

- 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.

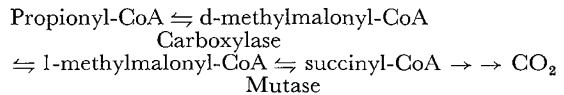
- 16 *Unstable Galactose-1-phosphate Uridyl Transferase: A New Variant of Galactosemia.* CLARAMMA M. CHACKO, JOE C. CHRISTIAN and HENRY L. NADLER, Northwestern Univ. Med. Sch., Children's Mem. Hosp., Chicago and Univ. of Indiana Med. Sch.

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 $^{14}\text{CO}_2$, but oxidized methylmalonate and succinate normally.



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