100 G.J. BRUINING*, G.B. FORBES*, M.F. BRYSON* (Intro. by H.K.A. Visser). Sophia Children's Hospital, Rotterdam and University of Rochester, U.S.A. Effect of estrogen on metabolic response to growth hormone.

Reports by others suggest that estrogen (E) administration blunts the usual metabolic response to growth hormone (GH). We gave GH (5 mg/day x 3), both with and without E (premarin 2.5 mg or ethinyl estradiol .04 mg x 3), to 2 pre-adolescent patients with GH deficiency and diabetes insipidus and to 3 patients with Turner's syndrome. All were fed a constant diet on the metabolic ward and E treatment was omitted for 1 month prior to the test. This research was approved by our Human Experimentation Committee. As judged by urinary excretion of Ca, Mg, P, N, Na, K, and OH-proline concomitant E administration did not consistently produce a response different from that observed from GH alone. In none of the patients was the hypercalcuria accompanied by a change in serum PTH levels.

101 B. Halikowski, M. Rybakowa^x, J. Frendo^x, E. Sołtysik-Wilk^x, Kr. Drop^x - Paed. Inst. Med. Acad. Kraków, Poland - Sulfate and taurine urinary excretion in hypothalamo-hypophyseal dwarfs /HSD/ before and after hGH treatment
The up-take of sulfates by growing cartilage is on the high level. Cysteine /Gaulle et al.; Raiha, 1971/ and homocysteic acid /McCully, 1971/ are the main sources of active sulfate. In order to see if growth hormone interferes with metabolic steps initiating cartilage growth the study of urinary sulfate and taurine excretion has been performed in 17-hypothalamo-hypophyseal dwarfs /HSD/, aged 6-15 yrs. 7 of them have subsequently been treated with 6 mg/week of hGH. After 3 days of standard diet average from 3 days sulfate S in the urine was 234.2 ± 94.9 mgs S/day and taurine excretion 292.8 ± 122.6 μ M/day in 9 normal controls. In 17 HSD the same values were 522.3 ± 27.3 mgs S/day /P<0.001/ and for taurine /n=5/ 302.4 ± 258.4 μ M/day /P>0.5/. After 4-6 week hGH therapy the respective values were: 320.2 ± 113.8 mgs S/day for sulfates /P<0.005/ and 626.8 ± 197.6 μ M/day for taurine /0.1 < p < 0.05/. Cysteine oral loading /0.130 g/kg/day during 3 days/ increased sulfate excretion in the urine in HSD, but methionine /0.225 g/kg/day/ did it to the lesser degree. hGH treatment increased in HSD taurine excretion and considerably diminished urine sulfate. The effect is more pronounced after methionine. hGH might activate the oxidation of cysteine from methionine precursor.

102 P.E. GARNIER* and J.C. JOB, Hosp. St-Vincent de Paul, Paris, France. Study of serum GH by radioreceptor assay in normal and constitutionally short children.

A growth hormone radioreceptor assay (RRA) was developed using the 100,000 g pellet from pregnant cortisone-treated rabbit liver homogenate, the 125 I hGH binding being specifically and completely displaced by unlabelled hGH. Sensitivity was 0.2 ng. Comparing the GH levels found by this method and by radioimmunoassay in normal sera, the mean RRA/RIA ratio was $0.84 \pm \text{SEM } 0.07$ (range 0.70/0.93). In 11 out of 16 primordial dwarfs the RRA/RIA ratio was in the normal range; it was lower in 2 and higher in 3. But in a number of samples from primordial dwarfs the curve of displacement of labelled GH from the receptor did not parallel the standard curve. This phenomenon was not due to anti-hGH antibodies, it was not induced by addition of insulin and was not modified when variable amounts of hGH were added to these sera. It may be concluded 1/ that serum levels of GH estimated by RRA are generally lower than by RIA; 2/ that individual discrepancies are found in primordial dwarfs; 3/ that the serum from certain primordial dwarfs modifies the affinity of GH for the receptors.

103 M.C. POSTEL-VINAY* and P. CZERNICHOW. INSERM U 30, Hôpital des Enfants Malades, Paris, France.
Radioreceptor assay for plasma human growth hormone; problem of specificity.

A radioreceptor assay (RRA) for human growth hormone (hGH) was set up using the microsomal fraction of female rat liver membranes. Sites which bind specifically hGH are present in these membranes; hGH but also prolactin (PRL) inhibits [¹²⁵I]-hGH binding with an apparent affinity of 2×10^{-10} M and 3×10^{-10} M respectively. The assay is performed at 23°C in 50 mM phosphate buffer pH 7.4, for 20 h. 250 μ l of plasma are added in a total volume of 1.5 ml. Under these conditions, 180 μ g of membrane proteins bind 13.8% of the total radioactivity. Assay sensitivity is 2.5 ng hGH per plasma ml. Values for hGH as measured by radioimmunoassay (RIA) and RRA in eight normal children, in basal conditions and during an arginine and an insulin stimulation test, are as follows (mean values, in ng/ml $\pm$ SEM):

	basal	arginine peak	insulin peak
RIA	4.05 ± 0.95	10.62 ± 3.99	13.61 ± 3.17
RRA	3.86 ± 1.53	11.84 ± 5.19	8.50 ± 2.84

There are no significant differences between RIA and RRA hGH values both in basal state and under stimulation. For some specimens high values measured in RRA could be due to high plasma PRL.

In conclusion, a sensitive RRA for plasma hGH is presented. Cross-reactivity with PRL can induce false interpretation of hGH values.

104 M.T. CORVOL (Intro. by R. Rappaport) INSERM U.30, Hôpital des Enfants-Malades, Paris, France.
A sensitive technique for the measurement of the sulfation of purified proteoglycans in cultured epiphyseal growth plate chondrocytes.

Biological somatomedin (SM) activity is usually assessed on sulfate incorporation in costal or pelvic cartilage. The present study evaluates the responsiveness of rabbit cultured growth plate chondrocytes. The specific radiolabeled incorporation into proteoglycans was measured after extraction and purification on DEAE cellulose column. The distribution of the labelled proteoglycans between the cell pellet, the cell coat and the medium was evaluated. This method is very reproducible (inter-assay variation coefficient 3.4 %) and was applied to the bioassay of purified human plasma fractions with insulin-like activity (ILA) expressed as the ability to compete for 125-insulin on human placental membranes. CMC small peptides fraction had a potent sulfation activity, 4.56×10^4 dpm/flask (control 3.13×10^4) for 4 ng/ml ILA. RRA compared to insulin with 4.16×10^4 dpm/flask for 3000 ng/ml ILA RRA. This technique provides a new sensitive tool for bioassay on epiphyseal growth plate chondrocytes. (CMC fractions and ILA RRA in collaboration with B. Posner and H. Guyda).

105 J. SACK*, Z. KRAIEM*, D. KATZNELSON* and B. LUNENFELD* (Intro. by: A. Pertzelan). Dept. of Pediatrics and the Institute of Endocrinology The Chaim Sheba Medical Center, Tel Hashomer/Israel.
THIRTEEN-YEAR PERSISTENCE OF AN ADRENAL 18-OXIDATION DEFICIENCY IN TWINS.

Identical male twins of consanguineous Jewish Persian parents developed dehydration, hyponatremia, hyperkalemia and failed to thrive during the first months of life. Two siblings with similar symptoms died in infancy. Lack of both pigmentation and virilization, low (<1) ratio of cortisol $\div$ corticosterone before and after ACTH, and failure of subnormal aldosterone levels to increase significantly in response to salt restriction, indicated a rare enzymatic impairment localized between corticosterone and aldosterone (i.e. an 18-oxidation defect). Seven years treatment with mineralocorticoid resulted in normal growth rate. When re-examined 5 years after mineralocorticoid withdrawal, a marked decline in their growth rate was observed. Despite a normal plasma Na:K ratio (30:1), the enzymatic deficiency, although less severe, could nevertheless be unmasked by low aldosterone and high PRA levels, and by the continued low - although increased over the years - ratio of cortisol $\div$ corticosterone metabolites. Thus, the 13-year follow-up revealed a persistence of this enzymatic defect never yet observed in adults.