

OXIDANT-INDUCED INJURY IN PYRUVATE KINASE (PK) DEFICIENT ERYTHROCYTES. Bertil E. Glader (Intr. by Herbert Schwartz), Department of Pediatrics, Harvard Medical School, Children's Hospital Medical Center, Boston, Mass. 02115

Oxidant-induced hemolysis, the hallmark of hexose monophosphate (HMP) shunt abnormalities, generally is not appreciated in PK deficiency. *In vitro*, however, some patients with PK deficiency manifest oxidant sensitivity (increased sulfhemoglobin formation) in the presence of cyanide (CN) and ascorbate (Asc). The HMP shunt (glucose-1-¹⁴C oxidation to ¹⁴CO₂) of these PK deficient RBC's was stimulated by Asc to the same extent as were normal cells. CN enhanced the Asc-induced HMP stimulation in control cells, but inhibited the Asc effect in PK deficient RBC's. ATP was decreased significantly in PK deficient RBC's incubated with CN + Asc. Therapeutic salicylate levels (2 mM) also inhibited the Asc-induced HMP stimulation in PK deficient RBC's, but not in normal cells. These experiments suggest that inhibition of mitochondrial oxidative phosphorylation in PK deficient reticulocytes deprives these cells of adequate ATP for accelerated glucose phosphorylation in the presence of an oxidant stress. Certain drugs (salicylates) or prolonged stasis in a hypoxic splenic environment presumably could inhibit oxidative phosphorylation, impair the ability of PK deficient RBC's to detoxify oxidants produced by bacteria and macrophages during infection, and thereby induce oxidant cell injury. Furthermore, this partially may explain the exacerbation of hemolysis seen in PK deficiency during infection.

METABOLIC REQUIREMENTS OF THE MOTILE FORM OF HUMAN LYMPHOCYTES. Armond S. Goldman, H. Beth Rudloff, Randall M. Goldblum and Michael H. Chamales, Dept. of Pediatrics, University of Texas Medical Branch and Shriners Burns Institute, Galveston, Texas.

Motile lymphocytes are characterized by a hand-mirror configuration and a regionalization of cellular functions. We found when lymphocytes were washed free of plasma, the relative frequency of motile forms rose from a mean \pm SD of 6.6 ± 2.9 percent to 45.5 ± 9.1 percent. By incubating lymphocytes in the absence of plasma, large populations of motile lymphocytes were obtained to study the metabolic requirements for the motile configuration. Motile forms were determined by interference contrast microscopy. Protein synthesis was inhibited by puromycin or cycloheximide, glycolysis by iodoacetamide and mitochondrial respirations by azide. The requirements for microtubules and for microfilaments were ascertained by use of colchicine and cytochalasin B, respectively. Isoproterenol was used as a β -adrenergic stimulant and phenylephrine as an α -adrenergic stimulant.

Protein synthesis, glycolysis, mitochondrial respiration or microfilaments were essential for the motile configuration, whereas colchicine-sensitive microtubules were not. α -adrenergic stimulation depressed the motile form. Thus, it would seem that cyclic 3'5' adenosine monophosphate (CAMP) is required for motility. This hypothesis is being tested by measuring CAMP in lymphocytes in the presence or absence of the plasma inhibitor of motile lymphocytes.

UPTAKE OF SERUM BILIRUBIN-¹⁴C (SB) BY ERYTHROCYTES (RBC) FROM NEWBORNS AND ADULTS. Abraham Gotlieb, Michael N. Applebaum, M. Michael Thaler (Intr. by Louis K. Diamond). University of California, Department of Pediatrics, San Francisco.

Tissue damage in jaundiced newborns is due to excessive intracellular deposits of SB. Radio-labeled SB was used to measure uptake of SB from serum by a readily accessible tissue (RBC). RBC and plasma from 0.5 ml heparinized blood were separated, and RBC resuspended in the original plasma diluted to different bilirubin/albumin ratios (B/A) with buffer and SB. After incubation, RBC's were washed and RBC cpm measured to determine RBC-B. Uptake of SB by RBC was complete in 15 minutes at all B/A (0.4-4.0). At B/A < 1, RBC-B was < 10% SB. At B/A 1.0-1.6, RBC-B increased 5-fold. RBC were saturated at B/A \geq 2.0. Individual uptake patterns characterized each infant. Membrane-bound SB was \sim 20% of RBC-B. Newborn and adult RBC had similar affinities for SB. These results indicate that some SB enters RBC at relatively low B/A, and increases rapidly in a narrow B/A range peculiar to each infant. This direct, rapid and sensitive microassay of RBC-B may be useful in predicting the possibility of damage to newborn tissues from excessive SB. (Supported by The Cerebral Palsy Foundation)

PLASMA MEMBRANE ULTRASTRUCTURE OF HUMAN LEUCOCYTES. Martha F. Greenwood and Phillip Holland, Dept. of Ped., Univ. of Ky. Sch. of Med., Lexington, Ky.

The surface of human blood neutrophils (PMN), monocytes, B and T lymphocytes, lymphoblasts from acute lymphoblastic leukemia (ALL) and macrophages transformed in-vitro from blood monocytes was compared using critical point drying and scanning electron microscopy (SEM). Elongated, veil-like extensions of the plasma membrane cover the entire surface of the monocyte and macrophage and are characteristic of the mononuclear phagocyte cell line. In contrast, numerous short microvilli and serpentine ridges cover the surface of the PMN. Following PMN attachment to glass the central portion of the cell becomes smooth and microrundulations of plasma membrane activity are noted only at the cell periphery. Numerous microvillous projections on the normal human B lymphocyte surface in contrast to the smooth surface and receptors for sheep erythrocytes (SRBC) on the normal blood T lymphocyte surface, as previously reported, were confirmed. SEM offers an additional tool for characterization of each of the normal blood leucocyte cell types. SEM observations of blood lymphoblasts in 3 children with ALL prior to treatment revealed greater than 90% of cells with a smooth surface identical to normal T lymphocytes. However, SRBC rosette formation was present in 6%, 7% and 91% respectively. Correlation of membrane ultrastructure and SRBC receptor sites appears less consistent following malignant lymphoid transformation.

RHEOLOGIC PARAMETERS IN DISEASE STATES OF INFANCY AND CHILDHOOD. Gary P. Gross and William E. Hathaway. University of Colorado Medical Center, Dept. of Pediatrics, Denver, Colo.

Alterations in rheologic parameters may play an important role in many disease states. Hyperviscosity secondary to polycythemia, leukocytosis or abnormal blood proteins may contribute to the pathogenesis of serious symptomatology due to sludging of blood flow. Decreased erythrocyte (RBC) deformability may contribute both to hyperviscosity and to the premature removal of sclerocytic erythrocytes by the spleen.

In a study of 68 patients during 1970-73 the following rheologic abnormalities were found.

- (1) Hyperviscosity due to polycythemia in: neonatal hyperviscosity, cyanotic heart disease and cystic fibrosis.
- (2) Decreased RBC deformability alone in: respiratory distress syndrome, infants of diabetic mothers, immune hemolysis, infantile pyknotocytosis, advanced liver disease, nephrosis, renal failure and intrinsic RBC defects.
- (3) Hyperviscosity of whole blood due to both polycythemia and decreased RBC deformability in: neonatal hyperviscosity syndrome, infants of diabetic mothers and cystic fibrosis.
- (4) Hyperviscosity due to increased leukocytes in: the myeloproliferative syndrome of trisomy 21 and acute leukemia.
- (5) Hyperviscosity due to parenteral protein concentrates in: hemophilia after intensive transfusion.

Investigation into the role of altered rheology in these conditions may increase our knowledge of disease mechanisms and associated laboratory and clinical findings.

HEMOGLOBIN S-J-BANGKOK DISEASE: A NEWLY IDENTIFIED SICKLING DISORDER. Unsal Gunay and George R. Honig. The Abraham Lincoln School of Medicine, University of Illinois Hospital, Sickle Cell Center and Department of Pediatrics, Chicago, Illinois.

Several members of a Negro family from Southern Illinois were found to be heterozygous for Hb J-Bangkok ($\alpha_2\text{A}_8\text{S}_6\text{gly}^+\text{asp}$), a rare hemoglobin abnormality previously reported only in individuals of Thai or Chinese ancestry. In 2 children (ages 3 and 8) Hb J-Bangkok was present in combination with sickle hemoglobin. Both children exhibited mild hypochromic, microcytic anemia, apparently due to iron deficiency. Neither child demonstrated hemolytic disease, enlargement of the liver or spleen, or symptomatic sickle crises. Hb J comprised 54-59% of the total Hb in all family members having this Hb, in common with previous reports of this variant. Hb A₂ and alkali-resistant Hb were present in normal concentrations. The interaction between sickle Hb and the Hb J in the doubly heterozygous subjects was evaluated by measurement of minimum gelling concentrations of deoxygenated mixtures of the two Hbs. Mixtures containing 40% sickle hemoglobin gelled at 32.5-33.7 gm./dl., values similar to those obtained with a 40:60 mixture of Hbs S and A. The pathologic potential of the combination of Hbs S and J-Bangkok therefore appears to be comparable to sickle cell trait.