643

ALTERATIONS IN RED CELL NAD/NADH RATIOS PRODUCED BY VARIATIONS IN GLUCOSE CONCENTRATION. Frank A. Oski.

Department of Pediatrics, SUNY, Upstate Medical

Center, Syracuse, New York.

The polyol or sorbitol pathway has been demonstrated in human erythrocytes and accounts for 2 to 3 percent of glucose metabolism at physiologic glucose concentrations. The flow through this pathway is regulated by the ambient glucose concentration. At high glucose concentrations, the accumulation of sorbitol and fructose is associated with a decreased concentration of pyruvate, increase in the lactate/pyruvate ratio and accumulation of the triose phosphates. These changes are felt to reflect alterations in the NAD/NADH ratio. To directly test this hypothesis red cells from 12 normal adults and 12 newborn infants were incubated at 5 and 50 mM glucose for periods of 3 hours and NAD and NADH concentrations were determined by the method of Sander et al (Anal. Biochem., 71:29, 1976) in conjunction with measurements of lactate and pyruvate. At 0 time NAD/NADH ratios averaged 5.9 in the adults and 6.8 in the newborns. After 3 hours of incubation the NAD/NADH ratios rose to 11.2 and 16.0 respectively at 5 mM glucose but were 24.7 and 47.2 at the 50 mM glucose concentration. Changes in NAD/NADH ratios paralleled changes in the pyruvate to lactate ratios. These observations validate the use of the lactate/pyruvate ratio as an indirect measure of the NAD/NADH ratio and demonstrate the effects of glucose concentration on the redox potential of the cell as mediated via the sorbitol pathway.

644

RED CELL SEQUESTRATION IN PRETERM INFANTS. Charles L. Paxson, Jr., Jay W. Dirksen, Merton A. Quaife. UNMC Omaha, Neb. (spon. by G.C. Rosenquist)

No previous studies have evaluated the response of red cell volume (RCV) to blood transfusion (Tx) in hypotensive preterm infants. We measured RCV in 69 hypotensive infants with respiratory distress syndrome (RDS) using Tc-99m and 51 Cr red cell leveling. All patients received Tx in amounts estimated by initial RCV to be sufficient to restore the volume to normal (55-60 ml/kg). Following Tx, RCVs were repeated in 27 patients; 2-7 serial values were obtained.

Sixty-five infants responded to Tx with a rise in blood pressure (BP) and/or RCV; 4 had no rise in RCV (3 expired). Serial measurements in non-asphyxiated infants demonstrated a rise to expected RCV. However, the rise observed in asphyxiated preterm infants was disproportionate to the volume Tx, and only 37% of the Tx appeared in the circulating RCV, as shown in the table:

	RCV-1	Tx	RCV-2	RCV-2	P
No asphyxia	34.9	28.9	53.8	49.3	
Asphyxia	25.0	29.7	54.7	36.2	0.005

Conclusions: Asphyxiated preterm infants have RCVs lower than those born without asphyxia. Tx to all hypotensive preterm infants produces a rise in BP and/or RCV but infants subjected to asphyxia may sequester a portion of the Tx. We speculate that the hypotension observed in patients with RDS results from red cell sequestration as a result of perinatal asphyxia.

645

INTERFERENCE PHASE MICROSCOPIC ENUMERATION OF PITTED RBC AND SPLENIC HYPOFUNCTION IN SICKLE CELL ANEMIA. Howard A. Pearson, Sue McIntosh, Yolanda Rooks and Dorothy Johnston, Yale U. Sch. of Med., New Haven.

Functional hyposplenism contributes to overwhelming infection in SS disease and may be assessed by Howell-Jolly bodies and 99mTc scans. H.J. bodies are rare (<1/10⁵ RBC), and scans involve radiation. By interference phase microscopy, many RBC of asplenic individuals show surface craters or "pits." 31 children with SS disease, 0-20 years of age, and various controls were studied.

Controls	Norm.	Hem. anem. c spleens	Hem. anem. s spleens	Hgb SC; S thal
% pitted RBC	0.007	0.94	19.0	0.36
SS patients	0-1 yrs.	1-4 yrs.	5-9 yrs.	10-20 yrs.
% pitted RBC	1.2	5.7	8.9	16.6

Preliminary results suggest that >3.0% pitted RBC correlate with negative scans and H.J. bodies. Negative correlation between HbF and % pits and positive correlation between age and % pits were noted. In patients with Hb SS disease, there is a gradual, rather than abrupt, loss of splenic activity over many years. Functional hyposplenism is associated with an intermediate number of pitted RBC compared to anatomic asplenia. This technique is non-invasive, non-isotopic and semi-quantitative. It may help date the onset of risk of severe infection and may be useful in determining a strategy for prophylaxis against severe bacterial infections.

646

FETAL AND NEONATAL CORRELATES OF SICKLE CELL ANEMIA, Howard A. Pearson, James C. Parke, Richard Ehrenkranz and Yolanda Rooks, Depts. of Pediat., Yale U. Sch. of Med. and Charlotte (N.C.) Memorial Hospital.

In the Hgb S homozygote, sickle hemoglobin is present at 12-16 weeks of gestation and accounts for 10-20% of the Hb at birth. In the neonate with sickle cell anemia, about 10% of circulating RBC can be induced to sickle and isolated instances of neonatal morbidity and mortality have been ascribed to sickling. 34 infants with SS disease were diagnosed by cord blood Hb electrophoresis, performed on all black infants at our hospitals. At Yale, stillbirths are also studied. The number of patients exceeded that predicted by gene frequency, making it unlikely that any cases were missed. Neonatal physical measurements and clinical courses were reviewed retrospectively. 32/34 infants were AGA with respect to birthweight, length and head circumference. 5/34 infants had gestational ages <38 weeks and weighed <2500 gm. One postmature and one premature were SGA. 4/34 had 1 min. APGAR's <8, but all were >8 at 5 min. 7/34 developed clinical jaundice. In 2 cases, ABO EBF was proven; 4 others were premature. Phototherapy was used in 4 cases. No regular transfusions or exchange transfusions were performed. No infants developed sepsis. Hospital stay was prolonged only in the prematures. None of these patients had significant morbidity which could be ascribed to sickling, and there were no neonatal deaths. Therefore, these data do not indicate very important consequences of SS disease during gestation or in the neonatal period.

647

EFFECTS OF MASTOID RADIATION ON THE HEARING FUNCTION OF CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA (ALL). Waldir V. Pereira, Gregory M. Thibadoux, John M. Hodges, and Rhames J.A. Aur. St. Jude Children's Research Hospital, Memphis, Tennessee 38101. (Sponsored by Alvin M. Mauer).

Co 60 cranial radiation, 1500 to 2400 rads plus intrathecal methotrexate for childhood ALL became an integral part of our "Total Therapy" program. Radiation is delivered to the mid-plane of the skull in 14 to 15 sections in 18 days. The ports include the temporal bone with all structures of the auditory system. This study was designed to detect any immediate or long-term ionizing effect on the hearing function. Pure tone audiograms were obtained before radiation and at 6 and 12 months. The following frequencies were tested: 500, 1000, 2000, 4000, 6000 and 8000 Hz. For younger children unable to respond to pure tone testing, stapedial reflexes were obtained for 500, 1000, 2000 and 4000 Hz. Testing was done using a Beltone 200 C audiometer and an American Electromedics Impedance Bridge Model 81 and test scores were obtained for ears free of outer or middle ear disease. One hundred children registered into the current "Total Therapy" from January '76 to July '77 were studied. Audiograms were obtained for 180 ears pre-treatment, 132 ears at 6 and 102 ears at 12 months post-radiation. A sign test for statistical significance was run to compare combined test scores for each frequency at 6 and 12 months after radiation to combined test scores for each frequency before radiation. No statistically significant reduction in hearing levels was noted for any test frequency at either 6 or 12 months post-radiation. Individual audiograms indicated essentially normal hearing levels for all patients at 6 and 12 months.

In summary, at 1 year post-radiation, 1500 to 2400 rads of cranial radiation resulted in no statistically significant reduction in hearing levels.

648

TRANSFUSION OF YOUNG RBCs WITH IMPROVED SURVIVAL: A NEW APPROACH TO THE THERAPY OF COOLEY'S ANEMIA. S. Piomelli, C. Seaman, L. Corash, A. Tytun, J.H. Grazi-ano, NYU Med Ctr & Cornell Med Ctr, NYC; NIH, Bethesda, Md.

In Cooley's anemia, maintenance of a mean Hgb level of 12g improves quality of life and prevents cardiac dysfunction. The resulting Fe overload (FO), which ultimately leads to death, could be reduced by transfusion of RBCs with improved survival. Age-dependent separation of RBCs can be obtained by buoyant density centrifugation on isotonic solutions of arabinogalactane (AG). Rabbit RBCs were divided into 2 equal parts: light-young (LY) and heavy-old (HO) RBCs, by centrifugation through a single layer of AG. LY-RBCs, HO-RBCs, mixtures of the two and unfractionated RBCs were washed, labelled with 51Cr and reinfused into the donors. RBCs survival was corrected for 51Cr elution and random cell loss by an appropriate mathematical model. Mean survival (MS) of LY-RBCs was 28d; for HO-RBCs, 14d; for both and unfractionated RBCs, 19d, indicating excellent age-dependent separation. (AG toxicity was negligible: LD50 in mice > 10g; AG antigenicity was undetectable in guinea pigs.) These results indicate that human LY-RBCs with MS of 88d (vs 60d in unfractionated RBCs) can be prepared. A mean Hgb of 12g can be maintained by transfusion of LY-RBCs, with less fluctuation and with only 2/3 of the Fe. This avoids transfusion of HO-RBCs that contribute as much to FO as LY-RBCs, yet offer the patient short and inefficient oxygen transport. Combination of improved transfusion modality with modern chelation therapy may lead to Fe balance in Cooley's anemia, which ultimately may permit prolonged survival.