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GALACTOSE METABOLISM IN GALACTOSEMIC LYMPHOID LINES. N.G. Beratis, and L. Wilbur. Inst. Basic Res. Mental Retdn., and Mt. Sinai Sch. Med., New York, New York.

The activity (mean ± SD) of galactose-1-phosphate uridyl transferase in two lymphoid lines from two patients with galactosemia, a heterozygote, and eight normal subjects was 0, 78, and 168 ± 55 μmoles UDPG consumed/mg protein/hr, respectively. Absence of enzyme activity also was found in RBC and skin fibroblasts of the galactosemic (glc) patient. The glc lines failed to grow in medium in which galactose was substituted for glucose. No difference in the total radioactivity present in the cells was found between normal, glc, and heterozygous lines cultured in the presence of (^3H)-galactose. The radioactivity incorporated into TCA-precipitated cellular material of the glc lines was 6.9% (3.5×10^3 CPM/mg protein/hr) of the normal (51.4×10^3) and heterozygous (49.6×10^3) lines. Normal and glc lines incubated with (^{14}C)-1-galactose produced 218 ± 66 and 18 pmoles $^{14}CO_2$/mg cellular protein/6hrs, respectively. The production of $^{14}CO_2$ from (^{14}C)-1-glucose was similar in normal and glc lines. Most of the radioactivity in normal cells was incorporated into molecular species with MW > 400,000. The glc cells did incorporate a small amount of radioactivity into macromolecules. Similar molecules were identified in the cell-free medium of both normal and deficient cells. In addition, a molecular form with MW < 25,000 was released in the medium of the normal cells but not of the glc cells. These findings indicate that a small amount of galactose is metabolized in glc lines even in the apparent absence of enzyme activity. Furthermore, these lines are suitable for studying galactose metabolism and treatment of patients with galactosemia.

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PRENATAL DIAGNOSIS OF LYSOSOMAL ENZYME DISORDERS USING MICROASSAYS. Mariluci T. Bladon and Aubrey Milunsky. Eunice Kennedy Shriver Ctr. and Harvard Medical School.

Efforts at prenatal diagnosis of biochemical disorders are often prolonged and may fail because of time and culture problems. We have used a microfluorimetric technique which assays only a few (100-300) cultured amniotic fluid cells or skin fibroblasts. Cell lines are matched for passage number and confluency and each line is assayed 5-10 times. Enzyme activity is expressed in nmoles of umbelliferone released/cell/hr. (± S.D.).

Tay-Sachs Disease

Skin Fibroblasts		Amniotic Cells	
Total Activity	Inactivated Form	Total Activity	Inact. Form
Controls (3)		Controls (6)	
0.0967±0.0353	0.0283±0.0206	0.0710±0.0262	0.0317±0.0116
Heterozygotes (3)		Heterozygotes (3)	
0.0583±0.0249	0.0320±0.0085	0.0253±0.0040	0.0150±0.0044
Homozygotes (2)		Homozygotes (4)	
0.0355±0.0417	0.0280±0.0311	0.0708±0.0930	0.0685±0.0897

GM_1-Gangliosidosis (Skin Fibroblasts)

Controls (3) 0.0360±0.0100, Heterozygotes (3) 0.0217±0.0015, Homozygotes (4) 0.0110±0.0061

Fabry's Disease (Skin Fibroblasts)

Controls (5) 0.0020±0.0006, Heterozygotes (3) 0.0012±0.0002, Homozygotes (4) 0.0006±0.0002. These preliminary studies suggested that microassays of lysosomal enzymes for prenatal diagnosis are feasible.

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CAYMAN DISEASE AND A NEW STORAGE DISEASE IN A WEST INDIES ISOLATE. Arthur D. Bloom, William G. Johnson, Mary Murphy, William I. Murphy, Nora Lindheim and Patricia Smith. College of Physicians and Surgeons, Columbia University, Depts. of Pediatrics, Human Genetics and Development, and Neurology, New York.

With a reported 1975 newborn malformation rate of 8.3%, and with isolation and inbreeding of its racially admixed population (now 13,260) for over 200 years, the Cayman Islands, B.W.I. have been the site of an intensive genetic study. To date, we have found an increased incidence in many, particularly recessive, disorders, including: sensorineural deafness (27 cases), with and without retinitis pigmentosum; congenital cataracts (14 cases); congenital ichthyosis (2 cases); and two new disorders, designated Cayman Disease (25 cases) and a new Storage Disease (6 cases). Twenty-two of the CD patients are clearly related, and all come from the town of West Bay (pop'n: 2715) as do the SD cases. All CD patients have congenital ataxia, MR, and ocular movement abnormalities. The SD (?MPS) patients are all related, and while normal at birth, develop abdominal protuberance by 18 mos., MR by 3-4 yrs., contractures thereafter, and die by 9-13 yrs. These diverse disorders appear to be the result of the combination of consanguinity, founder effects, drift, and selection.

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CYSTIC FIBROSIS FIBROBLASTS SHOW ENHANCED DEXAMETHASONE RESISTANCE. Jan L. Breslow and James Epstein. (spon. by Park Gerald) Harvard Medical School, Child. Hosp. Med. Ctr., Dept of Pediatrics, Boston.

We have shown previously that fibroblasts from cystic fibrosis patients are more resistant to ouabain (OB) cytotoxicity than fibroblasts from normal individuals. Although OB is generally assumed to act on the cell membrane and to cause killing of cells by inhibiting ion transport, the OB resistance of CF cells was seen only in K^+ deficient medium and was not associated with a diminished ability of the drug to inhibit ion transport. These findings suggested that OB may act in human diploid fibroblasts by a mechanism other than ion transport inhibition. We therefore examined the ability of normal and CF cells to survive exposure to ethacrynic acid, another inhibitor of ion transport, and to colchicine and aminopterin, resistance to which has been associated with membrane alterations in other cells. After exposure to these drugs, there were no differences in survival between normal and CF fibroblasts. This suggests that normal and CF cells do not differ in terms of a generalized resistance to ion transport inhibitors or to drugs which must pass through the membrane to be active. In further survival studies with structural analogues of OB, the effects of dexamethasone (DEX), which has a sterol nucleus similar to that of OB but is thought to have a different site of cellular action, was also tested. CF cells survived exposure to DEX significantly better than did normal cells in both K^+ deficient and K^+ containing medium. These results raise the possibility that CF cells have an enhanced resistance to drugs which have the sterol molecular structure found in OB and DEX.

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HOMOZYGOUS β-THALASSEMIA WITH HIGH LEVEL OF Hb A_2. Oswaldo Castro, William P. Winter and Donald L. Rucknagel (Spon. by Roland B. Scott). Depts. of Med. and Peds., Howard Univ. Washington, DC and Dept. of Human Genetics, Univ. of Michigan, Ann Arbor, MI.

A 15 y o Black male with severe anemia, splenomegaly and typical thalassemic blood picture had 12.4% Hb A_2. Family studies (table) were consistent with homozygosity for the β-thal.

	Hb(g%)	MCV(μ^3)	Hb A_2(%)	Hb F (%)	α/non-α
Proband	7.4	52	12.4	12.1	1.81
Mother	10.4	70	5.4	0.4	1.81
Father	14.4	79	4.4	1.4	0.88
Sibling	13.6	69	6.1	1.0	-
Sibling	10.2	68	6.4	1.0	-
Sibling	11.4	74	6.3	1.0	-

gene. The α/non-α globin synthesis ratio of the father's reticulocytes revealed the presence of α-thal. Analysis of the proband's A_2 Hb showed asparagine at δ Tp 2 and one methionine residue at δ Tp 13 suggesting a normal δ chain structure. The α/non-α ratio and % Hb F in the proband were lower than usual for homozygous β-thal. These values and the marked increase in Hb A_2 are probably due to the ameliorating effect of an associated α-thal. gene rather than to the presence of a Miyada-like hemoglobin, as has been postulated for a similar kindred (Biochem. Genet.: 10, 135, 1973).

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METHYLATED 5'-TERMINI OF GLOBIN mRNAs IN β^+-THALASSEMIA. Sechin Cho, Tu-chen Cheng, John Fox and Haig H. Kazazian, Jr., Johns Hopkins University School of Medicine, Department of Pediatrics, Baltimore.

Modified 5'-terminal structures (caps) of mRNA play an important role in the initiation of protein synthesis. A deficiency of 5'-capping and methylation has been suggested as a possible cause of β mRNA deficiency in β-thalassemia (β-thal). After incubating peripheral blood from β^+-thal major, α-thal, nonthal adults with reticulocytosis, and erythroblastotic infants with [methyl-^{3}H] methionine, the 5'-terminal structures of human α and β globin mRNA were labeled. To separate the 5'-terminal oligonucleotide of α mRNA from that of β mRNA, [^{3}H]-labeled poly(A^+) RNA was digested with RNase T_1 and alkaline phosphatase, and the digest was fractionated by DFAE-Sephadex chromatography in 7 M urea. After sequence analysis, we found that the methylated 5'-termini of the α and β mRNAs are identical through the first three nucleotides: $m^7Gpppm^6A^mpC^mp$. Our data also indicate that addition of the four methyl groups to the 5'-end follows an orderly sequence. The rate of methylation of the 5'-terminus of β mRNA was compared to that of α mRNA in a patient with β^+-thal major and in nonthal patients. No major differences in methylation were observed between the β^+-thal and nonthal samples. We conclude that 5'-capping and methylation is not defective in our β^+-thal patient.