744 CLEAVAGE EN BLOC OF N-ACETYLGLUCOSAMINE-6-SULFATE AND N-ACETYLGALACTOSAMINE-6-SULFATE BY HEXOSAMINIDASE A: DEFICIENCY OF BOTH ACTIVITIES IN TAY-SACHS DISEASE. Yoav Ben-Yoseph, Michael S. Baylerian, and Henry L. Nadler. Wayne State Univ. School of Medicine, Dept. of Pediatrics, Detroit.

Current assays of hexosaminidase A (Hex A) are either indirect (heat-inactivation method) or complex (GM_2 ganglioside substrate). The finding in Sanfilippo syndrome type D (N-acetylglucosamine-6-sulfatase deficiency) that Hex A can bypass the blockage in keratan sulfate degradation by cleavage en bloc of β-N-acetylglucosamine-6-sulfate, led to the present approach. 4-methylumbelliferyl derivatives of β-N-acetylglucosamine-6-sulfate (MUβGlcNAc-6-S) and β-N-acetylgalactosamine-6-sulfate (MUβGalNAc-6-S) were prepared from the commonly used unsulfated derivatives. Sera (n=5), leukocytes (n=3) and fibroblasts (n=2) from patients with Tay-Sachs disease (TSD) demonstrated markedly deficient activities toward both sulfated substrates, 2-4% of control activities (n=12), and samples from obligate heterozygotes for TSD (n=4) had intermediate activity values, 43-62% of control activities. Fibroblasts (n=2) from patients with Morquio syndrome type A (N-acetylgalactosamine-6-sulfatase deficiency) had normal activities toward both sulfated substrates but the characteristic urinary excretion of chondroitin sulfate in this disease, indicates that there is no analogy with Sanfilippo syndrome type D and Hex A is incapable of cleaving en bloc βGalNAc-6-S from the natural substrate. In contrast to natural substrates, the synthetic sulfated substrates are both specific for Hex A and thus, can provide direct and simple methods for diagnosis as well as for antenatal and carrier detection of TSD.

● 747 CARRIER FREQUENCIES FOR SANDHOFF DISEASE (SHD) AND TAY-SACHS (TSD) IN AMERICAN JEWISH (J) AND NON-JEWISH (NJ) POPULATIONS. R. Cantor, J. Lim, C. Roy, M. Kirk, M. Kaback, Harbor-UCLA Med. Ctr., Dept. Pediatrics, Torrance, CA.

Serum hexosaminidase (HEX) profiles from 59,805 J and 42,477 NJ individuals screened for TSD heterozygote status were used to estimate the SHD and TSD carrier frequencies in these populations. Mean levels of total HEX (T-HEX) and % heat labile HEX (% HEX-A) in sera differ among noncarriers (NC), obligate TSD carriers (TSD C) and obligate SHD carriers (SHD C) as follows:

	NC(n=200)	TSD C(n=85)	SHD C(n=31)
T-HEX* ± sd	732 ± 190	636 ± 146	400 ± 69
% HEX-A ± sd	63 ± 6	41 ± 5	81 ± 4

* nMoles 4MU produced/hour/ml. serum

A 95% bivariate isodensity ellipse has been constructed with T-HEX and % HEX-A values from 31 obligate SHD heterozygotes. The ellipse identifies suspect SHD C with 95% sensitivity and a 1-3% false positive rate. Cluster analysis and a derived linear discriminant function from retest serum and leukocyte T-HEX and % HEX-A values discriminates between true SHD C and NC. The SHD C frequency is 1/801 in J and 1/414 in NJ. These estimates are consistent with SHD incidence data from our national surveillance program. The TSD C frequency in J is 1/28, while a TSD C rate in NJ of 1/90 has been observed. The NJ TSD C rate is not consistent with disease incidence data. Rather, it is probable that 70% of the NJ identified as TSD C may be heterozygotes for other partial HEX-A mutations, and therefore at risk for other GM_2 ganglioside storage disorders.

● 745 I-CELL DISEASE AND PSEUDO-HURLER POLYDYSTROPHY: RADIOMETRIC ASSAYS OF N-ACETYLGLUCOSAMINYLPHOSPHOTRANSFERASE AND α-N-ACETYLGLUCOSAMINYL PHOSPHODIESTERASE WITH COMMERCIALLY AVAILABLE SUBSTRATES. Yoav Ben-Yoseph, Michael S. Baylerian, Henry L. Nadler, Michel Potier, and Serge B. Melançon. Wayne State U. School of Medicine, Dept. of Pediatrics, Detroit, and U. of Montreal, Hosp. Ste-Justine, Sec. Medical Genetics.

UDP-N-acetylglucosamine (UDP-GlcNAc) is the donor of N-acetylglucosaminyl-1-phosphate (GlcNAcP) in the reaction catalyzed by GlcNAcP transferase, the enzyme deficient in patients with I-cell disease (ICD) and pseudo-Hurler polydystrophy (PHPD). The use of commercially available UDP-[^{3}H or ^{14}C]GlcNAc rather than the synthetically made [β-^{32}P]UDP-GlcNAc was inadequate because of high background. We have overcome this in the assay of GlcNAcP trasferase with α-methylmannoside acceptor by removal of free [^{3}H or ^{14}C]GlcNAc which appeared to be the major breakdown product. In addition, the α-methylmannose-6-phospho-1-[^{3}H or ^{14}C]GlcNAc product of the transfer reaction was then isolated and following desalting could be used as a substrate for the assay of αGlcNAc phosphodiesterase. Using these relatively simple methods, deficiency of GlcNAcP transferase activity could be demonstrated in fibroblasts from patients with the classical forms of ICD (n=4; less than 4% of control activity) and PHPD (n=4; 3-33% of control activity). αGlcNAc phosphodiesterase activity was within the normal range. In contrast, in three related adult patients with what appears to be a very mild form of PHPD, both activities were normal and utilization of natural lysosomal enzyme acceptor (β-glucuronidase) was required to reveal deficiency of GlcNAcP transferase activity.

† 748 DYSMORPHIC SYNDROME, SPHEROCYTOSIS AND PARTIAL DELETION OF THE SHORT ARM OF CHROMOSOME 8. Robert R. Chilcote, Barbara Jones, Carlton Dampier, Michelle LeBeau, Janet Rowley, Yury Verlinsky, and Eugene Pergament (Spon. Marc O. Beem), The University of Chicago, Wyler Children's Hospital and Michael Reese Hospital, Chicago, IL

Congenital spherocytic anemia is a relatively common disorder but the genetic defect has not yet been definitively mapped to a chromosome. We studied a family in which 2 of 3 children were dysmorphic, affected by severe spherocytosis requiring intermittant transfusions, and showed deletion of a portion of the short arm of chromosome 8 (8p-). Both parents and the sibling were normal and showed no evidence of hemolysis or the deletion. Assays of carbonic anhydrase II, factor VII, thryroglobulin, and glutathione reductase, genes previously assigned to 8p were unremarkable. In order to elucidate the membrane defect, red cell membranes were electrophoresed using 1-D Fairbanks SDS polyacrylamide gels and a modified O'Farrell 2-D technique. Lipids were examined using thin-layer chromatography. Coomasie blue stained 1-D and silver stained 2-D electrophoretograms were normal; in particular members of the ternary binding complex, spectrin (α and β), band 4.1, and actin were normal as were membrane cholesterol and phospholipids. The association between the gene deletion and spherocytosis in the affected family members suggests that a gene for this defect lies on 8p, but does not affect the quantities of membrane proteins conventionally associated with rec cell membrane stability.

† 746 INBORN ERRORS OF UREAGENESIS; RESULTS OF THERAPY IN 44 PATIENTS. Saul Brusilow, Johns Hopkins Dept. of Ped, Balt. MD.

13 patients with complete deficiencies of CPS or OTC were treated for with a low protein, essential amino acid (EAA)diet plus arginine (Arg) or citrulline(Cit)(1 mmol/kg)plus 250 mg/kg of benzoate(B)(Regimen I). 5 of these patients died during 11 patient years of therapy. Phenylacetate(P)(250 mg/kg)was added in 12 patients (Regimen II) for 14 patient years, during which one patient died due to an overdose of B and P. Partition of urinary nitrogen (PUN) in 5 such patients revealed that hippurate-N(HAN) and phenylacetylglutamine-N (PAGN) respectively accounted for(m±SD) 21.8±4.2 and 38.2± 8.1% of effective urinary waste nitrogen (EWN)for a total of 60%. 7 patients with ASA synthetase deficiency (AS) were treated with Regimen I plus additional Arg to supply 2-4 mmol kg/d (Regimen III) for 18.4 patient years; one patient died. Regimen III was modified by substituting protein for the EAA and adding P for 3 patient years with no deaths. PUN in 3 AS children revealed that HAN, PAGN and Cit-N accounted for 17, 22 and 12% of EWN for a total of 51%. 17 patients with AL deficiency were treated with 1.5-2.0 g/d of protein supplemented with 2-4 mmol/kg Arg for 37 patient years; one died. ASA-N accounted for 54% of EWN in 2 patients. Thus HAN, PAGN, Cit-N and ASA-N are adequate EWN substitutes for urea and support life in these otherwise fatal diseases.

● 749 DEFECTIVE CHYLOMICRON REMNANT CLEARANCE IN PATIENTS WITH FAMILIAL DYSBETALIPOPROTEINEMIA. Jean A. Cortner, Paul M. Coates, Dennis R. Cryer. Lipid-Heart Research Center, Children's Hospital of Philadelphia, Philadelphia, PA

Familial dysbetalipoproteinemia results in part from a genetic defect in apolipoprotein E (apoE) which prevents normal binding of chylomicron remnants to a specific hepatic apoE receptor. We have developed a technique for measuring the consequent abnormality in chylomicron remnant clearance in these patients. Oral administration of retinol and Lipomul permits the labelling of nascent chylomicrons with retinyl palmitate (RP). RP is measured by high pressure liquid chromatography in plasma and in isolated lipoprotein fractions from 10-12 hourly blood samples. Clearance of chylomicron remnants ($T_{½}$) is calculated from the rate of disappearance of RP from chylomicrons and very low density lipoproteins. The method was found to be safe and without side effects in 45 adults. Chylomicron remnant $T_{½}$ was 1.5±0.5 hours in 7 people with normal fasting lipids. There was no change in low density lipoprotein RP; no RP was found in high density lipoprotein; no exchange of RP between lipoproteins was found. Five patients with familial dysbetalipoproteinemia were studied, including a teenage boy homozygous for the apoE 2 genetic variant (apoE 2/2) with severe type III hyperlipoproteinemia, generalized pruritic tuberoeruptive xanthoma and xanthoma striata palmaris. All patients failed to clear RP during the test, consistent with the failure of hepatic upake of chylomicron remnants carrying this apoE variant.