420 LATE ONSET CONGENITAL ADRENAL HYPERPLASIA (LOCAH) IN TWO GENERATIONS. Gertrude Costin, Univ. of So. Calif. Sch. of Med., Childrens Hospital of Los Angeles, Dept. of Peds., Los Angeles.

A 6 yr old girl (Pt1) presented with pubic hair, acne and body odor for 1 yr. Ht was at the 95th centile and bone age (BA) was 8 yrs; she had stage II pubic hair, acne and mild clitoromegaly. Serum 17-hydroxyprogesterone (17-OHP), androstenedione, and testosterone (T) were 218, 46 and 40 ng/dl; LH, FSH and E2 were prepubertal. Her sister (Pt2) was asymptomatic at 2½ yrs; at 3½ yrs her ht had increased from the 75th to the 90th centile and her BA was 4½ yrs. She had occasional acne and body odor. Their 9 yr old brother (Pt3) was at the 50th centile for ht with a BA of 9 yrs and no signs of virilization. The mother (Pt4) was 162cm tall, obese and hirsute; she had clitoromegaly and irregular menses. The father (Pt5) was 170 cm tall and apparently normal. Basal (B) and Cortrosyn® stimulated (S) serum adrenal steroids are listed below.

Pt	Age	17-OHP		P		T	F(ug/dl)	HLA		
#	Yrs	B	S	B	S	B	S	A	B	C
1	6	1449	3742	159	761	40	23.2	(3,24)	(14,14)	DR1
2	2½	785	2735	45	232	15	22.9	(3,33)	(14,14)	DR1
3	9	611	4500	<10	283	38	14.2	(24,28)	(14,14)	DR1
4	39	199	3224	<10	84	67	18.6	(3,28)	(14,14)	DR1
5	40	494	3699	<10	56	470	24.2	(24,33)	(14,14)	DR1

The findings in this family illustrate the spectrum of LOCAH ranging from no symptoms (Pts 3,5), to rapid growth (Pts1,2), premature pubarche and clitoromegaly (Pt1), and post pubertal hirsutism and oligomenorrhea (Pt4). There appears to be no correlation between the biochemical abnormalities and the clinical manifestations of the disease.

421 HYPOPITUITARISM AND PRECOCIOUS PUBERTY (PP). G. Costin and F. Kaufman. Univ. of So. Cal. Sch. of Med., Dept. of Peds., Childrens Hosp. of Los Angeles, Los Angeles.

Three girls and one boy with hypopituitarism owing to histiocytosis (#2,3) and to cranial radiation for a craniopharyngioma (#1) and a thalamic tumor (#4) developed PP. Signs of sexual precocity were noted prior to treatment with somatotropin (HGH) in 3 patients, and during the course of therapy in one. Clinical and laboratory data are indicated below.

Pt/	CA	HA	BA	Tanner	Peak GH	SmC	LH	FSH	E2/T
sex		(Yrs)		(stage)	(ng/ml)	(U/ml)	(mIU/ml)		(ng/dl)
1/F	7	6½	5½	II	3.7	0.2	6.1	6.2	4.0
2/F	7½	6	4½	II	5.0	0.9	2.0	4.4	3.0
3/F	8	5½	7	II-III	4.3	0.5	8.0	ND	5.0
4/M	8½	4½	7½	II-III	3.5	0.9	5.2	12	100

While in patient #2 there was a normal growth velocity associated with puberty, in patient #3 the growth spurt was subnormal;in both patients menses started at 11-11½ yrs. Five yrs after the onset of PP the CA/HA/BA ratios in patients #2 and 3 were 12½/11½/14 and 13½/10½/15, respectively. In the other 2 patients, the CA/HA/BA ratios 1½ yrs after onset of PP were 8½/7½/7½(#1) and 10/5½/10(#4).

These findings indicate that 1) sexual precocity does occur in the presence of hypopituitarism, 2) there is a rapid skeletal maturation before clinical signs of PP are apparent, and 3) the pubertal growth spurt even if adequate does not result in a normal adult height if there is an abnormal HA/BA ratio at the onset of PP. We submit that in these patients for achievement of an average height PP must be curtailed by pharmacologic means until the HA/BA ratio is normalized by treatment with HGH.

† 422 CLONING OF cDNA ENCODING BOVINE 21-HYDROXYLASE. Bon-Chu Chung, Karla J. Matteson, John E. Morin, & Walter L. Miller. Department of Pediatrics, University of California, San Francisco, CA

Over 90% of cases of congenital adrenal hyperplasia are due to disordered 21-hydroxylation, which converts progesterone and 17-hydroxyprogestone (17-OHP) to DOC and 11-deoxycortisol. 21-hydroxylase is a single 49000 dalton microsomal cytochrome P450, termed $P450_{C21}$, which represents <0.1% of adrenocortical protein. We prepared polyadenylated messenger RNA (mRNA) from demedulated bovine adrenals and used this mRNA as template for the synthesis of double stranded (DS) complementary DNA (cDNA). The 3' ends of the DScDNA were extended with deoxycytosine using terminal deoxynucleotidyl transferase; this "tailed" DScDNA was inserted into the Pst I site of pBR322 similarly tailed with deoxyguanosine. Transformation of E. coli 294 yielded >60,000 clones. Based on a partial porcine amino acid sequence, we used the manual phosphoramidite method to synthesize a family of 32 15-base oligonucleotides and used these to prime the synthesis of a specific cDNA probe. Clones hybridizing to the 15mer-primed cDNA were then probed with a different family of 64 17-base oligonucleotides we synthesized based on a different portion of the amino acid sequence. Of 10,000 clones containing cDNA inserts longer than 800 bases, 5 hybridize efficiently with both probes, indicating they carry bovine $P450_{C21}$ cDNA sequences. These DNA clones will permit the determination of the structure of bovine $P450_{C21}$ as well as human $P450_{C21}$ and its pathogenic variants.

423 THE EFFECT OF GLUCOSE (G) AND ESSENTIAL AMINO ACIDS (EAA) ON INSULIN (I) BIOSYNTHESIS AND RELEASE IN HUMAN FETAL PANCREAS CULTURES(HFPC).Agnes A. Cser,R.D. G. Milner,Dept.Ped.,Univ.Sheffield,England. (Spon. by T. Heim).

Fetal pancreatic tissue (FPT) has a great potential for proliferation and may provide a suitable source for transplantation in treating diabetes mellitus. In order to characterize its insulin producing capacity, 8 human fetal pancreases of 13-20 wk. gest. were studied in organ culture for 7 days. DNA and insulin content and release into the culture-medium from day 3 to 7 were measured. Two types of medium both supplemented with 20% (W/V) heated fetal calf serum were used: 1) Eagle's minimum essential medium (MEM) was supplemented with 6 times the basal concentration of EAA containing 5 (7EAA1G) or 16.5 (7EAA3G) mmol/l glucose and 2) "RPMI 1640 medium" contained 11.6 (R) or 23.2 (RG) mmol/l glucose. Glucose enrichment increased tissue insulin content (μU/μg DNA) by 44 to 263% in 7EAA (20.5±1.5 in 1G vs 38.9±2.6 in 3G;$p < 0.01$) and by 34 to 276% in RPMI (16.9±1.5 in R vs 36.2±2.9 in RG;$p < 0.01$). High EAA concentration itself favoured tissue insulin synthesis (7EAA1G larger than R;$p < 0.001$ and RG 31.1±5.7 vs R 12.1±2.6 μU/μg DNA;$p < 0.001$). The insulin specific activity (μU I/μg DNA) of FPT increased with gest. age being 0.24 at wk. 13 and 1.62 at wk. 20 in the fresh tissue and after 7 days of culture 25.2 at wk. 13 and 61.3 at wk. 20. Results indicate: 1) both insulin synthesis and release of FPT and HFPC increased in response to glucose and essential amino acids and 2) the combination of high glucose and high essential amino acids containing medium can potentiate the fetal pancreatic tissue capacity for insulin synthesis in a transplant.

424 GROWTH HORMONE RELEASE FOLLOWING ADMINISTRATION OF GROWTH HORMONE RELEASING FACTOR TO GROWTH HORMONE DEFICIENT AND NORMAL MALES. Floyd L. Culler and Kenneth Lee Jones, UCSD School of Medicine, Dept. of Pediatrics, La Jolla, California.

We have studied 4 males with idiopathic growth hormone (hGH) deficiency and 8 normal adult males comparing their responses to a 100 μg bolus infusion of the 44 amino acid synthetic growth hormone releasing factor (GRF-44). Responses were compared for changes in hGH, prolactin (PRL), insulin (I), glucagon (G) and glucose (Glu) concentration over a 2-hour period after injection of GRF-44. hGH deficiency was initially documented using standard testing procedures. No patient had received hGH replacement for at least one month prior to testing.

The hGH deficient patients had a significant ($p<0.05$) GRF-induced increase in hGH concentration from a baseline of 2.7±0.7 ng/ml (M±SEM) to a peak of 10.3±3.2 ng/ml. The normal males showed a much greater ($p<0.005$) change in hGH levels with a peak response of 46.0±5.8 ng/ml from a baseline of 2.98±0.1 ng/ml. There were no changes in PRL, Glu, I or G in either group.

We have demonstrated that patients with subnormal hGH responses to traditional stimuli can generate hGH levels in the normal range (>7 ng/ml) following GRF stimulation. The mean increase of the hGH deficient group, however, was only 22% of the mean increase of the group of normal male controls. This confirms that patients with idiopathic hGH deficiency may have hGH release, though it is subnormal, following single bolus GRF-44 testing.

425 CONGENITAL HYPOTHYROIDISM: THE EFFECT OF STOPPING TREATMENT AT 3 YEARS OF AGE. Till Davy, Denis Daneman, Paul G. Walfish, Robert M. Ehrlich, University of Toronto, Hosp. for Sick Children, Toronto, Canada.

69 children were detected by a low thyroxine (T4) and/or elevated thyrotropin (TSH) on neonatal screening to have congenital hypothyroidism (CH). Of these, 55 are 3 years of age or older. Thyroid scanning at diagnosis showed 16/55 were athyrotic (A), 21/55 had goiters (G) and 18 with ectopic (E) glands. E were excluded because of the anatomic abnormality. 22 of the remaining 37 showed a secondary rise of TSH to >10 mU/L confirming the diagnosis during the first 3 years. In 15 with continuous TSH suppression, thyroxine was discontinued at age 3 years or more. In the first 6, TSH was measured at 6 weeks: in 5/6 it was >60, in 1 with G-CH, TSH was 7 at 6 weeks and 15 at 8 weeks. Symptoms of hypothyroidism occurred in 4/6. TSH was then measured at 3 weeks and was >60 in the remaining 10. When the TSH was elevated, T4 was 40±39 nmol/L ($\bar{X}$±SD). In all patients with A-CH or G-CH, the diagnosis was confirmed either by a secondary rise of TSH while on treatment or by a TSH rise at 3-6 weeks after discontinuing therapy. In this group, thyroid screening appears to be 100% specific. Hypothalamic-pituitary responsiveness and hypothyroid symptoms return very rapidly after stopping L-T4 therapy. We recommend TSH measurement 3 weeks after stopping L-T4 in children with CH >3 years in whom confirmation of the initial diagnosis is desirable.