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ADRENAL CORTICAL FUNCTION IN ABNORMAL NEWBORN INFANTS*

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SUMMARY

The serum cortisol levels of premature infants with hyaline membrane disease (HMD) were compared to cortisol levels of prematures with benign respiratory distress (BRD). Infants (1–3 days old) with fatal HMD had higher ($P < 0.001$) cortisol levels than infants of the same age with BRD. Responses of serum cortisol to ACTH were adequate in all groups. The urinary excretions of THE, 6β-hydroxy-cortisol (6β-OH-F), 16α-hydroxy-dehydroepiandrosterone (16α-OH-DHA) and 16α-hydroxy-pregnenolone (16α-OH-PG) were studied in 23 normal full term newborns, eight normally grown prematures, eight newborns with intrauterine growth retardation (IUGR) and five infants exposed to diphenylhydantoin and barbiturates *in utero*. The excretions of 16α-hydroxy-pregnenolone and 16α-hydroxy-dehydroepiandrosterone were significantly reduced ($P < 0.02$, $P < 0.01$, respectively) in infants with IUGR compared to normal controls, while excretions of THE and 6β-OH-F were normal. Fetal exposure to anticonvulsants had no consistent effect on neonatal steroid excretions.

Hyaline membrane disease

Recent studies of human fetal and perinatal cortisol metabolism have been stimulated by evidence from experimental animal studies that histologic maturation of fetal lungs can be accelerated by glucocorticoid administration to the fetus [1, 2]. Functional maturation of the fetal lung occurs in parallel to the histologic maturation as indicated by prolonged postnatal survival of prematurely born fetuses and evidence of enhanced stability of the air filled lungs from the fetuses [3, 4]. This latter finding indicates that there is an increased amount of surfactant in the alveolar lining layer of the lungs of the treated fetuses, and indeed, glucocorticoids have been shown to increase the activity of the enzyme choline phosphotransferase, critical in the biosynthesis of the principal phospholipid component of surfactant, lecithin [5]. Evidence that the fetal lung has the subcellular components necessary for mediating a response to glucocorticoids comes from three groups who have demonstrated a high concentration of a cytoplasmic glucocorticoid receptor protein in fetal lung [6–8].

These findings in experimental animals led to an examination of the levels of serum cortisol in human premature infants who suffered from hyaline membrane disease [9]. As hyaline membrane disease (HMD) is a result of inadequate alveolar surfactant concentrations and as HMD is a major cause of death in the neonatal period, it was important to establish whether or not a potentially treatable postnatal adrenal cortical hypofunction was associated with HMD. Blood samples were obtained from 15 infants with fatal HMD, 13 infants with nonfatal HMD and 16 infants with benign respiratory distress (BRD), a mild neonatal pulmonary disease characterized by tachypnea and interstitial pulmonary edema. Total corticoids were measured in the ethanol extracts of the serum samples by a competitive protein binding assay (CPB). Cortisol was isolated from the serum samples on LH-20 Sephadex columns and measured by the CPB assay.

Infants in the first three days of life with fatal HMD were found to have significantly greater total corticoid levels ($P < 0.05$) and significantly greater cortisol levels ($P < 0.001$) than were found in infants of the same age with BRD (Fig. 1). Significantly greater circulating total corticoid and cortisol levels were also found in patients with fatal HMD who were 7 days of age and older. The differences between the total corticoid and the cortisol levels, in serum samples where both were measured, were significantly greater ($P < 0.01$) in infants in the first 3 days of life than in infants older than 6 days. This latter finding points up the relative nonspecificity of total corticoid measurement by CBG assay in the first days of life, because of competition with cortisol by other steroids derived from the feto-placental unit. Nine patients underwent intravenous cosyntropin stimulation tests. Pre- and post-stimulation cortisol levels showed adequate responses to cosyntropin in all of the patient categories studied.

The above studies [9] showed that newborn infants with HMD do not manifest adrenocortical insufficiency, but rather have high circulating cortisol levels indicating a probably appropriate response to the severe stress of their illness. Similar conclusions were drawn by another group, who, in addition, found that cortisol concentrations were significantly greater in infants with HMD who were less than 33 weeks

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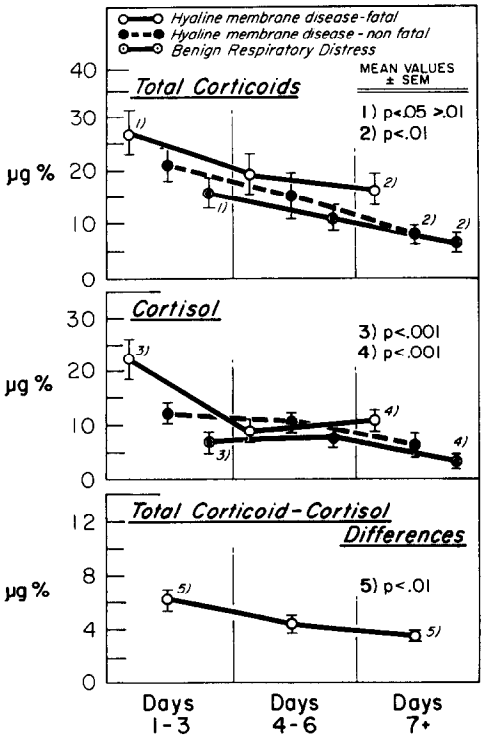

Fig. 1. Mean serum levels of total corticoids and cortisol in premature infants with fatal HMD, nonfatal HMD and BRD. Total corticoid-cortisol differences include data from all three diagnostic groups (from Ref. 9).

gestation as compared to infants with HMD who were 33 weeks or greater [10, 11]. However, their data were not separated into fatal HMD and nonfatal HMD categories.

When our patients with HMD are grouped by gestational age, we find 2 things:

(1) All but one of the patients with fatal HMD were less than 33 weeks gestation.

(2) Of the 13 infants with nonfatal HMD, five were less than 33 weeks gestation and eight were 33 weeks or more. The mean total corticoid levels were identical in the two age groups, 21 µg%. The mean serum cortisol concentration in infants with nonfatal HMD less than 33 weeks was 13·3 µg%, not significantly different from the mean level of 11·6 µg% in infants 33 weeks gestation or greater.

Thus, our data do not show an enhanced cortisol response in the more immature group, if the severity of the HMD is taken into account.

Intrauterine growth retardation

Another group of abnormal newborn infants that might be expected to have abnormalities of adrenocortical function is that with intrauterine growth retardation (IUGR). Women bearing growth retarded fetuses excrete subnormal amounts of estriol and the estriol excretion has been found to be low even taking into account the subnormal fetal size [12]. In addition, infants with IUGR are well known to be prone to hypoglycemia and often respond favorably to glucocorticoid therapy. Fetuses with IUGR have adrenal glands that are relatively more reduced in weight for gestational age than is the body weight, with the fetal zone being more affected than the permanent zone [13].

The few studies of adrenal function in IUGR infants have shown that they have abnormally low cortisol secretion rates [14] and that they have a decreased steroid response to stress [15]. In infants without IUGR but who were born to women with low estriol excretions, Cleary, *et al*, have found lower than normal urinary 16α-OH-DHA excretions [16] and low cord blood levels of DHA and 16α-OH-DHA [17].

As a means of examining the status of both cortisol and 5-ene-3β-hydroxysteroid metabolism in IUGR infants, daily urinary excretions of 6β-hydroxy-cortisol (6β-OH-F), tetrahydrocortisone (THE), 16α-OH-DHA and 16α-OH-pregnenolone (16α-OH-PG) were measured in eight infants with IUGR, eight normally grown premature infants and 23 healthy full-term infants [18]. The free and conjugated steroids were extracted on Amberlite XAD-2 resin, the steroids were eluted, and the conjugates were hydrolysed by β-glucuronidase followed by ethyl acetate solvolysis. Labeled tracer amounts of each steroid to be measured were then added to the extracts in order that procedural losses might be estimated. The individual steroids were isolated by use of paper chromatography and quantified by colorimetric procedures. The excretion values were expressed as µg steroid per 10 mg creatinine.

The study [18] showed that as a group, newborn infants with IUGR excreted significantly lower

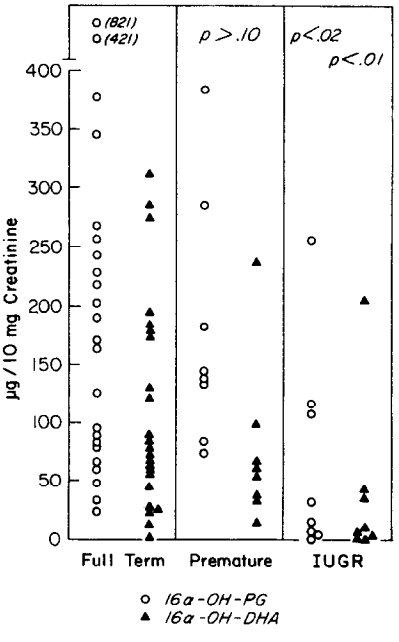

Fig. 2. Urinary excretions of 16α-OH-PG and 16α-OH-DHA by normally grown and IUGR infants. The p values represent the significance of differences from the full-term group by Mann-Whitney test (two-tailed) (from Ref. 18).

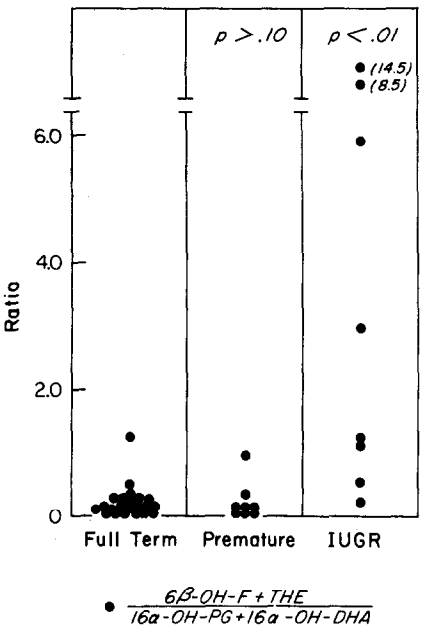

Fig. 3. The points represent the ratios of urinary cortisol metabolite/5-ene-3β-hydroxysteroids in individual samples from normally grown full-term or premature infants, and from infants with IUGR. The p values represent the significance of differences from the full-term group by the Mann-Whitney test (two-tailed).

amounts of 16α-OH-DHA and 16α-OH-PG ($P < 0{\cdot}01$, $P < 0{\cdot}02$, respectively) than did normal full-term newborns (Fig. 2). 6β-OH-F and THE excretions were not significantly different in the two groups of infants. The premature infants who were an appropriate size for gestation had urinary steroid levels similar to the normal full-term group. The differences in steroid excretion patterns among the three groups of infants are best expressed by the ratio of the urinary cortisol metabolites to the 5-ene-3β-hydroxysteroids, 6β-OH-F + THE/16α-OH-DHA + 16α-OH-PG (Fig. 3). The ratio in the IUGR infants was $4{\cdot}37 \pm 1{\cdot}8$ (SEM), in the prematures was $0{\cdot}23 \pm 0{\cdot}11$ and in the normal full terms was $0{\cdot}23 \pm 0{\cdot}05$.

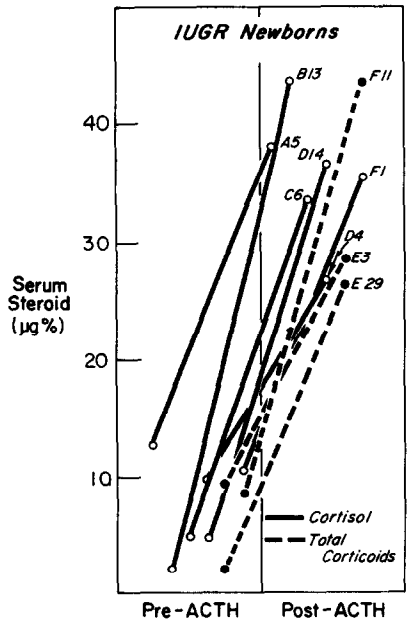

Fig. 4. Serum cortisol or total corticoid levels before and one hour after adrenal stimulation of infants with IUGR by I.V. cosyntropin. The letters by the post-stimulation values identify the patients and the numbers refer to the age in days at time of testing.

Cosyntropin stimulation tests of another group of IUGR infants was carried out and serum cortisol or total corticoid measurements before and after the stimulation showed the infants to have a normal response to the adrenal stimulation (Fig. 4).

Taken together, the studies of IUGR infants discussed above indicate that they have a normal capacity for cortisol secretion. However, it appears clear that there is a significantly reduced capacity for 5-ene-3β-hydroxysteroid secretion in IUGR infants. This latter finding fits well with the observed diminished estriol excretion by women bearing fetuses with IUGR [12], as 16α-OH-DHA is the principal neutral steroid substrate for placental estriol synthesis.

The intriguing question raised by these studies concerns the basis for the dissociation of the capacities for cortisol secretion and 5-ene-3β-hydroxysteroid secretion. One explanation may be that both capacities are reduced in the fetus and that postnatally, endogenous ACTH stimulates only cortisol secretion. This theory would be supported by the observation that ACTH administered to normal newborn infants for three days caused a 10-fold greater rise in cortisol metabolite excretion than in 5-ene-3β-hydroxysteroid excretion [19]. Another explanation may be that 5-ene-3β-hydroxysteroids are secreted principally by the fetal zone of the adrenal cortex. The fetal zone is known to be more atrophied than the permanent zone in newborn infants with IUGR [13]. However, past work from this laboratory [20] has demonstrated that 5-ene-3β-hydroxysteroid excretion in premature infants frequently rises over the first five weeks of life, long past the time when the fetal zone is reported to be atrophic in premature infants coming to autopsy.

Fetal exposure to diphenylhydantoin and barbiturates

There is much interest in the effects on the fetus and newborn infant of pharmaceutical agents administered to the pregnant woman. Two medications with known effects on cortisol metabolism in the adult, and frequently given throughout pregnancy to women with convulsive disorders, are diphenylhydantoin and phenobarbital. In order to examine the effects of fetal exposure to these medications on neonatal steroid metabolism, we measured the daily urinary excretions of 6β-OH-F, THE, 16α-OH-DHA and 16α-OH-PG by five infants born of women who received diphenylhydantoin • and barbiturates throughout pregnancy [21]. Of particular interest was the relative prominence of urinary 6β-OH-F, since both drugs are known to promote the excretion of this cortisol metabolite in adults [22, 23].

The study [21] showed that fetal exposure to these anticonvulsants had no significant effect on the ratio 6β-OH-F/6β-OH-F + THE in the urines studied. In addition, there was no consistent effect of these medications on the 5-ene-3β-hydroxysteroid excretions. A major conclusion is that one cannot necessarily predict the fetal or neonatal effect of a drug on the basis of known effects in the older child or adult.

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