DETECTION OF LEIGH'S DISEASE IN FIBROBLASTS. Jerome V. Murphy, Warren F. Diven and Linda Craig, University of Pittsburgh, School of Medicine, Children's Hospital of Pittsburgh, Department of Neurology, Pittsburgh, Pennsylvania.

When tested, all autopsy-proven patients with Leigh's disease have had an inhibitor to the synthesis of thiamine triphosphate in their urine. The inhibitor is also present in obligate carriers and thiamine treatment produces a drop in the inhibition (Peds 51: 710, 1973).

Normally the inhibitor is not detectable in fibroblasts. However, when fibroblasts are grown in low thiamine media (dialyzed calf serum and no added thiamine), the inhibitor is demonstrable. These fibroblasts were washed, sonicated and deproteinized. 0.1 ml. of the deproteinized supernatant from normal fibroblasts inhibited 14.9 to 26.0%. The identically treated fibroblasts from four patients with Leigh's disease inhibited 51.1 to 67.8%. Fibroblasts from obligate carriers of Leigh's disease behaved like controls. The inhibitor from Leigh's disease fibroblasts was biochemically indistinguishable from the urine inhibitor.

This fibroblast assay offers a method to distinguish Leigh's disease patients from carriers and suggests a technique for prenatal diagnosis.

HETEROZYGOTE DETECTION IN FABRY'S DISEASE. M. Philippart, E. Kamensky, P. Cancilla, R. S. Sparkes and M. Cotton. Mental Retardation Unit, Neuropsychiatric Inst., Los Angeles.

While screening families with hemizygotes for Fabry's disease, it became apparent that there were three useful criteria to detect female heterozygotes: corneal opacities, glycolipid levels in urine or serum, α-galactosidase activity in plasma or cultured skin fibroblasts. A few obligatory heterozygotes cannot be distinguished from normal subjects by any of these criteria. We have developed 2 techniques to improve screening of apparently normal females in families at risk. In agreement with Romeo and Migeon (Science:170, 180, 1970) it is possible to recognize two phenotypes by cloning cultured fibroblasts. Among 21 clones obtained from an obligatory heterozygote, only 4 had normal α-galactosidase activity. A faster technique is to use electron microscopy to search for characteristic lysosomal inclusions. We were able to demonstrate a significant proportion of abnormal cells in cultured fibroblasts as well as in the original skin biopsy. These findings confirm the anomalous inactivation of the X-chromosomes suggested by variability in the clinical phenotypes. This must be taken into account when counseling possible carriers. (Supported in part by PHS Grant HD-04612).

A t(14q22q) CHROMOSOME IN A LARGE KINDRED SPANNING FOUR GENERATIONS. Richard L. Neu, Lytt I. Gardner, Margaret L. Williams, Mary L. Voorhess, State University of New York, Upstate Medical Center, Dept. of Ped., Syracuse, New York.

Translocations between D and G group chromosomes are not rare events, and usually involve chromosome numbers 14 and 21. The case presented here is that of a family with a t(14q22q) chromosome. The complete pedigree consists of 41 family members, 21 of whom are balanced t(14q22q) carriers. There have been no cases of Down's syndrome in the family. The index case was a 33 month old female who was seen because of physical and mental retardation. The proband was the product of a full term pregnancy and had an uncomplicated neonatal period; the mother was 34 and the father 35 years of age at her birth. The proband had a 45,XX,-D,-G,+t(DqGq) chromosome complement. The chromosomes involved in the translocation were numbers 14 and 22, which were identified by autoradiographic and G-banding techniques. All of the other balanced translocation carriers are phenotypically normal and in good health. There have been numerous miscarriages in the family, and 3 of the carrier males have had childless marriages. All 8 children of the first complete generation karyotyped carried the translocation which had been inherited from the father. Eleven of the next generation out of a total of 24 carried the translocation chromosome. There was no indication in this generation that the sperm carrying the translocation had a selective advantage; the carrier males produced 15 children, 6 of whom were carriers.

SYNTHESIS OF AN ABNORMAL GANGLIOSIDE BY SKIN FIBROBLASTS IN SANDHOFF'S DISEASE. M. Philippart, S. Nakatani, M. Vidailhet, and G. Grignon. Mental Retardation Unit, Neuropsychiatric Inst., Los Angeles and University of Nancy, France.

In Sandhoff's disease, following a complete β-hexosaminidase deficiency, there is an accumulation of at least 3 different glycolipids. Upon electron microscopy, cultured fibroblasts from a patient with this condition contained a small number of lamellar inclusions. Major glycolipids in normal confluent cultured fibroblasts are ceramide trihexoside and hematoside. In 2 fibroblast lines from unrelated children with Sandhoff's disease, we detected a ganglioside with an Rf similar to GM_2-ganglioside. Complete characterization has not yet been completed. Both ^{14}C-mannosamine and ^{14}C-glucosamine were incorporated into this lipid which thus contains sialic acid and hexosamine. On the basis of this composition, this glycolipid may represent GM_2-ganglioside. This compound has never been detected in normal or pathological control fibroblasts, including infantile Tay-Sachs disease, or the other sphingolipidoses. (Supported by the Dept. Mental Hygiene, State of California and PHS Grant HD-04612.)

GENERALIZED N-ACETYLNEURAMINIC STORAGE WITH SIALURIA. M. Philippart, E. Kamensky, P. Cancilla, J. Callahan, K. Zeilstra, S. Nakatani and J. P. Farriaux. Mental Retardation Unit, Neuropsychiatric Inst., Los Angeles and University of Lille, France.

This case has remained unique (Fontaine, et al, Helv. Paed. Acta:23, Suppl. 17, 1968). The patient presented with mental retardation, seizures, mildly dysmorphic features, and sialuria, reaching several grams per day. The biochemical defect has not been identified. We studied biopsies from liver, kidney and skin. Hematoside level was normal in all these specimens as well as in erythrocytes. No abnormal ganglioside was detected. Significant increase of free N-acetylneuraminic acid was observed in liver, kidney and cultured skin fibroblasts. The ultrastructure of skin fibroblasts revealed scarce inclusions containing lamellar tubular cores, but no mitochondrial abnormalities such as were previously found in the liver. Skin fibroblasts were submitted to a loading test by incubation in a medium containing from 100 to 400 µg neuraminic acid per ml. This caused an increase in membrane-bound inclusions. When incubated with ^{14}C-mannosamine, the precursor was normally incorporated into hematoside and glycoproteins. This suggested that these cells were capable of synthesizing CMP-neuraminic acid. A deficiency of neuraminic aldolase could not be disproven owing to an insufficient amount of available tissue. (Supported in part by PHS Grant HD-04612.)

ABNORMAL PERIPHERAL BLOOD FLOW IN FABRY'S DISEASE: ITS RELATIONSHIP TO PAIN. M. Philippart, J. K. Vyden and S. Funderburk. Mental Retardation Unit, Neuropsychiatric Inst., Los Angeles.

Most children with Fabry's disease experience bouts of excruciating pain with a causalgic character. Many of them also present with a chronic state of discomfort, making them fear to engage in any physical activity. Most analgesics fail to offer significant relief. The mechanism of pain is not understood. Lipid storage involves dorsal root ganglia and the autonomous nervous system. Peripheral vascular investigations were undertaken in 3 males and 3 females with Fabry's disease. Significant abnormalities were observed, including stenosis at the level of the hands and feet, increased arteriolar resistance, decreased skin blood flow and markedly increased venous tone. Phenoxybenzamine (10-20 mg/day) gave prompt relief from the pain and discomfort, while peripheral blood flow appeared significantly improved. Diabetes, which can give rise to similar disturbances, can be excluded in our patients. Despite its promising action on pain in patients with Fabry's disease, phenoxybenzamine should be used with caution since one of our patients developed priapism shortly after a low dosage was started. It is advisable to try diphenylhydantoin initially (Lockman et al, Neurology: 21, 423, 1971) to relieve the pain. (Supported in part by PHS Grant HD-04612.)