A. SHORE, BENJAMIN LANDING, Childrens Hosp. of Los Angeles, and CLARK W. HEATH, Jr., Communicable Disease Center, Atlanta (introduced by George W. Clayton).

In the last 2 generations of a Latin American family, 17 males have died prior to 6 years of age. All were related in the family through their mothers. Clinical information on 16 of the boys indicated that the illness was characterized by fever, pallor, jaundice, hepatosplenomegaly, and lymphadenopathy with the median age of onset of illness being 14 months (4–62 months) and the median duration of illness being 22 days (1–50 days). Histologic studies on 12 of the children revealed bone marrow plasmacytosis and a mononuclear cell infiltrate of the liver, spleen, and lymph nodes, and less frequently of the brain, kidney, heart, and lungs, consistent with a malignant reticuloendotheliosis.

The most recently affected child was found to have anemia, thrombocytopenia, elevated IgG, IgA and IgM, normal phytohemagglutinin stimulation, *in vitro* transformation of peripheral blood lymphocytes, and an abnormal karyotype.

All first-degree relatives of affected children have normal peripheral blood counts, karyotyping, delayed hypersensitivity and lymphocyte proliferation studies, normal to elevated IgG and IgM levels with normal IgA, and absence of *in vitro* transformation of peripheral blood lymphocytes.

This is the first reported instance of a familial malignant reticuloendotheliosis occurring in a pattern consistent with an X-linked recessive mode of inheritance.

12 *Clinical and Enzymatic Variation in G_{M1} Gangliosidosis.* HARVEY S. SINGER and IRWIN A. SCHAFER, Dept. of Ped., Case Western Reserve Univ. Sch. of Med. at Cleveland Metropolitan Gen. Hosp., Cleveland, Ohio (introduced by R. Schwartz).

One patient presented at 3 months with Hurler facies, hepatomegaly, hypotonia, developmental retardation, lumbar beaking, and normal urinary mucopolysaccharides. The second patient presented at 23 months with progressive psychomotor deterioration, spasticity, lumbar beaking, but no Hurler features, organomegaly or mucopolysaccharidurie. B-galactosidase activity was virtually absent in WBC and fibroblasts in both patients.

Biopsied liver tissue showed striking morphologic and enzymatic differences. The early onset liver was enlarged, firm, and histologically showed marked vacuolization of most hepatocytes. The late onset liver was normal grossly and histologically. B-galactosidase activities were measured and expressed as nmoles of p-nitrophenyl-B-D-galactopyranoside hydrolyzed/min/mg protein. Enzyme activity in normal livers showed a pH optimum of 4.0–4.5, while the pH optimum was 6.5 in both patients. Activity at pH 6.5 was strikingly elevated in the late onset case (21.8) while the early onset patient (1.5) was below normal (2.0–5.2). Starch gel electrophoresis of normal liver showed one fast and two slow isozymes. In both patients only a single rapidly moving isozyme was visualized.

These patients despite distinctly different clinical phenotypes showed similar pH optima and isozymic patterns implying a similar gene mutation. We are currently studying the possibility that differences in visceral storage in the early and late G_{M1} gangliosidoses may be related to the activity of the pH 6.5 isozyme.

13 *Expression of G_{M1}-gangliosidosis Types I and II in Fibroblast Cell Culture.* J. CALLAHAN, L. PINSKY, E. POWELL and L. S. WOLFE, Montreal Neurological Inst., and Dept. of Genetics, McGill Univ. and the Lady Davis Inst., Jewish General Hosp., Montreal, Canada (introduced by Charles R. Scriver).

G_{M1}-gangliosidosis Type I resembles Hurler's disease whereas Type II manifests principally as psychomotor deterioration. Biochemically they share a general β-galactosidase deficiency, accumulation of G_{M1}-ganglioside in neural and to a lesser extent in extraneural tissues, and storage and excretion of undersulfated keratan sulfates. Fibroblast cell strains were established from a patient with each type. β-galactosidase activity at pH 5.0 in Type II cells was 3% of normal whereas it was less than 1% in Type I cells, a disparity confirmed in a separately developed strain from the same Type I patient. Other acid hydrolases were normal or elevated but the pattern was different in the two types. In addition to G_{M3} and D_{D1a} gangliosides normally present, G_{M1} and a component like G_{M2} were identified in Type II cells. Residual β-galactosidase activity in crude extracts of both mutant cell strains was more thermostable than control. The radioactivity from ^{14}C-galactose was 7–8 fold higher than normal in non-CPC precipitable glycosaminoglycans of Type II cells. Chromatographically and electrophoretically these substances behaved like the undersulfated keratan sulfates previously isolated from liver. Despite major biochemical similarities of both clinical types, the constancy of their respective intrafamilial phenotypes and their different degrees of β-galactosidase deficiency in culture suggest that different mutant genes are responsible for the two types of G_{M1}-gangliosidosis. (Supported by MRC grants: MT-1345, MA-2830.)

14 *Spontaneous Hybridization of Human Leukocytes With a 3T3 (TK-) Mouse Cell Line.* ANNE M. HAGEMEIJER and PARK S. GERALD, Children's Hosp. Med. Center, Boston, Mass.

The formation of hybrid cells by the fusion of nuclei has usually required use of a fusing agent, such as Sendai virus, although the virus itself may cause undesirable chromosomal rearrangements. A 3T3 (thymidine kinase deficient; TK-) mouse cell line has been found to differ from other tested mouse lines (A9, CL1D) in fusing spontaneously with human cells, including human leukocytes and fibroblasts. Hybrids derived from 3T3-human leukocyte combinations can be easily identified as early as 10 to 12 days after culture in selective (HAT) medium. Hybridization has been confirmed both by chromosomal analysis and by electrophoretic examination of enzymes. Chromosomal rearrangements are relatively rare in these hybrids (0–3%). The ability to fuse spontaneously with other cells has been studied with clones derived from the stock 3T3 line. Marked variation in numbers of heterokaryons formed, and in number of nuclei per heterokaryon, was observed for these clones, suggesting that the property of spontaneous fusion may be intrinsic to the particular cell type, although presence of a latent virus cannot be excluded. Spontaneous fusion of 3T3 cells with leukocytes has been achieved using 8 different human donors. This system can be used with leukocytes possessing chromosomal abnormalities for gene-chromosome correlations or with leukocytes possessing aberrant cytoplasmic organelles to elucidate their genetic control.