937 INTERACTION OF PYRUVATE/LACTATE AND NAD/NADH RATIOS IN THE REGULATION OF RED CELL (RBC) GLYCOLYSIS. Carol Seaman, William Tilton, Martha Smith, and Sergio Piomelli. Columbia University, College of Physicians and Surgeons, Division of Pediatric Hematology, NY.

In vivo, the final products of RBC glycolysis, pyruvate (PA) and lactate (LA), diffuse into the plasma, and a steady level of glycolytic intermediates is maintained within the cell. In vitro, when RBCs are incubated with glucose (GL) in a closed system, an increase in fructose-1,6-diphosphate (FDP) and triose phosphates (TP) rapidly takes place, rising twenty-fold in 3 hours. This makes in vitro studies of glycolytic intermediates impossible. We have incubated RBCs in an open system that allows physiologic diffusion of the PA and LA produced. In this system, in the presence of GL alone, the accumulation of FDP and TP is markedly reduced, with a six-fold increase over normal. On addition of PA only, the accumulation of FDP and TP is suppressed, and steady state concentrations are observed. On addition of LA only, however, the accumulation of FDP and TP is again twenty-fold as in the closed system. On addition of both PA and LA, at the physiological ratio of 1/19, regardless of concentration, the accumulation of FDP and TP is suppressed, with levels similar to those observed with PA only. If LA is increased and the PA/LA ratio is significantly reduced, then accumulation of FDP and TP occurs. When LA increases in the RBCs, an accumulation of NADH takes place at the LDH step, and thus glycolysis is slowed at the level of the 3-phosphoglyceraldehyde dehydrogenase (3-PGAD) step, through NADH inhibition. An excess of pyruvate lowers the NADH concentration at the LDH step, in turn releasing the inhibition of 3-PGAD. These data suggest that the orderly flow of glycolysis in RBCs depends on the diffusion of the PA and LA produced, to maintain a constant intracellular PA/LA ratio, necessary to regulate the intracellular NAD/NADH ratio. An open system of incubation approximates closely physiologic conditions and its use allows control of glycolysis in vitro. With this technique it is possible to study in vitro glycolytic intermediate levels, in normal and abnormal conditions.

● 938 LACK OF SUPPRESSOR CELL ACTIVITY IN CHILDREN WITH HISTIOCYTOSIS-X (H-X). Barry Shannon, William Newton, Debra Jacobs (Spon. by Grant Morrow). Ohio State University School of Medicine, Children's Hospital, Departments of Pediatrics and Pathology, Columbus, Ohio.

H-X consists of a spectrum of diseases of unknown etiology with variable clinical expression. The histology of lesions suggests this disease is immunoreactive and not neoplastic. In order to determine whether patients with active disease lack suppressor cells and/or activity, we examined children with active and inactive disease. The percentage and absolute number of T lymphocytes and their subsets (T_{11}, T_4, T_8, T_{10}, T_6), B lymphocytes and natural killer cells (Leulla) were examined in addition to suppressor activities of mononuclear cells. No significant differences were noted on any parameter between patients with inactive disease (n=14) and age-matched controls (n=22). However, the following was noted between patients with active disease (n=7) and age-matched controls (n=7).

	Active Disease	Controls
T_{11}^+ %, no./mm^3	63±16*, 1982±905*	82±4, 2525±302
T_4^+ %, no./mm^3	45±16, 1435±750	51±7, 1571±421
T_8^+ %, no./mm^3	16± 9*, 573±453	26±5, 803±228
T_4/T_8	3.5±1.6*	2.0±0.4
Indomethacin stimulation index	1.17±0.20*	1.40±0.02
Concanavalin-A suppressor index	0.80±0.23*	0.69±0.09

*$p<0.05$, x±SD

These results show a lack of lymphocyte and monocyte suppressor function in patients with active disease.

939 PHENOTYPIC AND FUNCTIONAL CHANGES OF LYMPHOCYTES IN HEMOPHILIACS. Barry Shannon, Jane Roach, Melissa Luten, Fred Ruymann (Spon. by Grant Morrow). Ohio State University School of Medicine, Children's Hospital, Departments of Pediatrics and Pathology, Columbus, Ohio.

Changes in cellular immunity have been reported in hemophiliacs receiving lyophilized factor concentrate. T cell subsets, mitogenic responsiveness, and degree of hypergammaglobulinemia in healthy hemophiliacs were examined in order to determine the effect of product therapy on immune responsiveness of children with hemophilia (age range, 3-21 years). The study population was comprised of 21 hemophilia A patients receiving factor VIII concentrate, 10 hemophilia A patients receiving cryoprecipitate, 8 hemophilia B patients receiving factor IX concentrate and 20 healthy children receiving no blood products. The percentage of T_4^+ lymphocytes was decreased in all hemophiliacs regardless of therapy ($p<0.05$). Only patients with hemophilia A receiving factor VIII concentrate exhibited a concomitant increase in the percentage of T_8^+ lymphocytes and showed a significantly decreased T_4/T_8 ratio ($p<0.05$). Depressed mitogenic responsiveness to phytohemagglutinin and concanavalin-A was observed in both hemophilia A and B patients receiving factor concentrate ($p<0.05$). No correlation was observed between changes in the T_4/T_8 ratio or mitogen responsiveness relative to the amount of product received. Hypergammaglobulinemia of the IgG class was demonstrable in all hemophilia groups and correlated with age ($p<0.05$). Phenotypic and functional alternations of lymphocytes demonstrated in hemophiliacs are probably the result of chronic factor exposure, which becomes more pronounced with increasing age.

† 940 ERYTHROPOIESIS IN INFANTS OF DIABETIC MOTHERS. Kevin Shannon, Jack Davis, John Kitzmiller, Gisela Clemons, Sam Fulcher, John Pacely, Harold Koenig. Depts of Pediatrics and OB/GYN, Naval Hospital, Oakland; Children's Hospital of San Francisco, U of Calif, Berkeley.

Polycythemia is frequent in the infants of diabetic mothers (IDM). We enumerated erythroid progenitor colonies (BFU-E) and determined cord blood erythropoietin (EP) levels in IDM and controls. Fifteen of 18 diabetic mothers were insulin dependent and were maintained on a protocol of strict glycemic control throughout gestation. Growth of BFU-E in methylcellulose supplemented with 0.1 to 2.0 units/EP/ml followed identical dose-response curves in IDM and controls. Although numbers of BFU-E varied widely among both IDM and controls, for individual infants there was a highly significant correlation between growth at the lowest and highest doses of exogenous EP tested ($r= 0.91$; $p < 0.0001$). Mean cord blood EP values were 56 ± 21 in IDM and 44 ± 11 in controls. We observed no relationship between BFU-E growth and cord blood EP; however, cord blood pH independently correlated with both EP ($r= -0.72$; $p= 0.006$) and with the number of BFU-E observed at optimal EP ($r= 0.40$; $p= 0.016$) in IDM. The association of poor BFU-E growth with low pH and elevated EP in some infants suggested depletion of the erythroid progenitor pool in response to hypoxemia. The low incidence of elevated cord blood EP (1 of 18) in the IDM perhaps reflects a beneficial effect of strict biochemical control of maternal diabetes on fetal hypoxia. Erythroid proliferation is intrinsically normal in IDM, supporting the hypothesis that polycythemia in these infants is a secondary phenomenon.

941 PREVALENCE OF VITAMIN K DEFICIENCY IN NEWBORN INFANTS: INFLUENCE OF PERINATAL RISK FACTORS. Amy D. Shapiro, Peter Hulac, Linda J. Jacobson, Peter A. Lane, Marilyn J. Manco-Johnson, Wm. E. Hathaway, University of Colorado School of Medicine, Department of Pediatrics, Denver.

Measurement of noncarboxylated prothrombin (PIVKA-II assay) was used to study 559 infants for vitamin K (vit K) deficiency. This test, performed by immunoelectrophoresis of plasma before and after $BaCO_3$ precipitation, is sensitive to 0.03 u/ml PIVKA-II, has an intra- and inter-test coefficient of variation of 3-5%, and is negative in liver disease. Catagories of infants studied and results are as follows. As part of an ongoing study of 1,000 cord bloods, 534 cords were assayed and 2.6% were PIVKA-II positive (0.03-0.15 u/ml). The PT of the PIVKA-II positive samples ranged from 11.5-23 seconds and correlated inversely with prothrombin activities of 10-55%. SGA infants were at increased risk for K deficiency (14%); however, other groups were not (preterm, post-term, infants with fetal distress, and infants of mothers with hypertension or third trimester infection). Interestingly, of 16 infants born to mothers on chronic anticonvulsant therapy, only one showed PIVKA-II. Eight breast-fed infants who were given neonatal vit K did not develop a deficiency by two months of age. A K deficient infant given 1 mg vit K and then fed entirely by parenteral nutrition without supplemental vit K showed absence of PIVKA-II for five weeks.

In summary, 2.6% of all newborns may be vit K deficient at birth. While most K deficient infants were born of normal pregnancies and deliveries, SGA infants were more likely to be PIVKA positive. Vit K is stored in newborns to an appreciable degree.

942 IN VITRO OXIDATIVE METABOLIC FUNCTION OF HUMAN CORD NEUTROPHILS POTENTIATED BY EXPOSURE TO THE CHEMOATTRACTANTS FMLP AND ZYMOSAN ACTIVATED SERUM. Ann O. Shigeoka, Univ. of Utah School of Medicine, Salt Lake City, Utah.

We previously observed that neutrophils(PMN) from well neonates had oxidative responses similar to PMN from well adults but stressed neonates' PMN exhibited decreased chemiluminescence and superoxide (O_2^-) responses to opsonized zymosan(OpZ). The present studies were designed to evaluate oxidative responses to OpZ and phorbol myristate acetate(PMA) from cord PMN exposed to zymosan activated serum(ZAS) which contains C5a or to FMLP, a synthetic analog of a bacterial product. Since the chemotactic function of cord PMN is decreased, we expected such treatment of cord PMN might reproduce the oxidative abnormalities in stressed neonates' PMN. Aliquots of each PMN sample were preincubated in the presence or absence of the chemoattractant at 22° or 37° for 15 or 30 min. Exposure of cord PMN to ZAS decreased O_2^- formation in response to OpZ (ZAS$\bar{x}$=19nmoles cyt c red/10^6 PMN/30 min; no ZAS 28 nmoles) but O_2^- formation by adult PMN also decreased (ZAS 19 nmoles; no ZAS 24 nmoles). Using the stimulus PMN, O_2^- by cord PMN also decreased (ZAS 37; no ZAS 46) but adult PMN responses were unchanged (46 nmoles). HMPS activity by ZAS treated cord or adult PMN significantly increased in response to PMA but not OpZ. Exposure of cord PMN to FMLP had little effect upon O_2^- formation and O_2 consumption in contrast to the increased responses of adult PMN. These results suggest that adult or cord PMN exposed to activated complement fragments may respond less efficiently to particulate stimuli. In contrast, the effects of FMLP to potentiate oxidative responses of adult PMN were not observed with cord PMN.