPA axis in preterms (27). Although a complete understanding of the adrenal cortical function requires the simultaneous determination of several hormones (*e.g.* dehydroxyepiandosterone sulfate and 11-deoxycortisol), blood sampling in preterm infants is limited by their small blood volume and frequent blood sampling needed during their neonatal intensive care stay.

Traditionally, ACTH stimulation tests in term and preterm infants were performed with high dose ACTH ranging from 36 μg/kg body weight to 250 μg (27–30). The ACTH stimulation test of 1 μg has been evaluated in healthy adult volunteers, adult and pediatric patients with suspected or verified endocrine disorders, and in adults, children, and preterm infants treated with steroids (6, 22, 31–34). Stimulation tests using these lower doses of ACTH have been reported to be more sensitive in detecting insufficient adrenal function in critically ill preterm infants compared with the standard test (22). We found increments of cortisol and 17-OHP in response to 1 μg/kg ACTH stimulation in the preterm infants studied. Dickstein *et al*. have shown that doses lower than 1 μg fail to induce a significant rise in cortisol in adults (34). However, others have indicated sufficient responses in plasma cortisol with even lower doses (33, 35). Because of large differences in body size in neonates, infants, and children it seems reasonable to adjust doses of ACTH to weight or body surface. We have chosen to correct the dose of ACTH for weight because weight measurements are readily available during the 1st week of life, in contrast to length measurements (necessary to calculate body surface).

Some remarks should be made with regard to the technical and practical aspects of an ACTH test. We tried to reduce technical errors that could influence the effect after ACTH stimulation and used freshly prepared ACTH and neonatal indwelling i.v. cannulas without additional plastic tubing. The bioavailability is much lower when ACTH is administered intramuscularly (36). Therefore, especially in "low-dose" ACTH tests i.v. administration is necessary to reach sufficient circulating levels. Some authors have raised concerns about loss of material because of poor i.v. technique resulting in incomplete injection or because of binding of ACTH in plastic giving sets (37).

Several other factors may influence the response after ACTH stimulation in preterm infants. Differences in cortisol and 17-OHP concentrations have been observed earlier between small-for-gestational age (SGA) infants and AGA infants (38, 39). It has also been shown that the cortisol response to ACTH increases in the 1st month of life in preterms compared with the cortisol response directly after birth (28). To minimize the influence of all these factors we studied the effect of a "lower dose" ACTH stimulation test only in AGA preterm infants born before 33 wk of gestation between d 5 and 10 after birth. Furthermore, antenatal steroids administered to mothers at risk of preterm birth may suppress the HPA axis in preterms directly after birth but not after the 1st week of life (40). Exclusion of preterm infants who did not receive antenatal glucocorticoid treatment from the study did not significantly change the results [data not shown]. With respect to the small number of infants not treated with antenatal glucocorticoids, the present study does not provide information on the suppressive or nonsuppressive effect of antenatal steroids on the function of the HPA axis in preterm infants after birth.

CONCLUSION

In conclusion, the effect of gestational age on the response to ACTH stimulation and the different response to ACTH stimulation in preterm infants born before 30 wk of gestation compared with preterms born between 30 and 33 wk of ges-

tation indicate a gradual maturation of the HPA-axis during the third trimester of gestation. This suggests that the HPA-axis function may be immature in infants before term which is expressed by decreased cortisol synthesis and/or a decreased responsiveness to ACTH. A difference in the ratio of cortisol and a cortisol precursor, 17-OHP, suggest an immaturity of enzymes in the adrenal cortex that are necessary for the production of cortisol. The lower levels of cortisol after ACTH stimulation found in preterms with a gestational age below 30 wk indicate that the adrenal cortex in these infants may inadequately respond to stress, which may contribute to the high rate of pulmonary and circulatory morbidity in these infants compared with older preterms (41, 42). The low-dose ACTH test could be a valuable tool in assessing the HPA-axis in sick very preterms (40). However, care should be taken to perform the test technically correct in well-defined pediatric or neonatal populations with as few confounding factors as possible.

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