15 *Molecular DNA-RNA Hybridization as a Means of Chromosome Mapping.* Roy Schmickel and Dorothy Vegter, University of Michigan, Ann Arbor (introduced by W. Oliver).

We have used molecular DNA-RNA hybridization as a direct means of assaying a human gene. This assay provided a logical method of chromosome mapping. Comparison of rRNA hybridization with normal and mongoloid DNA was used to measure ribosomal RNA genes on the 21st chromosome. The DNA-rRNA reaction was chosen because of the relative abundance of rRNA and its characteristic sedimentation in sucrose. Mongoloid tissue was used since the 21st chromosome is known to contain a nucleolar organizer. The nucleolus is the site of ribosome synthesis in other eucaryotes.

The assay of the rRNA genes required purification of the DNA and rRNA. We found that approximately 14×10^{-5} of the human DNA hybridized with rRNA under conditions where the specific complementary sites were saturated. Assuming a DNA content per cell of 9.2 pg enough DNA complementary to rRNA was present in each cell to code for approximately 300 ribosomes.

In two different mongoloid patients in whom we assayed for rRNA genes, we found a 14% and a 20% increase in hybridization. These initial studies provide a foundation for increasing the sensitivity of the reaction. We should be able to utilize tissue with specific deletions of the 21st chromosome for fine mapping of the chromosome and exact quantitation of the genes. This type of study also provides a method of mapping other human genes for which we can obtain purified gene products. These include hidden viral genomes, transfer RNA's and purified messenger RNA's such as hemoglobin messenger.

16 *Unstable Galactose-1-phosphate Uridyl Transferase: A New Variant of Galactosemia.* Claramma M. Chacko, Joe C. Christian and Henry L. Nadler, Northwestern Univ. Med. Sch., Children's Mem. Hosp., Chicago and Univ. of Indiana Med. Sch.

Galactosemia, a recessively inherited disorder, is caused by a deficiency of galactose-1-phosphate uridyl transferase (transferase). Variants of transferase which are not associated with classical galactosemia have been described. A patient with the classical clinical manifestations of galactosemia has been studied and was shown to have one-third of normal transferase activity in heparinized blood. Transferase of this patient is highly unstable and no activity could be detected after 72 h of storage in heparin whereas normal enzyme is stable for months. Transferase activity of the patient's red blood cells immediately falls to zero upon exposure to isotonic phosphate buffer, pH 7.4. Transferase activity in heparinized blood from both parents is approximately three-quarters of normal and exposure of their red blood cells to isotonic phosphate buffer results in 50% inhibition. Starch gel electrophoresis of hemolysates from the patient had no detectable activity while hemolysates of the mother and maternal grandmother have shown a decreased mobility as compared to normal.

These data are interpreted as evidence for a new variant of 'classical' clinical galactosemia. The presence of transferase activity in patients with clinical manifestations of galactosemia may not exclude the diagnosis of this condition and may in fact represent a generalized phenomenon applicable to many familial metabolic disorders.

17 *Inherited Propionyl-CoA Carboxylase Deficiency in 'Ketotic Hyperglycinemia'.* Y. Edward Hsia, Katherine J. Scully and Leon E. Rosenberg, Depts. of Ped. and Med. Div. of Med. Genetics, Yale Univ. Sch. of Med., New Haven, Conn.

Infantile 'ketotic hyperglycinemia' is an inborn error of metabolism leading to severe protein intolerance, ketoacidosis, and developmental retardation. The biochemical basis for this disorder was obscure until we found in 1969 that peripheral leukocytes from a girl with this condition failed to catabolize propionate-^{14}C to $^{14}CO_2$, but oxidized methylmalonate and succinate normally.

$$\text{Propionyl-CoA} \rightleftharpoons \text{d-methylmalonyl-CoA} \xrightarrow[\text{Carboxylase}]{} \rightleftharpoons \text{l-methylmalonyl-CoA} \rightleftharpoons \text{succinyl-CoA} \xrightarrow[\text{Mutase}]{} \rightarrow \rightarrow CO_2$$

Propionyl-CoA Carboxylase

Normal	46.6±11.9	(20)*
Patient	0	(10)
Mother	19.8± 5.1	(12)
Father	16.5± 5.4	(9)

* pmole/min/mg protein.
mean±1 s.d. (observations).

Since this catabolic defect was also present in her cultured skin fibroblasts, we used extracts of these cells for specific enzyme assays. Propionyl-CoA carboxylase activity was absent, and was not restored by added cofactors. Neither was any enzyme inhibitor detectable. Methylmalonyl-CoA mutase, however, was normal. Significantly, propionyl-CoA carboxylase was clearly reduced in both parents' fibroblasts.

Therefore, 'ketotic hyperglycinemia' is actually propionyl-CoA carboxylase deficiency, and this explains the protein intolerance and the remarkable similarity of this condition to methylmalonicaciduria. Finally, partial deficiency of this enzyme in her parents proves autosomal recessive inheritance.

18 *Studies of the Protein Defect in Tangier Disease.* Samuel E. Lux, Robert I. Levy, Antonio M. Gotto and Donald S. Fredrickson, NIH, Bethesda, Md.

Tangier disease is an autosomal recessive disorder characterized by absence of normal high density lipoproteins (HDL), storage of cholesterol esters in foam cells, and neuropathy. Small amounts of an abnormal HDL (HDL_T) having only partial immunochemical identity with normal HDL occur in Tangier plasma. HDL has recently been shown to contain two major protein subunits, one with C-terminal threonine (R-Thr), the other with C-terminal glutamine (R-Gln). R-Thr and R-Gln are immunochemically different and occur in normal HDL in a ratio of 2–4:1 (R-Thr:R-Gln). We now report investigations of these two moieties in HDL_T isolated from a Tangier homozygote.

R-Gln was identified in delipidated HDL_T (apo HDL_T) by polyacrylamide gel electrophoresis (PGE), DEAE cellulose chromatography and immunodiffusion with antisera to R-Gln and normal HDL (anti-HDL). An antiserum to HDL_T (antiHDL_T) reacted with R-Gln and absorption of antiHDL with apoHDL_T removed all reactivity to R-Gln, confirming the presence of R-Gln in HDL_T. R-Gln from apoHDL_T was electrophoretically and immunochemically identical

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 dentical 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 $MKPO_4$ 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. KABAEK, 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 lysozomal 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 (^{3}H-glucosamine and $^{35}SO_4$) 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, relativ 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