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EFFECT OF CHRONIC ADMINISTRATION OF CLONIDINE IN NORMAL CHILDREN WITH SHORT STATURE: RATE OF GROWTH AND SOMATOMEDIN C LEVELS. Mariano Castro-Magana,

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The oral administration of Clonidine, (C) a selective Alpha-Adrenergic agonist has been shown to increase growth hormone (GH) secretion in children and has been proposed as one of the reliable tests of GH reserve. The effects of chronic administration of C on growth have not yet been reported. We have now evaluated the effects of C administration on the rate of growth (RG) and somatomedin C (SM-C) levels in normal prepubertal children with short stature. Ten children (4-9y) with normal RG and normal GH reserve as assessed by the C test and SM-C determination were the subjects of this study. C was administered orally to these children as a single dose (0.15mg/m²/body surface area) every night during a 6-month period. Height, weight and blood pressure were measured. Plasma SM-C was determined before and one week after the last C dose. No side effects were observed during the 6-month period.

The administration of C during that period produced a significant increase in the RG and SM-C levels.

	Before C		After C	
	RG (cm/6m)	SM-C (U/ml)	RG (cm/6m)	SM-C (U/ml)
$\bar{x} \pm SD$	2.41 $\pm$ 0.27	0.36 $\pm$ 0.15	4.45 $\pm$ 1.15	0.54 $\pm$ 0.16

We conclude that C-administration could be used as an alternative treatment modality for normal children with short stature

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THE ROLE OF ADRENAL STEROIDS IN PSEUDOPRECOXIOUS PUBERTY DUE TO LARGE OVARIAN FOLLICULAR CYSTS.

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Ovarian secretion of trophic steroids, or their precursors, such as 17-hydroxyprogesterone (17P), increases during puberty. Many of these steroids are also secreted by the adrenal. We have shown that girls with premature adrenarche have increased 17P levels after ACTH stimulation. In order to study the role of adrenal androgens in other forms of precocious puberty, we measured 17P after dexamethasone (DEX) suppression (1mg) and an IV bolus of ACTH (0.25 mg) in 5 girls with premature thelarche who also had small ovarian cysts documented by ultrasound (PT) and 2 girls with pseudoprecocious puberty due to a large follicular ovarian cyst (PP). The girls with PT had normal 17P levels which were suppressed completely after DEX and increased to 61 $\pm$ 16 (X $\pm$ SD) ng/dl after ACTH (normal 71 $\pm$ 20ng/dl). Although basal 17P levels were normal in the girls with PP, they were not completely suppressed by DEX and increased to levels over 3-times normal after ACTH (360,540,ng/dl). Excision of the cysts abolished pubertal progression and restored DEX suppressibility of 17P. Although the 17P responses to ACTH were substantially decreased (160,350 ng/dl), they were still supranormal. Suggestion: Excess steroid production by the adrenal may stimulate development of small ovarian cysts into large cysts and thus cause PP.

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ENHANCED RESPONSE TO hpGRH-40 IN GROWTH HORMONE NEUROSECRETORY DYSFUNCTION. Stuart A. Chalew,

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In growth hormone neurosecretory dysfunction (GHND), poor growth is due to low 24-h integrated concentration of GH (IC-GH) even though the response to paired pharmacologic stimulation (PS) of GH is normal. We compared the GH response to 1 μ g/kg hpGRH-40 of 8 GHND children, 4 children with subnormal GH responses to paired PS tests and subnormal IC-GH (GHD), and 3 short children with normal PS and normal IC-GH (NORMAL SHORT). The peak GH response to hpGRH-40, PS stimulation and IC-GH of the 3 groups is presented as mean $\pm$ 1SD in ng/ml:

TEST	GHND	GHD	NORMAL SHORT
hpGRH-40	69.2 $\pm$ 39.4	13.1 $\pm$ 9.6**	22.7 $\pm$ 16.1*
Pharm.Stim.	20.4 $\pm$ 6.4	5.9 $\pm$ 1.5**	24.5 $\pm$ 15.2
IC-GH	1.8 $\pm$ 0.7	1.7 $\pm$ 1.1	6.8 $\pm$ 2.1

*P=less than 0.05, **P=less than 0.01 compared to GHND

The response to hpGRH-40 of GHND children was significantly higher than the other groups.

Conclusion: 1/GHND children have an increased reserve of releasable GH. 2/GHND lack the neurologic GRH releasing mechanism to maintain adequate spontaneous GH release. 3/The increased response of GHND to hpGRH-40 may be of diagnostic value.

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MOLECULAR CLONING OF THE BOVINE 21 HYDROXYLASE GENE.

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About 1 in 5000 people has congenital adrenal hyperplasia due to disordered 21 hydroxylation. 21 hydroxylase is a single specific 49000 dalton microsomal cytochrome P450, (P450c21) which is less than 0.1% of adrenocortical protein. Most *in vitro* studies of steroidogenesis have been bovine adrenals. We manually synthesized 23- and 30-base oligonucleotides corresponding to different regions of the bovine P450c21 cDNA sequence. After screening 2 x 10⁵ bovine genomic DNA clones in λ EMBL3A we identified a single plaque hybridizing to both oligos. This phage (λ El1) was plaque-purified and the 17 kb insert was characterized by restriction endonuclease mapping. The whole 50 kb phage was denatured and hybridized to each synthetic oligo and used to initiate dideoxy DNA sequencing, proving that λ El1 contains bovine P450c21 gene sequences. Hybridization of ³²P-labeled total bovine genomic DNA to restriction fragments of λ El1 suggests some, but not all of the noncoding regions contain repetitive DNA. A 1.2 kb EcoRI fragment lacking repeats was sequenced in M13, revealing 3 exons and 3 introns with classic splice sites. Southern blots of genomic DNA indicate the bovine and human genomes contain two P450c21 genes (or pseudogenes). Northern blots of bovine and human adrenocortical mRNA indicate P450c21 mRNA is ~ 2 kb long and arises from precursors as large as 9.5 kb. This cloned bovine 21 hydroxylase gene will permit detailed characterization of the structure and regulated expression of the gene(s) for this important steroidogenic enzyme.

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DEVELOPMENT OF STEROIDOGENIC ENZYMES IN PIG TESTES DURING THE FIRST TWO WEEKS OF LIFE. Fred I. Chasalow

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To evaluate androgen synthetic pathways in newborn pigs, we isolated microsomes from testes of 2, 6, 10 and 14 day old pigs. Microsomes were incubated with (7-³H) pregnenolone and/or (4-¹⁴C) progesterone. After extraction with ethyl acetate products were identified by co-chromatography with authentic steroids. In contrast to rat and human testicular microsomes, the major products were 5,16-androstadien-3 β -ol (I) and 4,16-androstadien-3-one (II) from pregnenolone and progesterone respectively. The ratio of I to II decreased with age indicating that 3 β -hydroxysteroid dehydrogenase activity had increased during the first 2 weeks of life. Other products included dehydroepiandrosterone and androstenedione, but not testosterone. Although 17-ketosteroid reductase was found in testicular microsomes from boars, it was not present in testicular microsomes from newborn pigs.

Conclusion: The enzymes for the production of I and II are present in testes from newborn pigs. Thus these compounds, which represent 75% of the androgens produced by boars, can also be produced during the newborn period. The production of these compounds and the difference in subcellular localization of 17-ketosteroid reductase are two significant differences in the steroidogenic processes in the testes between piglets and boars or humans.

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SERUM OSTEOCALCIN: RESPONSE TO EXOGENOUS PARATHYROID HORMONE (PTH) IN X-LINKED HYPOPHOSPHATEMIA (XLH). David E.C. Cole and Caren M. Gundberg.

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Osteocalcin (OC) is a bone-specific protein that can be quantitated in serum by radioimmunoassay. Serum OC is a sensitive measure of bone formation, but its response to calcitropic hormones *in vivo* is still poorly understood. We observed the effects on serum OC of a 5 min i.v. infusion (mean 5.0 U/kg) of PTH in 7 XLH patients (3 males, 4 females; ages 8-35 yr) who had been treated with 1,25(OH)₂D₃ and phosphate (P_i). Pre-infusion renal P_i reabsorption (TRP_i) was determined as a mean of three values from sequential 30 min collection periods (0900 to 1030 hr). Post-infusion clearances in 3 subsequent periods (1030 to 1200 hr) were examined separately. The large variation (5-116 ng/ml) in pre-infusion serum OC among individuals was related to age differences and treatment status. Urinary cAMP response to PTH was maximal in the first post-infusion period (mean increase: 7.6-fold); the phosphaturic response (blunted in XLH) was greatest in the second (Δ TRP_i=14%). Serum OC decreased in all 7 patients. At 15 and 45 min, OC concentrations were 63 $\pm$ 5% and 69 $\pm$ 7%, respectively, of pre-infusion ones ($\bar{x} \pm$ SE, p<.01); but by 75 min, mean OC values were no longer significantly different from pre-infusion (87 $\pm$ 14%). We suggest that serum OC responds rapidly to PTH and may therefore be useful in the study of skeletal sensitivity to the hormone.