with normal R-Gln. At protein concentrations at which R-Gln was readily apparent, PGE, DEAE cellulose chromatography and immunodiffusion demonstrated R-Thr in apoHDL but *not* in apoHDL_T. However, antiHDL_T reacted weakly with R-Thr suggesting the presence of traces of R-Thr in apoHDL_T. Using antiR-Thr sera and increasing the amount of apoHDL_T 10 to 100-fold, an antigen was seen that migrated on PGE and eluted from DEAE the same as normal R-Thr and was immunochemically identical with R-Thr.

These results indicate a markedly disproportionate decrease in R-Thr compared to R-Gln in Tangier disease and suggest the hereditary defect is one of control of R-Thr synthesis.

- 19 *Lp System of Plasma in Individuals at Risk of Developing Coronary Disease.* EVERETT W. LOVRIEN and LYLE J. FAGNAN, Crippled Children's Div., Univ. of Oregon Med. Sch., Portland, Oreg. (introduced by Martin Lees).

The Lp system of human plasma represents a genetic polymorphism of beta lipoprotein (BLp). KAHLICH-KOENNER showed an increased BLp in Lp^a(+) individuals compared to Lp^a(-) persons. We have produced an antiserum to Lp^a in order to investigate the possibility that Lp^a(+) children are more likely to develop coronary artery disease as adults than Lp^a(-) children.

Lp^a factor was isolated from a single normal Lp^a(+) donor, an Lp^a(-) donor and from an Lp^a(+) donor who has type II hyperbetalipoproteinemia (II BLp). The procedure for Lp^a isolation included: (1) a modification of the method of Berg using 0.65 MKPO₄ elution from hydroxalapatite and (2) ultracentrifugation flotation gradient at a density of 1.100 in KBR modified from Schultz. Anti-Lp^a was produced by immunizing rabbits followed by absorption with Lp^a(-) serum. Immunelectrophoresis revealed a pure antiserum. Anti-BLp was also prepared in order to quantitate BLp by radial immunodiffusion. Sera were typed for Lp^a by agarose immunodiffusion.

Investigation of 240 members of a family with II BLp revealed that segregation of Lp^a was independent of the segregation of II BLp. This indicates that the BLp associated with Lp^a(+) factor and the BLp associated with II BLp was controlled by different genetic loci. The presence of Lp^a(+) was less of a determinant of coronary disease than II BLp. The production of anti Lp^a now makes it possible to investigate other populations with various risks of coronary disease.

- 20 *Intrauterine Diagnosis: Comparative Enzymology of Fibroblasts Cultivated From Maternal Skin, Fetal Skin, and Amniotic Fluid Cells.* MICHAEL M. KABAEEK, CLAIRE O. LEONARD and TIM H. PARMLEY, Depts. Ped. and Ob.-Gyn., Johns Hopkins Univ. Sch. Med., Baltimore (introduced by R. Rodney Howell).

We have compared the specific activities of several lysosomal enzymes in skin fibroblasts cultured from 7 normal women (MAT), their fetuses (FET), and fibroblasts developed from corresponding amniotic fluid cells (AMN). All samples were obtained between the 17th-21st week of pregnancy. Highly significant differences in the mean specific activities of β -galactosidase, β -D-N-acetylglucosaminidase, and arylsulfatase A are evident between fibroblasts developed from each of these sources. In addition, the pattern of differences in the 3 fibroblast types is dissimilar for each enzyme:

β -galactosidase: AMN > MAT > FET
 β -D-N-acetylglucosaminidase: AMN = MAT > FET
 Arylsulfatase A: MAT > AMN > FET

Both the pattern and relative differences in enzyme specific activities are consistent in each of the 7 groups of cultures studied. If quantitative enzymologic data from cultured amniotic fluid cells is to be used for the antenatal diagnosis of specific inherited genetic disorders, it is essential that relative specific activities of the enzymes in question be established in fibroblasts developed from each of the pertinent cell types. These relationships are also critical if heterozygous fetuses are to be correctly identified *in utero* and if fetal tissue is to be used to corroborate intrauterine diagnoses based on amniotic cell analysis.

- 21 *Dependence of Epithelial Morphogenesis on Extracellular Acid Mucopolysaccharide (MPS).* MERTON BERNFIELD, Dept. of Ped., Stanford Med. Center, Palo Alto, Calif.

The control of morphogenesis is poorly understood, but may involve extracellular substances associated with cell surfaces (e.g. collagen, MPS). Epithelio-mesenchymal interactions provide a system for investigating the role of extracellular materials in organogenesis. Since epithelial morphogenesis is not exclusively dependent upon collagen [BERNFIELD, Dev. Biol., in press, 1970], the localization and morphogenetic significance of MPS was studied. The embryonic salivary epithelial-mesenchymal interface contains MPS as shown by (1) specific staining (Alcian blue at various Mg++ concentrations and Schiff reactivity after two-step periodate oxidation), (2) autoradiography (³H-glucosamine and ³⁵SO₄) and (3) removal of stain and radioactivity with MPSase. The MPS is localized almost exclusively at the surface of the growing and branching adenomeres. The role of MPS in epithelial morphogenesis was studied by autoradiography and organ culture after treating the rudiments with various enzymes (collagenases, proteases, sialidases and MPSases) to remove mesenchyme and MPS. Untreated rudiments undergo progressive branching and characteristic epithelial morphogenesis in organ culture. Single-step removal of mesenchyme and surface-associated MPS, followed by recombination with fresh mesenchyme, results in loss of adenomeres and a delay in epithelial morphogenesis. Removal of mesenchyme, but not surface-associated MPS, yields epithelia which maintain a characteristic contour and undergo continued morphogenesis when recombined with fresh mesenchyme. Removal of MPS from these epithelia causes a loss of contour and a delay in morphogenesis. The data indicate that epithelial surface-associated MPS (or MPS-protein complexes) are required for normal epithelial morphogenesis, and that this system may serve as a model for studies of morphogenetically active materials.

- 22 *A New Growth Disorder With Marked Acceleration of Skeletal Maturation and Relative Failure to Thrive.* RICHARD E. MARSHALL, DAVID W. SMITH, C. RONALD SCOTT, C. BENJAMIN GRAHAM, Dept. of Ped., Univ. of Washington Sch. of Med., Seattle, Wash.

Two infants are described with a new syndrome characterized by increased early linear growth, relative failure to thrive, and the most marked early acceleration of osseous maturation of any condition described to date: Bone age for both patients at 6 months of age

was in excess of 6 years. Additional features of this syndrome are significant motor and mental retardation, and an unusual facies including coarse eyebrows, prominent eyes, and low, upturned nose. Respiratory infections have been a major clinical problem. One patient died at 20 months of age from a hemorrhagic pneumonia and his autopsy did not reveal any cellular explanation for his condition. The other patient is having severe respiratory infections at 11 months of age. Both patients have been extensively investigated for the presence of endocrine or storage disorders, and no cellular or biochemical abnormalities have been detected. Although the etiology of this disorder remains unknown, the mechanisms underlying this syndrome may enhance our understanding of control mechanisms beside androgen, estrogen, or thyroid hormone which affect the rate of skeletal maturation.

Presented at the Western Society for Pediatric Research meetings. This material is of sufficient importance to be presented to a national audience.

- 23 *Genesis and Significance of Palmar and Digital Creases.* GREGORY A. POPICH and DAVID W. SMITH. Dept. of Ped., Univ. of Washington Sch. of Med., Seattle, Wash.

Our studies of skin creases in the early developing hand and in a variety of malformed hands show that these creases, which develop between 7 and 14 weeks of fetal life, are secondary to the early flexion planes in the developing hand. Thus with lack of development or flexion of a digital joint no underlying digital crease was found; and with lack of formation or oppositional function of the thumb there was no thenar palmar crease. The normal distal palmar sloping crease was found to be related to the sloping flexional plane of the 3rd, 4th, and 5th metacarpal-phalangeal joints and the usual mid-palmar crease represents the plane of skin folding between the sloping distal crease and the thenar crease. The occurrence of a single horizontal palmar crease, the simian crease, was found by roentgenographic studies to be related to a diminished slope of the 3rd, 4th, and 5th metacarpal-phalangeal joints such that only a single early horizontal plane of flexion existed in the skin of the upper palm.

Thus hand creases provide a record of the early flexional planes of function in the developing hand and any crease abnormality should always be interpreted as an indication of a more primary defect in form and/or function of the early developing hand.

- 24 *Severe Pyridoxine Deficiency: A Model for Ataxia Telangiectasia.* THOMAS E. NELSON, STARKEY D. DAVIS and THOMAS H. SHEPARD, Univ. of Wash. Sch. of Med., Dept. of Ped., Seattle, Wash.

The association of neural, vascular, and immunologic abnormalities in ataxia telangiectasia is unexplained. We studied the teratogenic effects of B_6 as an experimental model because it is associated with (1) neonatal convulsions and (2) impaired immunologic response in rats. Pregnant rats were given a B_6 -deficient diet and 4-desoxypyridoxine, an inhibitor of B_6 . The control group had the same plus excess pyridoxine. The fetuses were examined at 20 days. Treated rats developed clinical signs of B_6 deficiency and gained less weight than the controls. Mean weight of 108 B_6 -deficient fetuses was 1.63 g; mean crown rump length was 28.3 mm. The 61 control fetuses had a mean weight of 3.24 g and a mean length of 37.0 mm; no anomalies were found in the control group. Of the treated group, 48%

had digital defects, 20% had cleft palates, 12% had omphaloceles, 8% had micrognathia, and 6% had exencephaly.

Organ weights (as g % formalin fixed body weight)

Fetus	Kidney	Thymus	Spleen
Treated (21)	0.88	0.19	0.03
Control (25)	0.85	0.24	0.14

Though the thymus and spleen are both significantly smaller ($p < 0.001$) in the treated group, only the spleen is strikingly hypoplastic. We anticipate that intra-uterine B_6 deficiency will cause a lifelong immunologic defect.

- 25 *The Production of Multinucleated Lymphocytes by Cytochalasin B. An Electron Microscopic Study.* G.F. SMITH, P.O'HARA and M.A.C. RIDLER, Dept. of Ped., Loyola Univ. Stritch Sch. of Med., Maywood, Ill., and Kennedy-Galton Lab., St. Albans, England.

The cytochalasins are newly derived from moulds and are useful in studying nuclear division. Multinucleated fibroblasts were produced by CARTER [Nature 213: 261, 1967] and lymphocytes by us [Nature 216: 1134, 1967]. Lymphocytes with up to eight nuclei were seen. Progressive nuclear addition [1, 2, 3, etc.] was found instead of a doubling of the nuclei (1, 2, 4, etc.). The term 'pseudomitosis' was proposed for the process of synchronous mitosis, but which results in the division of only one nuclei.

Our observations, using the light microscope shows the nuclei of binucleated cells to be similar in size and shape; however, in cells with more than two nuclei there is marked variation in size and shape of each nucleus. What might have appeared, in many instances, as separate nuclei are seen by electron microscopy to be lobes of a rather large and irregular nucleus. In other instances, small nuclear bridges connected what might appear as separate nuclei. Variation exists in nuclear size from rather large nuclei to very small micronuclei. Serial sectioning of cell nuclei verified the morphological variability seen in single sections. Spindle fibers and kinetochore attachment to the chromosomes revealed no unusual features. Electron microscopy showed synchronous division of nuclei. Golgi apparatus remained as a single large unit. There was a doubling of centrioles in binucleated cells. What appears as separate nuclei under the light microscope were connected by nuclear bridges. The finding suggests that as nuclei accumulate that the spindle bridge is unable to accurately divide the large numbers of chromosomes present.

- 26 *Phocomelia With Mandibular Hypoplasia: A New Syndrome.* SALMA REGINA ASSEMAN, TADASHI KAJI and LYTT I. GARDNER, State Univ. of New York, Upstate Med. Center, Dept. of Ped., Syracuse, NY.

We have observed the association of non-thalidomide phocomelia and hypoplasia of the mandible (Pierre-Robin syndrome) in two unrelated infants. Baby boy C was born at term after a normal gestation. The mother was 29 and denied either illness or medication during pregnancy. Five months postpartum she had no glucosuria. The baby showed multiple deformities: both hands were hypoplastic with no visibly separate fingers. The right hand was more primitive than