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SEPARATION OF FETAL AND ADULT ERYTHROCYTES BY SELECTIVE HEMOLYSIS: AN AID TO PRENATAL DIAGNOSIS OF HEMOGLOBINOPATHIES. Blanche P. Alter, David G. Nathan,

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Prenatal diagnosis of hemoglobinopathies is difficult if placental blood samples are contaminated by maternal cells, because R/y ratios exceed the normal fetal range of 0.07 to 0.15. We have shown that fetal reticulocytes have less carbonic anhydrase than adult cells, and resist NH_4Cl -, NH_4HCO_3 -mediated (Ørskov) hemolysis. To test this approach in actual cases, pure fetal blood was obtained by fetoscopy from two midtrimester fetuses at risk for homozygous β -thalassemia (thal). 2.5% fetal blood was mixed with blood from the β -thal trait mothers. Samples were incubated with ^3H -leucine; an aliquot was treated in the Ørskov reaction. Red cell size distributions were analysed with a Coulter channelizer. Globin chains were separated on carboxymethylcellulose columns. In the mixed samples, only small maternal cells were seen with the channelizer. Following Ørskov hemolysis, only large fetal cells were detected. In case 1, β/γ in the mixed sample was 0.42. β/γ fell to zero following Ørskov hemolysis, and was zero in pure fetal blood. This fetus had thal major. In case 2, β/γ was 1.97 before and 0.04 after Ørskov hemolysis; it was 0.04 in pure fetal blood. This fetus has β -thal trait. Ørskov hemolysis provides pure fetal blood from samples with only 2.5% fetal cells and enables the appropriate diagnoses to be made even when low yield samples are obtained.

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PHOSPHORIBOSYL PYROPHOSPHATE (PRPP) SYNTHESIS IN CULTURED HUMAN FIBROBLASTS. Paul J. Benke and David Dittmar. Mailman Center and Department of Pediatrics, University of Miami School of Medicine, Miami, Fla.

An investigation of the regulation of purine metabolism is important in understanding the accelerated purine synthesis and abnormalities in PRPP metabolism that occur in the Lesch-Nyhan (LN) syndrome. PRPP levels in cultured human fibroblasts increased from 0.48 ± 0.11 nmoles/mg protein ($\pm 1\text{SD}$) to 0.75 ± 0.15 ($p < .01$) when hypoxanthine was removed from the medium. This is less than levels observed in LN cells (1-1.8). Without hypoxanthine, PRPP amidotransferase, and not hypoxanthine-guanine phosphoribosyltransferase is utilized to supply cellular purine needs. Two to three fold increase in PRPP synthetase occurred in cells starved for purines by growth in medium lacking purines and containing aminopterin, an inhibitor of purine synthesis. Enzyme activity did not increase when the aggregation of PRPP synthetase was prevented by homogenization in a buffer containing 0.05mM ATP and 1mM MgSO_4 . These results suggest that the aggregation of PRPP synthetase has physiological significance in providing higher PRPP levels for de novo purine synthesis. ADP inhibition of PRPP synthetase was the same in control and purine starved cells.

Purine levels and PRPP synthetase increased in a biphasic pattern following removal of thymidine inhibition of mitosis and at S phase of the cell cycle. Adjustment of PRPP levels by change in PRPP synthetase activity and aggregation is a sensitive cellular mechanism in the regulation of purine metabolism.

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IDENTIFICATION OF INHERITED PROTEIN VARIANTS IN INDIVIDUAL HUMAN RED CELLS. Stephen I.O. Anyaibe, Syama P. Bhattacharya, and Verle E. Headings.

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Identification of genetic phenotypes in individual red cells could elucidate intercellular variation in specific locus gene products as may accompany chromosome inactivation or allelic exclusion, cell-aging, and cell mixtures. Furthermore, concurrent identification of phenotype and either replicative or protein synthesizing stages would allow phenotype correlation with stage of cell history. Electrophoresis of the contents of individual erythrocytes from adults or cord blood in ultrathin layer agar plates, allowed microscopic identification of specific hemoglobins (F,A,S,C) and variants of glucose-6-phosphate dehydrogenase (A,B). Fetal red cells were sequentially incubated in Cl^{14} -amino acids, suspended in an ultra-thin layer of agar, incubated with fluorescein-conjugated antibodies against specific hemoglobins (F,A,S, or C), and covered with radiosensitive emulsion. Pericellular immunoprecipitates and autoradiographic grains were identified concurrently in individual synthetically active cells. Marrow erythroid cells cultured in plasma clots were incubated with H^3 -thymidine. Evidence for cell replication and specific Hb in the same cell were obtained by autoradiography and pericellular immunoprecipitates. These findings confirm the feasibility of phenotyping certain proteins in individual red cells.

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REVERSION OF THE GALACTOSEMIA TRAIT IN SV-40 TRANSFORMED HUMAN FIBROBLASTS. Peter A. Benn, Hollister K. Jameson, Frances S. Boches, Helene B. Markus, and William J. Mellman. University of Pennsylvania, School of Medicine, Department of Human Genetics, Philadelphia.

Patients with galactosemia have a deficiency of the enzyme galactose-1-phosphate uridylyl transferase (GALT) in the Leloir pathway. In vitro, normal cells can grow in media containing galactose as the sole sugar source while in cultures derived from galactosemia patients the majority of cells are unable to survive. In 2 lines of SV-40 virus transformed human fibroblasts derived from galactosemic patients, surviving populations of cells were isolated which were able to proliferate in galactose. Some of these cell populations appeared to be revertants with a measurable GALT activity while others had undetectable GALT activity. The latter group were able to grow in sugar free media. Cloned revertant lines were characterized by heat stability studies and starch gel electrophoresis. Study of these revertants provides additional insight into the molecular effects of mutation at the GALT locus.

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CORRECTION OF HYPERAMMONEMIA IN ARGININOSUCCINIC ACID EMIA BY STIMULATING ARGININOSUCCINIC ACID (ASA) SYNTHESIS. Mark L. Batshaw, Saul W. Brusilow, Johns Hopkins Medical Inst., Dept. Pediatrics & Kennedy Institute, Balto.

Hyperammonemia in urea cycle defects is a consequence of impaired waste nitrogen excretion (WNE). In contrast to other defects, ASAemia is not necessarily associated with impaired WNE. ASA contains 2 N atoms normally excreted as urea and 2 N atoms as ornithine (orn). If these are replenished stoichiometrically, either by arginine (arg) or by de novo orn synthesis, WNE could be normal and hyperammonemia prevented. This hypothesis was tested in a full-term 3 kg black female who became lethargic at 24 hrs of age and comatose at 48 hrs. At 7 days of age, the diagnosis of ASAemia was made based on a plasma NH_4^+ of 800 μM , and identification of peaks of ASA and its anhydrides in plasma and urine. Over a period of 24 hrs., she received two intravenous doses of Arg-HCl 5 mmol/kg. Twenty-four hours after the first infusion, there was a fall in NH_4^+ (837-112 μM), glutamine (1799-1081) and alanine (1566-188). There were increases in arg (40-883), orn (62-170) and ASA (466-1558). Subsequently the NH_4^+ fell to 62. Arg was then discontinued and the NH_4^+ rose and ASA fell. Thus, provision of orn skeletons as arg permitted ASA synthesis and a calculated 3-fold increase in WNE as ASA. As a consequence, NH_4^+ and its precursors (alanine and glutamine) fell to near-normal. Although ASA levels were increased, they may not be toxic (Clin. Res., 24:186A, 1976). Although this child had irreversible brain damage and died at 17 days, future cases, if diagnosed early, should respond to supplemental arg or orn with normal NH_4^+ and survival.

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GALACTOCEREBROSIDASE (GC) CROSS REACTING MATERIAL (CRM) IN GLOBOID CELL LEUKODYSTROPHY (GLD). Y. Ben-Yoseph, M. Hungerford and H.L. Nadler. Northwestern U. Med. Sch., Children's Mem. Hosp., Dept. of Pediatrics, Chicago, Ill.

The primary genetic defect in GLD is a deficiency of GC activity. In order to clarify the nature of the mutation in this disease, we have purified GC from placenta and used antibodies prepared against this normal enzyme to look for CRM in tissues of patients with GLD. Antigenic identity was observed between GC of normal brain, liver and skin fibroblasts. The antibodies were monospecific to this enzyme, precipitated only β -galactosidase activities toward galactosylceramide and lactosylceramide (assay I) and did not react with either GM₁ or neutral β -galactosidases.

Preparations of 1 brain, 2 livers and 4 fibroblast lines from GLD patients were examined by double gel immunodiffusion against the anti-GC antibodies and found to be CRM positive. The patients' CRM was identical to the normal enzyme and demonstrated a similar electrophoretic mobility on immunoelectrophoresis. However, activity towards naphthol-AS-BI- β -galactoside could be demonstrated in the immune precipitates of normal controls and other sphingolipidoses but not in those of the GLD patients. A single radial immunodiffusion assay was developed for quantitation of the CRM in the various tissues independent of its enzymic activity. Quantities of CRM within the normal range were determined in the brain, liver, and skin fibroblasts of the GLD patients.

The existence of normal amounts of CRM in the patient's tissues indicates a structural gene mutation producing a protein which has lost its enzymic activity but retained its antigenicity.