tion suggesting that the placental barrier is not impermeable to certain antigens. However, little is known about placental transfer of antigens in normal pregnancies. For this reason leukocyte cultures from random cord blood samples were incubated with various antigens, and transformation was assayed by tritiated thymidine (TdR³H) uptake.

Analysis of variance of the response of triplicate cultures revealed that a ratio of 3:1 or greater of uptake of TdR³H by stimulated to unstimulated cultures was statistically significant ($p < 0.01$). The number of newborns tested that manifested such a significant *in vitro* response was 5/21 with streptolysin O, 5/15 with Type I pneumococci, 4/17 with Group A type 12 streptococcal cell wall extract, 2/14 with *E. coli*, and 3/9 with *S. enteritidis* endotoxin. Examinations of metaphases in cord blood cultures from male infants revealed that the response of cord blood lymphocytes to these antigens could not have been due to the passage of maternal cells across the placenta. This 14–33% incidence of significant stimulation of cord blood lymphocytes by these common bacterial antigens is therefore either due to inborn cellular immunity or to transplacental transfer from the maternal circulation and prenatal sensitization of the fetus by these antigens. (Supported by: NIH Grant No. HD-04273.)

- 8 *Cell-mediated Immune Response in vitro*. SAMUEL P. GOTOFF and SOMSAK LOLEKHA. Dept. of Ped., The Abraham Lincoln Sch. of Med., Univ. of Illinois, Chicago.

Studies of the mechanism of cell-mediated immune responses have been hampered by the lack of *in vitro* systems. While the inhibition of macrophage migration model has advanced our understanding of delayed hypersensitivity reactions, the technique is complex and cumbersome. We have recently developed a simple test for measuring cell-mediated immune responses which depends on the aggregation of peritoneal exudate cells (PEC) in suspension cultures.

PEC from guinea pigs with delayed hypersensitivity aggregate when the cells are cultured with the appropriate antigen. Diphtheria toxoid, PPD, egg albumin and keyhole limpet hemocyanin have been used in this system. Aggregation appears at 6 h and reaches a maximum at 24–48 h which is comparable to the time course of cutaneous delayed hypersensitivity reactions. This *in vitro* model also correlates with another cell-mediated response, allograft rejection. PEC from strain 13 guinea pigs previously grafted with skin from strain 2 animals aggregate in the presence of strain 2 cells. Aggregation does not occur with mixtures of PEC from guinea pigs of the same strain or different strains without prior grafting.

Peripheral blood leukocytes, spleen cells or lymph node cells from sensitized animals cultured with antigen synthesize a factor which causes aggregation of PEC from nonsensitive guinea pigs. The aggregating cells are macrophages, and the titer of macrophage aggregation factor (MAF) is determined by serial dilution. Macrophage aggregation *in vitro* provides a simple semiquantitative test for cell-mediated immune reactions and permits further analysis of the mechanism involved.

- 9 *A Mechanism for Inhibitory Effects of Diverse Compounds on in vitro Antigenic Lymphocyte Stimulation and Histamine Release From Leukocytes*. CHARLES D. MAY, Dept. of Ped., New York Univ. Sch. of Med., New York, NY.

Ethanol (E), nicotinamide (N), cyclic 3',5'-adenosine monophosphate (AMP) and theophylline (T) are examples of dissimilar compounds found to inhibit the fundamental cellular responses of antigenic histamine release and lymphocyte stimulation. As glucose is a prime source of energy and intermediates essential to cellular responses, the effects of the compounds on glucose utilization by leukocytes were determined, and corresponding inhibition of glucose metabolism was revealed. The molar concentrations required for comparable degrees of inhibition were closely similar for the two cellular responses and glucose utilization. To ascertain loci of inhibitory actions, the effects on conversion of glucose labeled with ¹⁴C at various sites to ¹⁴CO₂ were compared in suspensions of intact leukocytes and extracts of leukocytes devoid of cell membrane or nuclei. E and N were inhibitory mainly with intact cells and appear to affect glucose transport across the cell membrane. AMP and T also inhibited conversion by cell-free extracts and thus enzyme systems within the cell. Inhibition of glucose utilization by leukocytes is a common property of inhibitors of cellular responses, including E, N, AMP, T, and we found likewise for cortisol, colchicine, and chloroquine. The loci of action of the diverse compounds in the complex steps in utilization of glucose by leukocytes differ.

The *in vitro* systems employed to compare cellular responses with glucose utilization are useful in fundamental studies and also convenient for screening of compounds for activity in these processes.

- 10 *Isoimmune Neonatal Neutropenia Due to a New Neutrophil-specific Antibody*. EVA RADEL, DAN G. HANDELSMAN and PARVIZ LALEZARI, Depts. of Ped. and Hematol., Montefiore Hospital and Medical Center and Albert Einstein Coll. of Med., New York (introduced by Laurence Finberg).

Maternal isoimmunization to fetal leukocytes has been implicated in instances of neonatal neutropenia. However, leucoagglutinins often exist in pregnant women and are not associated with clinical symptomatology in the newborn. It has therefore been suggested that when neutropenia, sepsis, and maternal leucoagglutinins coexist, the latter are coincidental and the neutropenia is the result, and not the cause, of sepsis.

A newborn infant, in whom sepsis was suspected, had almost complete absence of circulating polymorphonuclear neutrophils, persisting for 5 weeks. An antibody was detected in the serum of both mother and infant which agglutinated the neutrophils of the infant and father. It did not react with eosinophiles, lymphocytes, platelets, or with the mother's neutrophils. Two neutrophil-specific antigens have been described previously in association with neonatal neutropenia. Neutrophil typing of the family and unrelated donors demonstrates that this antibody is distinct and thus constitutes a third neutrophil-specific antigen. The more common leucoagglutinins which react with many types of cells may be benign, but antibodies specifically directed against neutrophils may produce profound neutropenia and may thus predispose the neonate to severe infection.

- 11 *An X-linked Recessive 'Malignant' Reticuloendotheliosis*. JOHN M. FALLETTA, DONALD J. FERNBACH, DON B. SINGER, Baylor Coll. of Med., NOME

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 mucopolysacchariduria. 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 under-sulfated 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 ¹⁴C-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.