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HUMORAL AND CELL-MEDIATED IMMUNE RESPONSE IN SHORT CHILDREN: EFFECT OF THERAPY WITH hGH.

In 18 short children treated with hGH we investigated the ability of this hormone to influence the serum levels of immunoglobulins, in vitro IgM production, mitogen-stimulated lymphoproliferation, Sm-C and serum growth-promoting activity (Thymidine Activity, TA). Blood was collected before treatment (Gr. A), then on the 5th day following a 4 day course of daily hGH (0.1 U/kg) i.m. injections (Gr. B), then again after a 3 month course of hGH three times weekly (Gr. C). Lymphocytes were separated by centrifugation of Ficoll-Isopaque. The IgM production from the patients' unstimulated lymphocytes in vitro decreased from 277±41 (Gr. A) to 168±38 (Gr. B) and 119±43 ng/ml (Gr. C) (p 0.05). Using stimulated lymphocytes it decreased from 2015±464 (Gr. A) to 116±316 (Gr. B) and then to 511±170 ng/ml (Gr. C) (p 0.02). The variation of this decrease is correlated with the variation of growth velocity during treatment (r = 0.619, p 0.05). In contrast no significant changes were found following therapy neither in the serum levels of IgA, IgE, IgG, IgM, Sm-C and TA, nor in PHA, ConA and PWM-stimulated lymphoproliferation. Our data suggest some relationship between growth hormone, growth and immunity.

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USE OF $^{13}$C-CORTISOL TO DETERMINE THE CORTISOL PRODUCTION RATE (CPR) IN CONGENITAL ADRENAL HYPERPLASIA DUE TO 17-ALPHA-HYDROXYLASE DEFICIENCY.

1,2,3,4-$^{13}$C cortisol($^{13}$C-F) was used to measure the CPR in two sisters(karyotype 46,XX)of 11(I) and 3(II) yrs of age with 17α-hydroxylase deficiency. The disease was diagnosed on hyper tension, low plasma conc. of 17β-estradiol,cortisol, PRA and aldosterone and high conc. of progesterone and in patient I of ACTH, FSH and LH. Both children excreted in the urine high amounts of tetra-and hexahydro-metabolites of corticosterone and deoxycorticosterone and (very) low amounts of metabolites of cortisol (F), androstenedione and testosterone.

After the i.v. dosage of 60 and 2.4 µg to patient I and II, urine was collected for 3 days. After extraction, hydrolysis and HPLC Chromatography the metabolites of F were oxidised to 11-oxo-etiocholanolone (11-OET) and -androsterone.The $^{13}$C-enrichments in the methoxime-tert.butyldimethylsily ethers were measured by gas chromatography mass fragmentography at the leading ions m/z 348 ($^{13}$C$_4$) and 344 ($^{12}$C). Calibration standards were prepared from mixtures of $^{13}$C-F and F, which were oxidised to cortisone (E), reduced to tetrahydrocortisone (THE) by Cl.paraputrificum and oxidised to 11-OET. The CPR (mg/day) was for I:0.28±0.003 (THE) and 0.19±0.007 (α+βcortolone) and for II:0.022±0.003 (THE). Owing to the very low amounts of cortisol metabolites the CPR determinations would not have been possible if tritiated cortisol was used. Financial support was obtained from the Netherlands Organisation for the Advancement of Pure Research (ZWO).

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AMNIOTIC FLUID 17OH-PROGESTERONE (17P) IS INCREASED ONLY IN SALT-LOSING FETAL 21-HYDROXYLASE DEFICIENCY (21 OHD).

Second trimester amniotic fluid was analysed from 54 pregnancies at risk of fetal 21-hydroxylase deficiency; the outcome is currently known in 46. Analysis of samples from a further 96 normal outcome pregnancies provided a reference range for 17P. The assay used an $^{125}$I-radioligand and an antiserum raised against a 17P-3-CMO-BSA conjugate coupled to a magnetisable, solid-phase support. Mean ± SD 17P values were: normals, 18.5 ± 5.2 nmol/l (range 9.8 - 30.7); predicted unaffected, 17.4 ± 6.5 (9.4 - 31.3, n = 37); predicted affected, 64.2 ± 46.3 (30 - 170, n = 9; p<0.001). Unaffected pregnancies produced all normal infants at birth, but a male non-salt loser had increased 17P levels at 3 months. Four of 9 predicted pregnancies were terminated, 1 affected male died at 2 weeks after preterm delivery; in the remaining 4 term pregnancies, 21OHD was confirmed postnatally in 3 and excluded in 1 by a normal plasma 17P. Family HLA typing showed he was a heterozygote. Information on 44 index cases showed 61% females and 77% salt-losers; 8 of these had died in infancy. All fetuses in this study predicted to be affected were salt-losers. Thus prenatal steroid analysis is reliable only for this vulnerable group. Preliminary data from the literature indicates that use of current 21OH gene probes may not be any more specific in the diagnosis of the precise phenotype.

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PITFALLS OF PRENATAL TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA (CAH) DUE TO 21-HYDROXYLASE DEFICIENCY

It has been reported that prenatal dexamethasone (DEX) treatment of women carrying female CAH fetuses suppresses fetal adrenal function and prevents virilization of external genitalia.In 2 CAH families,the mothers were treated in their 2nd pregnancies with DEX (1mg/d) from week 10.Dex was stopped 5 d before amniocentesis (wk 17),then resumed until delivery. Both fetuses (46,XX) had high amniotic fluid 17OHP levels (10.5, 10.4;normal range:1.2-3.8 ng/ml) suggestive of homozygous 21-OHase def.Pregnancies and deliveries (term) were normal.Both newborns were virilized (Prader IV).Plasma levels of progesterone (P),17-hydroxyprogesterone (17OHP),aldosterone (Aldo),cortisol,estriol and DEX were measured by specific RIAs in Pat.1 from wk 17,in Pat. 2 from wk 25 until delivery.Results (Pat.1/Pat.2) in ng/ml.

| wk | P | 17OHP | Aldo | Estriol | Cortisol | DEX |
|---|---|---|---|---|---|---|
| 17 | 25.6/ - | 4.01/ - | 0.036/ - | 33/ - | 229/ - | 0.2/ - |
| 20 | 28.2/ - | 3.21/ - | 0.113/ - | 51/ - | 259/ - | 2.1/ - |
| 25 | 46.6/31.9 | 3.34/2.67 | 0.119/0.200 | 74/ - | 310/113 | 9.7/11.3 |
| 29 | 60.4/20.9 | 1.99/2.06 | 0.078/0.500 | 66/97 | 108/ 38 | 9.9/ - |
| 36 | 80.2/39.4 | 11.3/4.17 | 0.171/0.138 | 156/221 | 216/ 49 | 10./4.87 |
| Term | 101.9/90.6 | 21.7/16.2 | 0.075/0.222 | 153/350 | 668/131 | 6.76/1.94 |

Whereas P,17OHP and cortisol levels were generally suppressed in comparison with normal pregnancies,estriol levels were not sufficiently suppressed throughout pregnancy, despite appropriate DEX levels.This suggests that fetal adrenal function was not adequately suppressed,possibly due to insufficient DEX dose and/or increased metabolic clearance rate.

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AGE-RELATED CHANGES IN ADRENAL SIZE DURING THE FIRST YEAR OF LIFE: COMPARISON OF ULTRASOUND EVALUATION AND DHEA-S IN NORMAL INFANTS AND A NEWBORN WITH CONGENITAL ADRENAL HYPERPLASIA (CAH)

In 48 newborns and infants of 3 age groups, plasma DHEA-S was measured by RIA, and adrenal size, expressed as an adrenal size index (ASI), was assessed by ultrasound.

| Group (age) | I (-14d) n=17 | II (>14d-3mo) n=13 | III (>3mo-1y) n=18 |
|---|---|---|---|
| ASI(mm$^2$) | | | |
| mean(range) | 62.4(31.4-107.4) | 46.0(25.9-75.0) | 46.0(21.6-79.4) |
| DHEAS(µg/dl) | | | |
| mean(range) | 55.2(5.8-219.2) | 29.4(6.3-56.7) | 10.2(0.1-36.8) |

ASI were high in the newborn period and decreased with age (group I vs. II and III: p<0.02). Concentrations of DHEA-S as a marker of the fetal zone of the adrenal showed a negative correlation with chronological age (r=-0.62; p<0.0001) and differed significantly in the 3 age groups (p<0.05).
In a boy with CAH due to 21-hydroxylase-deficiency, diagnosed prenatally, ASI were above the normal range for age during the first days of life and returned to normal with treatment.
In conclusion, ASI are helpful in the clinical diagnosis of adrenal diseases in the newborn period. Reference ranges are given for newborns and infants during the first year of life. Comparison of DHEA-S concentrations and adrenal size in vivo as determinad by ultrasound, is consistent with the fact that decrease in adrenal size shortly after birth is due to involution of the fetal zone.

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ONTOGENY OF PLASMA 5-ALPHA-ANDROSTAN-3-ALPHA, 17-BETA-DIOL GLUCURONIDE (Adiol-G) IN HUMAN.

Plasma Adiol-G values, considered as a good marker of peripheral androgen conversion in adults, have not yet been reported in children. Adiol-G was measured in plasma aqueous phase left after ether extraction of unconjugated steroids. The method included β-glucuronidase hydrolysis, ether extraction, celite column chromatography and specific RIA. The results, in 150 normal children and 33 adults, are given in ng/dl (mean±SD). In adults, Adiol-G levels were significantly higher (p <0.001) in males (436±174) than in females (135±74). In children 1-6 years old, Adiol-G was low without sex difference: 21.2±9.8 in boys, 19.9±6.6 in girls. Then, Adiol-G levels rose progressively: 43.9±13.8 in boys and 47.8±18 in girls 9-11 years old. After the age of 11, Adiol-G rose significantly and more rapidly in boys than in girls: 72.7±39 in boys vs 52.9±17 in girls from 11 to 13 years and 158.8±68 in boys vs 76±4.8 in girls from 13 to 15 years. Adult values were observed at age 15-16 in girls, while in boys, Adiol-G levels continued to rise till adulthood. In 3 boys, age 13.6 to 19, with complete adrenal insufficiency and normal pubertal development, values of Adiol-G were normal for age. The Adiol-G rise observed at the end of an hCG test (204±159) in 12 boys (1-11 yrs) and the high values found in untreated CAH, 528±267 in 12 infants and 216.6±102 in 7 girls (1-6 yrs) suggest that Adiol-G could be used as a parameter of androgen metabolism in childhood.