667 INTERDEPENDENCE OF RED CELL HEXOKINASE, INORGANIC PHOSPHORUS(Pi)AND 2,3-DIPHOSPHOGLYCERATE(2,3-DPG)IN THE DEVELOPING FETAL LAMB. Susan F. Travis, Cruz M. de Alvarado, Elizabeth Cannon, and Maria Delivoria-Papadopoulos. Thomas Jefferson Univ., Dept. of Pediatrics, Univ. of Pa. School of Medicine, Depts. of Physiology and Pediatrics, Phila., PA

Previous studies have demonstrated that 2,3-DPG inhibits red cell hexokinase activity in vitro and that the concentration of red cell 2,3-DPG may be influenced by serum Pi in vivo. Since the developing fetal lamb exhibits wide variation in its red cell 2,3-DPG content, hexokinase activity and serum Pi were studied in 7 lambs that were followed sequentially from birth until 8 weeks of life. The mean 2,3-DPG concentration on day 1 of life was 4389±994 mμmoles/ml RBC. There was a significant increase($p<0.005$) in 2,3-DPG from day 4 of life(mean:8249±1754) through days 10-11 (mean:7577±1670). By day 17 of life the mean 2,3-DPG concentration decreased to 4242±1832 mμmoles/ml RBC, and thereafter fell rapidly; by day 45 of life, 2,3-DPG was not detected. No measurable 2,3-DPG was found in 6 adult sheep. Red cell hexokinase activity appeared to be modulated by changes in the 2,3-DPG content. Hexokinase was 1.18±0.33 units/gm hemoglobin on day 1, fell to 0.94±0.05 by days 10-11, and increased to 1.16±0.13 on day 17. From days 1 through 60 of life, changes in red cell 2,3-DPG content correlated directly with the serum Pi($r = 0.82$; $p<0.001$). These data demonstrate an in vivo confirmation of inhibition of red cell hexokinase activity by 2,3-DPG and the regulation of red cell 2,3-DPG concentration by serum Pi in the developing fetal lamb.

668 A FETAL ISOZYME OF PHOSPHOFRUCTOKINASE IN NEWBORN ERYTHROCYTES. Shobhana, Vora, Sergio Piomelli; New York University Medical School, Department of Pediatrics, New York.

Phosphofructokinase (PFK) exists in isozyme forms in various human tissues. Erythrocytes of newborns have a relative PFK deficiency and an altered rate of glycolysis. PFK in the newborn also shows increased in-vivo lability.

Experiments were designed to elucidate the differences between adult and newborn erythrocyte PFK. The purified enzymes from human muscle and liver were separated by chromatography on DEAE-Sephadex A-25 columns equilibrated with 0.1M Tris-PO_4 pH 8.0, and elution with a salt gradient. Human muscle and liver PFK eluted as widely separated sharp peaks. Adult erythrocyte PFK (from either crude hemolysates or from purified preparations) eluted as a single broad peak in a position intermediate between those of the liver and muscle isozymes. Hemolysates from cord blood, examined by this technique, were found to consist of two clearly separated PFK peaks: one chromatographically indistinguishable from adult erythrocyte PFK, and a second peak chromatographically indistinguishable from liver PFK.

These data demonstrate that in fetal erythrocytes there is an isozyme presumably identical with liver PFK isozyme, which is probably responsible for the relative deficiency of PFK in newborn erythrocytes.

669 COOLEY'S ANEMIA: TRANSFUSIONS REGIMEN AND IRON BALANCE; Michael Weiner, Margaret Karpatkin, David Hart, Carol Seaman, Shobhana Vora, Sergio Piomelli; New York University Medical School, Department of Pediatrics, New York.

In Cooley's anemia, maintenance of a hemoglobin level$\geq$9.5 g/dl prevents anoxia and suppresses endogenous erythropoiesis. Eight patients have been maintained on this transfusion regimen since initial diagnosis, for 3 to 13 years. The patients have maintained excellent health and have been able to live normal lives, including school sports. Hepato-splenomegaly has been contained to a modest level; hypersplenism has not developed. Cardiac size and function have been normal; however, echo-cardiography revealed increased left ventricular wall thickness in children older than 8 years, suggesting increased Fe deposition. All children have received test doses of I.M. Desferrioxamine-B 20 mg/Kg 15-16 times each year. The results indicate that in the youngest children, 20% of the transfused Fe, and in the oldest children, 50%, could be eliminated by daily I.M. injections. Recently, the same dose of Desferrioxamine-B has been administered by intravenous or subcutaneous infusion overnight. By these routes, the excretion of Fe has increased fourfold in the youngest, and twice in the oldest children.

Thus, children with Cooley's anemia may be kept in excellent health by this transfusion regimen, and the iron overload prevented by daily subcutaneous chelation therapy at home. This should result in increased longevity.

670 ALTERED PYRIMIDINE METABOLISM IN RBC OF 4 PATIENTS WITH CONGENITAL HYPOPLASTIC ANEMIA (CHA). H. Ronald Zielke, Pinar T. Ozand, Ruth E. Luddy, William H. Zinkham*, and Marvin Cornblath. Univ. of Maryland School of Medicine, Univ. of Md. Hospital, Dept. of Pediatrics, Baltimore and *Johns Hopkins School of Medicine, Johns Hopkins Hospital, Dept. of Pediatrics, Baltimore, MD.

In prednisone-dependent CHA, the levels of orotate phosphoribosyl transferase (OPRT) and orotidine monophosphate decarboxylase (ODC) were significantly elevated in RBC. (OPRT: patients = 7.6-54.8 nmoles/hr/10^9 RBC; controls = 2.9 ± 1.8 (Mean ± S.D., n=30); ODC: patients = 26-118 nmoles/hr/10^9 RBC; controls = 10.6 ± 4.7 (n=30)). In 1 patient prior to prednisone therapy, the OPRT and ODC levels were 10-fold elevated and remained 3-fold elevated after one year of therapy. In 2 clinically normal individuals, one of whom was related to a CHA patient, the levels of OPRT and ODC in RBC were 1.6 to 3.1 standard deviations greater than the mean of the controls. Elevated OPRT and ODC did not occur in leukocytes, fibroblasts or short term lymphocyte cultures in CHA. The levels of 3 other pyrimidine synthetic enzymes, as well as purine and pyrimidine salvage enzymes, were within normal limits in all cells studied. OPRT and ODC activities were also elevated in the RBC of the father of 2 siblings (mother not tested), but were normal in 3 parents of 2 other patients. In contrast to CHA, all of the pyrimidine biosynthetic enzymes including OPRT and ODC were elevated in RBC of sickle cell anemia, DiGuglielmo syndrome and the newborn. It is postulated that factors which affect the levels of OPRT and ODC may also result in diminished erythropoiesis in CHA. (Supp. by John A. Hartford Fndn., Inc. #74266).

671 ERYTHROCYTE MORPHOLOGY IN NEWBORN INFANTS. A NEW LOOK. Alvin Zipursky, McMaster University, Department of Pediatrics, Hamilton, Ontario, Canada.

We have developed a simple technique for the three dimensional assessment of glutaraldehyde fixed erythrocytes partially immobilized in glycerol. In 24 adults, 70 ± 10% of erythroctes collected at room temperature were biconcave discs and 27 ± 10% were bowl shaped or stomatocytes. In 36 newborn infants (27 premature and 9 full-term) significantly fewer (43 ± 14%) cells were biconcave discs and 32 ± 13% were stomatocytes. Abnormally shaped erythrocytes were classified according to Bessis. In the infants, an average of 7.5% of the cells were echinocytes (range = 0-50%) as compared to significantly lower values in adults (mean = .75%; range = 0-4%). Abnormally shaped cells (including schizocytes, keratocytes, knizocytes and pitted cells) were significantly more frequent in infants (19 ± 8%) than in adults (2 ± 1.5%). We conclude, therefore, that erythrocyte morphology is distinctly different in infants as compared to adults. In addition, these findings suggest that erythrocyte morphology in disease can now be quantitated.

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672 ALTERATION OF CYTOPLASMIC ORGANELLES AFFECTS POLYMORPHONUCLEAR LEUKOCYTES ADHESIVENESS. John M. Allen, Laurence A. Boxer and Robert L. Baehner, Indiana University School of Medicine, James Whitcomb Riley Hospital for Children, Department of Pediatrics, Indianapolis.

Striking spontaneous polarization of surface glycoproteins following exposure to Concanavalin A occurs in polymorphonuclear leukocytes (PMN) in the Chediak-Higashi syndrome (C-H) which is associated with the absence of microtubules as visualized by the transmission electron microscope. We now show that impaired microtubule assembly also correlated not only with reduced chemotaxis and degranulation but with decreased granulocyte adherence (GA) as well. GA was measured by filtering purified PMN over nylon fibers contained in a pasteur pipette and quantitating the percentage of PMN adhering to the fibers. GA in normal mink and mice was 74±1% and 64%; whereas, it was reduced in C-H mink and mice to 40±1% and 32% respectively. Drugs previously shown to inhibit microtubule assembly in PMN were examined. GA was decreased to 27±6%, 29±2% and 19±9% with 10^{-5}M colchicine, 5 uM diamide, 500 uM tertiary butyrylhydroperoxide respectively in human PMN compared to normal values of 48±2%. When 5 ug/ml of cytochalasin B was incubated with human PMN, a drug known to inhibit microfilament polymerization, GA was also reduced to 33%. These studies support the notion that impairment of either cytoplasmic microtubule or microfilament assembly will lead to alterations in the surface properties of PMN membrane which, in turn, will affect the ability of the PMN to adhere to activated surfaces.