● **748** RELATIVE EXPRESSION OF THE α-GLOBIN GENES IN MAN DURING DEVELOPMENT. Stuart H. Orkin, Sabra C. Goff, and Alan L. Schwartz. Harvard Medical School, Children's Hospital and Sidney Farber Cancer Institute, Division of Hematology-Oncology, Boston, MA.

The structural genes for α-globin in man are duplicated. They encode identical polypeptides, but differ in sequence in the 3'-untranslated region of the gene. Because this segment is found in all mature mRNAs, detection of this sequence difference in mRNA would provide a means of assessing relative expression of the two α-genes independent of identifiable protein variants. We have devised a sensitive, quantitative assay for the two predicted α-mRNA species based on this principle. Using this approach we have investigated whether the relative expression of the α-genes changes during the transition from fetal to adult erythropoiesis in utero. Normally the mRNA derived from the more 5' gene (α2) predominates slightly relative to that from the more 3' (α1) gene: ratio 60/40. In fetal blood of 17-18 weeks gestation and newborn blood samples this ratio is preserved. During hepatic hematopoiesis (10-16 weeks gestation) the α-mRNAs are present in this proportion as well. Therefore, during the transition from γ to β-globin expression, the relative expression of the α-genes is unaltered. Furthermore, the relative expression of the α-genes is similar in hepatic and bone marrow phases of erythropoiesis.

749 TURNER'S SYNDROME: THE PATHOGENESIS OF THE PHENOTYPE. Lyman A. Page and Laurent J. Bearegard, Webber Hospital, Biddeford, Maine, Dept. of Pediatrics and Eastern Maine Medical Center, Genetics Laboratory, Bangor, Maine.

Two girls with Turner's phenotype and 45 X karyotypes in peripheral blood have good sexual development including menses. Buccal smears showed Barr bodies in 1/200 and 0/200 cells. Paraovarian fibroblasts from one girl gave 45 X on 40 spreads. Ovarian biopsies on both showed fibrous gonads with sparse follicles (est. total 8000/2 ovaries). Limited studies of estrogens and gonadotropins were normal. Traditional interpretation invokes mosaicism. A better interpretation is that these girls represent an expected extreme of a spectrum; that, because both X's are active in oogenesis before meiosis (Migeon and Jelalian, Nature 269:242, 1977), the XO complement causes an increased rate of atresia of follicles from the high fetal number, but does not prevent maturation. Somatic features of Turner's syndrome - almost uniform short stature and highly variable combinations of other features-can be explained by random inactivation during embryogenesis of the only X (Gartler and Sparkes, Lancet II:411, 1963) with ensuing cell death. This has never been seen because such cells are nonviable. This hypothesis would explain the high fetal wastage yet good viability of individuals who survive embryogenesis. The predicted reduction of cell number in early embryogenesis explains the uniform short stature in survivors. If such inactivation occurs, inactivation of the second X in normal cells must be prevented by interaction between the heterochromatic X(s) and the euchromatic X.

● **750** RESTRICTION ENDONUCLEASE STUDIES OF INACTIVE GROWTH HORMONE GENES. John A. Phillips, Leslie P. Plotnick, Peter H. Seeburg, Milo Zachmann, Robert G. Thompson, Allen A. Kowarski, Claude J. Migeon, and Robert M. Blizzard. Dept. of Peds. and Endocrinol., Johns Hopkins U., Baltimore; Genentech, San Francisco; Depts. of Endocrinol., Kinderspital, Zurich, U. of Iowa, Iowa City, and U. of Virginia, Charlottesville.

We have studied DNAs from 8 individuals whose clinical findings and pedigrees were consistent with familial isolated growth hormone deficiency (IGHD). Nuclear DNA was prepared from leukocytes, digested with various restriction endonucleases, subjected to electrophoresis, Southern transferred, and hybridized to ^{32}P-labeled growth hormone (GH) DNA sequences. Restriction patterns of samples from affected individuals in 3 different families with IGHD Type I (autosomal recessive) and 1 family with IGHD Type II (autosomal dominant) were normal; i.e., GH genes were present. Two common polymorphic restriction sites were detected in DNAs of various family members which could be used in linkage analyses. DNA from an individual with IGHD Type IA (autosomal recessive) yielded abnormal patterns following Bgl II, Eco RI plus Bam HI, Hinc II, Pst I, and Sst I digestion. In each digest, one or more fragments (~2.2 to ~3.3 kilobases) were absent. These findings suggest that some inactive GH genes are associated with deletions of GH or GH-like sequences.

● **751** ALKALINE PHOSPHATASE EXPRESSION IN CULTURED HUMAN SKIN FIBROBLASTS AND SOMATIC CELL HYBRIDS Kathleen W. Rao (Spon. by Henry N. Kirkman). University of North Carolina, School of Medicine, Department of Pediatrics, Chapel Hill.

We have developed an in vitro model system for studying the human hypophosphatasias, a group of familial diseases characterized by low serum alkaline phosphatase (ALP) levels and poor bone development. ALP electrophoretic and activity patterns were studied in human fibroblasts, mouse fibroblasts, and mouse-human hybrids. On polyacrylamide gels, human fibroblast ALP is seen as two zones of activity (a fast and a slow band), while the mouse fibroblast ALP appears as only one. Studies in human fibroblasts suggest that the ALP fast band is a product of modification of the slow band. In the hybrids, ALP fast band segregates with the gene for human malate dehydrogenase (MDH) which is located on chromosome 2 ($p<0.001$). This association has been confirmed by concordant segregation of ALP fast band and MDH in two hybrid subclones. ALP activity profiles were studied on 37 independently isolated hybrid clones. In one group of clones, human chromosome 19 was associated with high ALP activity levels ($p=0.04$), while in another group, chromosome 19 was missing from clones with repressed ALP activity levels ($p=0.005$). These observations suggest that a gene on human chromosome 19 may be involved in the regulation of ALP activity. The identification of genes which control ALP expression in cultured cells should help us to understand the genetic mechanism operating in hypophosphatasia.

● **752** ENZYME REPLACEMENT IN FELINE GM_2 GANGLIOSIDOSIS: CATABOLIC EFFECTS OF HUMAN β-HEXOSAMINIDASE A (HEX A). Mario C. Rattazzi, Alan M. Appel, Henry J. Baker, Dept. Pediatrics, Children's Hospital, SUNYAB, Buffalo, NY; Dept. Comparative Medicine, U. Alabama, Birmingham, AL.

To assess the feasibility of enzyme therapy in human GM_2 gangliosidosis, hampered by hepatic uptake of, and blood-brain barrier (BBB) impermeability to exogenous Hex A, kittens with GM_2 gangliosidosis (a model for human Sandhoff disease) were injected IV or intracarotid (IC) with ~5 mg purified human placental Hex A. Hepatic uptake was reduced with IV mannan; reversible BBB permeability compatible with survival without gross neurologic sequelae was induced by 1 ml oxygen IC. At 12 to 72 hrs, residual exogenous enzyme activity in liver, spleen, kidney and brain cortex was 100-50%, 14-5%, 22-5% and 50-10%, respectively, of normal endogenous activity. TLC quantitation showed time- and dose-dependent reduction of liver GL4 globoside and GM_2 ganglioside to 20% of affected controls, and increase of GM_3 ganglioside. A reduction of GL4 globoside to 65% of controls was observed in spleen and kidney. These results demonstrate for the first time a catabolic effect of Hex A in vivo at organ level, even at the relatively low extrahepatic levels obtained by hepatic uptake depression. Although oxygen-induced BBB permeability allowed delivery of comparable enzyme activity to the CNS, no effects on GM_2- or GM_3 ganglioside were evident in brain. Higher enzyme doses, longer exposure, and clarification of neuronal uptake specificity are needed to assess catabolic effects in CNS, a prerequisite for therapeutic attempts in humans.

753 AN INTRAORAL PROSTHESIS FACILITATES CONTINUOUS NOCTURNAL FEEDINGS (CNF) IN GLYCOGEN STORAGE DISEASES, TYPES I AND III (GSD I/III), William J. Rhead and William E. Lavelle, Univ. of Iowa, Depts. of Pediatrics and Otolaryngology, Iowa City, IA (Spons. by J. Robillard)

CNF is one of the mainstays of treatment of patients with GSD I/III. Previously, this goal has been achieved by infusing glucose-containing fluids intragastically via a nasogastric (NG) tube. However, the NG tube can be both difficult to insert and uncomfortable, is disliked by patients and parents, and occasionally leads to severe epistaxis. To avoid these problems, we have constructed an intraoral prosthesis (IOP) to facilitate CNF in GSD I/III. A piece of thin metal tubing 2mm (D) x 5mm (L) is attached to a circular orthodontic band 3mm in width and affixed to a superior 1st or 2nd molar with the tubing lying parallel to the aveolar ridge in the buccal-gingival sulcus. At night, a NG tube is pressed firmly through the IOP and its orifice positioned at the posterior end of the aveolar ridge. The CNF is then infused at the normal rate ($\leq$ 0.5 cc/min) and is swallowed readily during sleep. To date, one patient each with GSD I and III have been fitted with the IOP. The IOP has caused no local irritation or hemmorhage, even after nightly infusions of 40% dextrose for 6 months. Blood glucose levels and linear growth rates are equal to or better than those observed previously in the same patients using intragastric CNF. The IOP is well tolerated by both patients and parents because of its simplicity and comfort. The IOP may facilitate CNF in many patients with GSD I/III.