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To understand the early molecular events that lead to some of the effects of growth hormone on liver, a dose of ^{3}H-HGH (18×10^6 cpm, 60 μg) was given i.v. to hypophysectomized rats. After the injection, livers were homogenized and fractionated by centrifugation. Specific activity (cpm/mg protein) and total radioactivity expressed as percent of that in the homogenate were estimated (table). ^{3}H-HGH in the homogenate was estimated by precipitation with TCA, and with Na_2SO_4 as ^{3}H-HGH antibody complex with and without unlabeled hormone. Up to 10 min most radioactivity was protein bound and ^{3}H-HGH in the homogenate was antigenically similar to the injected dose. In contrast, when ^{3}H-HGH was added *in vitro* before homogenization most radioactivity (77%) appeared in the cytoplasm and the rest distributed almost equally among the other fractions. These results indicate (1) the early rapid uptake by the particulate fractions may be of functional significance and the important initial binding sites may be mitochondria and microsomes; (2) the movement of ^{3}H-GH in liver cell may be from the microsomal and mitochondrial fractions into cytoplasm; (3) after 10 min ^{3}H-HGH is degraded or deacetylated.

Fraction	2 min	5 min
Homogenate	1517 (100%)	2816 (100%)
Crude nuclear	866 (18.3%)	1232 (11.2%)
Mitochondrial	1638 (22.5%)	5043 (41.0%)
Microsomal	4776 (41.9%)	5841 (29.2%)
Cytoplasmic	1464 (27.5%)	2177 (23.2%)
Fraction	10 min	20 min
Homogenate	3246 (100%)	3901 (100%)
Crude nuclear	1707 (13.3%)	2042 (13.8%)
Mitochondrial	6308 (29.9%)	4622 (21.8%)
Microsomal	4754 (15.3%)	2954 (8.5%)
Cytoplasmic	3294 (23.5%)	7186 (46.1%)
Fraction	40 min	
Homogenate	1997 (100%)	
Crude nuclear	845 (13.5%)	
Mitochondrial	1442 (16.1%)	
Microsomal	1302 (7.9%)	
Cytoplasmic	4665 (65.3%)	

89 *Partial 3β-hydroxysteroid Dehydrogenase (3β-HSD) and 21-hydroxylase Deficiencies in a Family With Congenital Adrenal Hyperplasia, With Evidence for Increasing 3β-HSD Activities with Age.* FREDERIC M. KENNY, JOHN W. REYNOLDS and ORVILLE C. GREEN, Univ. of Pittsburgh, Univ. of Minnesota and Northwestern Univ., Depts. of Ped.

In this family, two affected boys have perineal hypospadias and bifid scrotum, and two affected girls have slight clitoral enlargement with otherwise normal genitalia. All are mild 'salt-losers' with spontaneous crises occurring late (3 months and 2 years) in the boys. The girls had negative Na+ balance only when stressed by salt deprivation at ages 2 months and 4 years. All had elevated 17-ketosteroid excretion when diagnosed and, in the 2 youngest, urinary DHA > androsterone. Cortisol production and/or 17-OH-corticosteroid excretions were normal. Steroid excretion patterns showed an increase of 3β-HSD activity with increased age, but with a persisting high excretion of Δ^5-pregnanetriol (Δ^5-p'triol). At 2 months, one girl excreted per 24 h: pregnanetriol (p'triol)-0.23 mg, Δ^5-p'triol-1.2 mg, 17α-OH-pregnenolone-2.6 mg, 16α-OH-pregnenolone-8.2 mg, 16α-OH-DHA-5.4 mg, DHA-0.5 mg. Her sister, at age 10 years, during withdrawal of cortisone therapy, excreted per 24 h: p'triol-24 mg, Δ^5-p'triol-14 mg, DHA-1.2 mg, no 16α-OH-pregnenolone or 16α-OH-DHA. We conclude that these cases have partial 3β-HSD deficiencies, on the basis of the inadequate fetal virilization and persistent post-natal excretion of large amounts of Δ^5-p'triol; and partial 21-hydroxylase deficiencies, on the basis of the high p'triol excretions. The low post-natal excretion of DHA, relative to Δ^5-p'triol and 17α-OH-pregnenolone, may be due to a late fetal appearance of the 3β-HSD for DHA or may be due to an underactivity of the 17–20 desmolase (side-chain splitting) enzyme.

90 *Sulfonylureas Amplify Cyclic 3',5' AMP Mediated Hormone Action.* HANS H. BODE, BARBARA M. HARLEY, ALLEN M. SPIEGEL and JOHN D. CRAWFORD, Harvard Med. Sch., Massachusetts Gen. Hosp., Children's Service and Shriners Burns Inst., Boston, Mass.

The reports that the sulfonylurea compounds have a vasopressin-like action in some patients with diabetes insipidus (DI) and MAHONEY and GOODMAN's observations of benefit in one patient with hypodipsia led us to a trial in three children with both deficiencies. Hitherto extraordinarily difficult management problems, these children have shown restoration of homeostatically appropriate thirst and facultative urinary concentrating ability during chlorpropamide (CPM) treatment.

Observations to provide insight into the mechanism of these clinical effects were as follows: much as in total diabetes mellitus, the sulfonylureas were without benefit in total vasopressin lack (Brattleboro rats) and in clinical nephrogenic DI Sulfonylureas, while inactive alone, augmented vasopressin stimulated water flux across toad bladder. Water flux induced by cyclic 3',5' AMP was not increased suggesting the compounds act neither by augmenting the influence of second messenger nor by phosphodiesterase inhibition. CPM treatment increased and prolonged parathyroid hormone provoked urinary excretion of cyclic 3',5' AMP in two patients with pseudohypoparathyroidism much as it extended and intensified antidiuresis due to exogenous vasopressin in water loaded normals.

The observations support the hypothesis that sulfonylureas act by amplifying the cyclic 3',5' AMP signal generated by hormones at their specific target tissues, possibly including CNS, where second messenger concentrations are high. If correct, the amplifier hypothesis implies extended applicability of sulfonylueras and their congeners in restoring homeostasis in states of attenuated endocrine secretion.

91 *Adverse Effects of Large Doses of Medroxyprogesterone Acetate (MPA) in Idiopathic Isosexual Precocity.* ROBERT A. RICHMAN, LOUIS E. UNDERWOOD, FRANK S. FRENCH and JUDSON J. VAN WYK, Univ. of North Carolina Sch. of Med., Dept. of Ped., Chapel Hill.

Three girls and one boy with isosexual precocity were treated with large intramuscular doses of MPA, 200–300 mg *q.* 7–10 d., in an attempt to control the rapid advancement of skeletal maturation. Although secondary sexual development regressed, all four developed signs suggestive of corticoid excess. These included rapid weight gain, mild hypertension, plethoric

facies, increased body hair, and inappropriate insulin responses to glucose and arginine. In the presence of these signs of corcoid exctiess, rapid linear growth and skeletal maturation continued in all patients. Fasting growth hormone levels and acute responses to insulin and l-arginine remained within normal limits. Urinary testosterone in the boy decreased from 15.1 μg/24 h before treatment to 1.2 μg/24 h; however, further testicular enlargement occurred during therapy.

Adrenal function in the two patients so studied revealed pituitary-adrenal suppression. Excretion of Porter-Silber (P-S) chromogens was within normal limits and not suppressed by dexamethasone. Neither patient responded to metyrapone or ACTH. Thus, it is likely that the urinary steroids measured as P-S chromogens were derived from MPA rather than from endogenous adrenal corticoids.

Experience with these patients suggests that bone advancement cannot be arrested with larger doses of MPA without producing potentially hazardous side effects.

92 *Causes of Failure of Catch-up Growth After Certain Forms of Growth Retardation.* H. David Mosier, Jr., Univ. of Calif., Irvine Coll. of Med., Dept. of Ped., Memorial Hosp. of Long Beach, Long Beach, Calif.

These experiments were carried out in order to elucidate regulation of catch-up growth and occasional failures in catch-up which are observed clinically. Growth retardation was produced in Long-Evans rats, 37 to 41 days of age, by fasting, by cortisone injections, or by propylthiouracil (PTU) feeding. The cortisone or PTU treatments were adjusted to produce maximum differences in body weight between control and experimental groups that were equivalent to the differences produced in fast periods. Light and electron microscopy of epiphyseal cartilage and tibial epiphyseal width determinations were performed in representative groups. Serial measurements of body weight and tail length were carried out for 60 to 80 days from the start of the experimental treatments. Food intake was recorded.

In the fast experiments refeeding was followed by nearly complete catch-up in body weight and tail length. After cortisone treatment there was no catch-up in body weight or tail length growth. After PTU there was a slight tendency to catch-up in body weight and tail length. The latter appeared to show a pattern of late catch-up. The results correlated with anatomic changes in cartilage.

The findings suggest that in the rat cortisone treatment in the post-weaning period permanently damages growth mechanisms and prevents growth recovery in certain circumstances. Hypothyroidism also results in a delayed catch-up in skeletal growth. These experiments illustrate certain complexities in the problem of elucidating the underlying basis of catch-up growth. The usefulness of this experimental model in studies of catch-up growth is demonstrated.

93 *The Prepubertal Androgenic Response to ACTH.* Robert L. Rosenfield, Burton J. Grossman and Natividad Ozoa. The Pritzker Sch. of Med. and LaRabida-Univ. of Chicago Inst., Dept. of Ped., Chicago, Ill.

The following studies were undertaken to explore in detail the androgenic secretory capabilities of the prepubertal adrenal. Five 6–11-year-old children with active rheumatic carditis, otherwise non-toxic, were given ACTH gel 2.2–2.9 μ/kg i.m. daily for one week as initial therapy. Plasma C 19 steroids and indices of testosterone binding protein concentration and unbound androgens were determined by competitive protein binding techniques developed in this laboratory.

Mean urinary corticoids rose from a mean (±SD) of 1.1±1.2 to 15.7±4.3 mg/day and 17-KS from 0.84± 0.69 to 3.4±0.98 mg/day. Baseline mean plasma concentrations (±SEM) were: testosterone (T) 6.7±2.4 (range 1.6±15.0), androstenedione (Δ) 15.2±4.4 (range 2.7–29.6), and dehydroepiandrosterone (D) 71.6±6.2 (range 39.8–124) ng%. Following ACTH, these were increased in each: T–22.3±3.9, Δ–149±22, and D–347±95 ng%. D-sulfate rose in each from 13.5±3.2 (range 2.3–19.8) to 62.8±24 μg%. These changes were accompanied by a fall in testosterone binding protein concentration in four. There was a concurrent rise in unbound androgen levels in all subjects as a consequence of ACTH. This steroidogenic pattern is characterized by a disproportionate increase in Δ concentration, the values sometimes exceeding those of the normal adult. Evidence, thus, has been gained that the adrenal cortex of the prepubertal child evidences a characteristic androgenic response to chronic ACTH administration.

94 *Effects of Human Growth Hormone (HGH) on 79 Hypopituitary Children.* Thomas Aceto, Jr., Alvin B. Hayles, Mary L. Parker, S. Douglas Frasier, Richard W. Munschauer and Giovanni Di Chiro, Depts. of Ped., Med., Rad., SUNY, Buffalo; Univ. of Minn., Rochester; Washington Univ., St. Louis; USC, L.A.; NIH, Bethesda, Md.

We have treated 58 idiopathic (IH) and 16 organic hypopituitary (OH) children for 12 months, using 5 treatment regimens, in order to determine optimum therapy with HGH and glucocorticoids. All patients had: growth hormone levels < 5 mμg/ml plasma during insulin induced hypoglycemia; bone ages of 12 years or less; sexual infantilism. In IH, height was –4 SD or further below the mean. In OH, pretreatment growth rate was below 2.5 cm/year. Listed are 5 treatment regimens and growth rates during therapy.

Dx	Subject No.	Age Years	HGH U; 3×/ week	Cortisone 20 mg/ M²/day	Thyroxine 0.2 mg/ M²/day
OH	10	14.9	2	+	+
OH	6	14.0	Emb. 2	+	+
IH	24	12.1	2	+	+
IH	19	11.5	2	0	0
IH	20	11.5	10	0	0

Dx	Subject No.	Age years	Growth cm/year	Failures < 5 cm/ year
OH	10	14.9	5.6±2.4	6/10
OH	6	14.0	6.8±2.0	1/6
IH	24	12.1	7.1±1.5	6/24
IH	19	11.5	9.4±0.9	1/19
IH	20	11.5	10.1±1.0	3/20

Conclusions: HGH stimulated linear growth of hypopituitary children to varying degrees; more effectively in younger smaller dwarfs, less effectively in the older