COMPARATIVE GAS CHROMATOGRAPHIC (GC) ANALYSIS OF SERUM AND URINE ORGANIC ACIDS IN HEALTHY TERM INFANTS. Serge B. Melançon, Bernard Grignon, Michel Potier, and Louis Dallaire. Dept. Ped., Univ. of Montreal and Ped. Research Ctr., Ste-Justine Hosp., Montreal, Québec, Canada.

In a search for the optimal time to apply GC screening procedures to newborn physiological fluids and to assess the effect of formula feeding on biologically important organic acids, we have surveyed serum and urine from 31 normal babies, at 1 and 5 days of age. Our results have demonstrated the presence of more than 50 organic acids, half of which bear some relation to the more common organic acidopathies. Their significant rise in serum and urine after formula feeding suggests that a comparable method of screening for those organic acids known to be associated with disease, should preferably be applied after 5 days of life. The usefulness of serial analysis was suggested by the presence of possibly harmful metabolites in 3 serum at day 1, with normal levels at day 5. These included isovaleric, methylmalonic, benzoic, phenylacetic and p-hydroxyphenylacetic acids. We have applied our method to the investigation of 4 patients presenting abnormal amino aciduria and found large amounts of those phenolic acids characteristic of tyrosinemia and hyperphenylalaninemia.

These findings support the reliability of our procedure in screening for metabolic disease in laboratories where mass spectrometry is unavailable. This work was supported by the Medical Research Council of Canada, Grant MA 4767.

SANDHOFF DISEASE: INCREASED β -D-N-ACETYLGLUCOSAMINIDASE ACTIVITY AFTER CORTICOSTEROID EXPOSURE IN CULTIVATED SKIN FIBROBLASTS. Serge B. Melançon, Michel Potier, Louis Dallaire and Guy Geoffroy. Dept. of Ped. and Neurol., Univ. of Montreal and Ped. Research Ctr., Ste-Justine Hosp. Montreal, Que., Canada.

We studied a 20 months old French-Canadian girl presenting the clinical picture of Tay-Sachs disease with progressive blindness, bilateral cherry red spots, hyperacusis, hepatosplenomegaly and hypotonia, with onset of symptoms by 9 months of life. Serum and WBC β -D-N-acetylglucosaminidase activities were reduced to levels less than 1% normal. Obligate heterozygotes showed reduced levels of total serum hexosaminidase activity and lower than normal % B form:

	SERUM HEXOSAMINIDASE (nanomoles/hr/ml.)	
	Total	% B form
Patient	3	2
Mother	394	14.5
Father	382	12.7
Controls	506-950	21-35

A similar reduction of total hexosaminidase to 8% normal values was found in cultivated skin fibroblasts from our patient. Interestingly, levels of hexosaminidase were raised up to 25% normal value when fibroblasts were grown in nutritional medium supplemented with prednisolone or cortisone (1 μ g/ml). This observation, if confirmed in other cases of Sandhoff disease might suggest a therapeutic approach in this rapidly progressive and always fatal disease. Supported by MRC grants MA-4767 and 5163.

EFFECTS OF OPIUM ALKALOIDS ON MITOSIS AND DNA SYNTHESIS Mona Milstein, Akira Morishima, Michael I. Cohen and Iris F. Litt Depts. of Ped., Col. of Physicians and Surgeons, Columbia Univ., and Albert Einstein Col. of Med., N.Y.N.Y.

The cytogenetic effects of opium alkaloids were investigated. When lymphocytes from adolescent heroin addicts were cultured *in vitro*, a strikingly high mitotic index was observed, reaching over 20% in some. The mean mitotic index of 11.82 ± 1.827 (S.E.)% in 9 patients was significantly higher than that of sex and age matched controls of 6.59 ± 0.970 (S.E.)% ($p < 0.05$). The addition of plasma from addicts to normal cultures caused no increase. This observation suggested that circulating metabolites of heroin were not responsible for the observed phenomenon. An *in vitro* model was then developed to study the effects of opium alkaloids on DNA synthesis. Adopting the method of Suciu-Poca, et al. (Cancer Res., 33:2373, 1973), microcultures of normal lymphocytes (200,000 T cells/cult.) were established and exposed to various concentrations of morphine sulfate. An enhancement of DNA synthesis was observed when cells were exposed to 10^{-4} μ g/ml of morphine for 72 hrs. as measured by ^3H -thymidine uptake. The observed increase in chromosomal replication might be directly related to addictive phenomenon at the cellular level. The effects of opium alkaloids appear to be opposite to that of marijuana.

(Supported by HRC, New York U-2215)

EXPERIENCE WITH α -FETOPROTEIN (AFP) IN PRENATAL DIAGNOSIS OF NEURAL TUBE DEFECTS (NTD), Aubrey Milunsky, Elliot Alpert, Harvard Med. Sch., E.K. Shriver Ctr., Mass. Gen. Hosp., Dept. Ped. & Med., Boston.

The use of AFP assays of amniotic fluid (AF) as a diagnostic adjunct to the prenatal diagnosis of these defects represents the most significant advance yet in prevention through early detection. AFP assays by electroimmunodiffusion were done on AF's derived from 525 cases (10-34 weeks gestation) with subsequent normal births. The observed normal range of AFP ± 2 S.D. is shown in the table. A narrow normal range of

Wks. Gest.	12	13	14	15	16	17	18	19	20
AFP(mg%)	2.09	1.94	1.92	1.96	1.89	1.83	1.75	1.74	1.65
± 2 S.D.	0.21	0.19	0.20	0.25	0.22	0.14	0.17	0.15	0.17

AFP concentration is evident. Additional assays were done on AF's in cases with fetal abnormalities (24), fetal death (8) and obstetric complications (9). A slight elevation in AFP concentration was noted in 1 fetus with Turner's syndrome. In studies prior to 20 weeks gestation 2 fetuses with anencephaly and 1 with spina bifida were detected. A striking fall in AFP concentration (16.5 to 4.55 mg%) was observed between 16 to 21 weeks in 1 case of anencephaly. In the overall total of 577 cases, 43 were mothers who had previously had an affected child with a NTD. No elevated AFP levels were found in this group. About 90% of all NTD's may be diagnosable through AFP assay between 14-18 weeks gestation.

FETAL SERUM LYSOSOMAL ENZYMES, Aubrey Milunsky, Julian N. Kanfer, Fredric D. Frigoletto, Harvard Med. Sch., E.K. Shriver Ctr., Boston Hosp. for Women, Mass. Gen. Hosp., Dept. Ped. & Obstet., Boston.

The difficulties and hazards of prolonged tissue culture (3-6 wks.) needed to establish the prenatal diagnoses of the hereditary biochemical disorders of metabolism are well recognized. Direct fetal blood sampling for study of lysosomal enzymes should allow the prenatal diagnosis of many 'storage' disorders within 24 hours of amniocentesis. The normal range of specific activity (SA) for these enzymes in fetal serum need be established. The minimum volume of fetal serum required for assay was 2.5-100 μ l. Assays using 4-methylumbelliferyl glycosides as substrates, for hexosaminidase, glucuronidase, arylsulfatase, and mannosidase, were performed on 5 fetal sera (12-20 wks. gestation), 3 second trimester pregnancy sera, and 4 healthy adult sera. The total fetal serum protein (mean 40.7 mg/ml) was less than half the level of normal adult serum (91.0 mg/ml) and lower than mid-trimester pregnancy serum (74.7 mg/ml). The SA's of hexosaminidase in fetal serum (mean 38.4) was more than 3 times the adult control SA (11.4) and significantly more than the pregnancy sera (23.5). Similar observations of increased SA for mannosidase were made in fetal serum. The SA of arylsulfatase was similar in fetal and pregnancy serum which were each almost 4 times the SA of normal adult serum. No difference was observed in the SA of glucuronidase in the 3 groups studied.

LEIGH'S DISEASE: BIOCHEMICAL NATURE OF THE INHIBITOR. Jerome V. Murphy, Linda Craig and Robert Glew. Children's Hospital of Pittsburgh and Departments of Biochemistry and Pediatrics, University of Pittsburgh, School of Medicine, Pittsburgh.

Only one biochemical factor has been common to all untreated and autopsy-proven patients with Leigh's disease; i.e., an inhibitor to the phosphotransferase responsible for the synthesis of cerebral thiamine triphosphate. The presence of such an inhibitor in urine is not unique to patients, but has also been found in all obligate heterozygous carriers of the disorder (Pediatrics 51, 709, 1973).

We have collected urine from Leigh's disease patients and their parents, dialyzed and concentrated the inhibitor and observed its biochemical behavior. The inhibitor is indefinitely stable when stored frozen in 50 millimolar 2-mercaptoethanol. The urine concentrate inhibits the cerebral phosphotransferase up to 90%, but it does not influence either hepatic phosphotransferase, or several other enzymes. Brief exposure to trypsin, followed by trypsin inhibitor, destroys the inhibitor. Acetone or chloroform extraction similarly inactivates the inhibitor. The inhibitor is eluted from a G-200 sephadex column in a sharp peak. Using appropriate markers its molecular weight is about 28,000.

These results suggest that the inhibitor in Leigh's disease is a low molecular weight protein, possibly a glycoprotein.