SPLenic "POLISHING": REMOVAL OF HIGH MOLECULAR WEIGHT RED CELL (RBC) MEMBRANE PROTEIN DETRITUS BY THE SPLEEN. Samuel E. Lux and Kathryn M. John (Intr. by David G. Nathan). Children's Hospital Medical Center, Dept. of Medicine, Boston, Mass.

To determine whether splenic "polishing" and "pitting" of RBC includes removal of agglomerated membrane proteins as well as membrane lipids and intracellular debris we examined the RBC membrane proteins of normal and splenectomized patients.

RBC ghosts were dissolved in sodium dodecyl sulfate (SDS) and chromatographed on Sepharose 2B (exclusion limit 40×10^6). Membranes from splenectomized patients contained an excluded macromolecular species not present in "normosplenic" individuals. This colorless material formed insoluble fibrils on removal of SDS and was proteinaceous as judged from amino acid analysis and ultraviolet spectroscopy. It comprised $4.2 \pm 1.3\%$ (range 2.4-6.1%) of the total membrane protein. Equal proportions were present in membranes from older (more dense) and younger (less dense) cells. When freeze-thawed ghosts were banded on a sucrose density gradient, this material remained with the membrane fraction, indicating it was not a particulate cytoplasmic contaminant. Reduction partially disaggregated the complex and revealed spectrin (a high molecular weight RBC membrane protein thought to be involved in red cell shape maintenance), and at least one other unidentified protein component on SDS-gel electrophoresis.

These studies suggest the spleen normally "polishes" or "pits" from RBC membranes an aggregated complex of RBC membrane proteins. Thus RBC membrane proteins join lipids and intracellular particles as targets for splenic cleansing.

NEUROTOXICITY IN LEUKEMIC CHILDREN RECEIVING PROPHYLACTIC CRANIAL IRRADIATION AND MAINTENANCE HIGH DOSE METHOTREXATE. S. McIntosh, G.T. Aspnas, R.T. O'Brien, E. Klatzkin, C. Snead and S. Kalavsky, Dept. of Ped., Yale Univ. Sch. of Med., New Haven (Intro. by H.A. Pearson).

Non-leukemic neurologic abnormalities may be produced or enhanced in children with lymphoblastic leukemia receiving prophylactic CNS therapy and high-dose maintenance methotrexate (MTX). 22 children given cranial irradiation and intrathecal MTX after remission induction were maintained for 1-3 years with biweekly intravenous MTX, 125-450 mg./m²; cyclophosphamide, 200 mg./m², and cytosine arabinoside, 100 mg./m². Eight children (36%) suffered transient encephalopathy, previously described by Aur, et al., during the second month after irradiation. Other neurologic abnormalities were observed 6-24 months after CNS prophylaxis, during which time dosage of MTX was increased to tolerance. Four children developed a seizure disorder and perceptual-motor deficits; two improved clinically after discontinuation of parenteral MTX. McCarthy tests administered to 18 patients revealed gross and fine motor or perceptual-motor deficits in 9 (50%). Insidious limping of uncertain etiology was persistent in 8 children (36%). CSF folate levels fell to less than 10 ng./ml. in 13 (59%), and transient, mild EEG changes occurred in 8 asymptomatic patients. The combination of high-dose parenteral MTX following CNS prophylaxis may be causally related to subsequent non-leukemic neurologic disturbances.

EFFECT OF Na BENZOATE ON SERUM BILIRUBIN IN THE GUNN RAT Gerald Nathenson, Michael I. Cohen, and Helen McNamara, Albert Einstein Col. of Med., Montefiore Hosp. & Med. Ctr., Dept. of Pediatrics, Bronx, New York

That Na benzoate will displace bilirubin from albumin has been demonstrated in vitro; however, bilirubin-albumin kinetics may not be altered in vivo by Na benzoate because of its rapid conversion to Na hippurate. Of particular concern is whether the available injectable diazepam, which contains Na benzoate in its liquid vehicle, is safe to administer to newborns for such conditions as seizures or narcotics withdrawal. The Gunn rat was used as an experimental model for the displacement of bilirubin from albumin by serially measuring the fall in serum bilirubin following I.P. or I.M. administration of Na benzoate. Control animals were injected with saline. 26 animals were given 7 or 35mg/kg of Na benzoate (equivalent to 2 or 10mg diazepam in a 3kg infant) as a single injection and 7 rats were given the same doses repeated 2 times at 1 hour intervals. Serum bilirubin was determined at 10, 20 and 60 minutes after injection. No differences were found in mean bilirubin levels between control and treated rats. With repeated benzoate injections of 35mg/kg or a single dose of 100mg/kg some depression of serum bilirubin occurred; but only at 200mg/kg did bilirubin levels fall to the extent observed in the Gunn rat with similar doses of sulfasoxazole. These data suggest that recommended doses of diazepam for neonates do not contain sufficient Na benzoate to alter bilirubin binding capacities in vivo and thus are safe.

COMPLETE RESPONSE OF INOPERABLE HEPATOBLASTOMA TO ADRIAMYCIN. Nancy B. McWilliams, Yao-Shi Fu, and Harold M. Maurer. (Intr. by William E. Laupus). Medical College of Virginia, Depts. of Pediatrics and Pathology, Richmond, Virginia.

Total surgical excision of hepatoblastoma, an uncommon malignancy of early childhood has been the only favorable therapy for this disease. The tumor is poorly responsive to both radiation and standard chemotherapy.

The case described is that of an 8 month old white male who presented with a huge abdominal mass. Laparotomy disclosed replacement of the entire liver with nodular tumor masses. Biopsy revealed mixed hepatoblastoma consisting of embryonal malignant epithelial cells and osteoid formation. No distant metastases were evident. Alpha-fetoprotein was negative.

1500 r Co⁶⁰ was delivered to the liver over 25 days without change in tumor size. This was followed after 2 weeks rest by 5 courses of Adriamycin 30 mg/m² in 3 daily doses at 3 week intervals or at marrow recovery. Serial physical examinations showed marked reduction in tumor size following chemotherapy. Radiologically, surgical clips migrated laterally and right upper quadrant calcification appeared. Five months after chemotherapy a second laparotomy was performed. The liver appeared normal and biopsies revealed no tumor. Adriamycin has been shown to have significant activity against a variety of tumors including osteogenic sarcoma. It is possible that the osteoid elements in this tumor were also sensitive as demonstrated by the calcifications seen radiographically. Further Adriamycin trials are warranted in this tumor.

INFLUENCE OF GESTATIONAL AGE(GA) AND BIRTH WEIGHT(BW) ON THE WHITE BLOOD CELL COUNT(WBC) IN THE NEONATE. Guillermo Martinez, Charles R. Bauer, Vichien Lorch and Thomas Noto (Intr. by W. W. Cleveland) Depts. of Ped. and Path., Univ. of Miami, Miami, Fl.

The clinical usage of the WBC in the neonate has been limited over the years because of their wide variation. This report demonstrates a possible correlation between GA, BW, chronologic age and WBC. The 38 healthy newborns with differing GAs and BWs were studied on the 1st and 3rd day of life. A direct relationship was found between the difference in the WBC on the 1st and 3rd day of life ($p < .001$). The WBC were shown to be lower in infants with lowest BWs and this relationship was statistically significant ($p < .001$). Within the full term group, those infants who were small for gestational age (TSGA), also demonstrated a statistically significant decrease in WBC compared to infants who were appropriate for gestational age (TAGA) ($p < .05$). The results are:

# of Infants	GA (weeks)	BIRTH WEIGHT Mean $\pm$ SD	WBC (DAY 1) Mean (range)	WBC (DAY 3) Mean (range)
25	37-40	3317 $\pm$ 249	21,258 (10,900-39,400)	11,208 (6,650-20,000)
7	38-40	2363 $\pm$ 106	15,935 (9,400-20,600)	10,307 (6,000-13,000)
6	33-36	2168 $\pm$ 392	16,066 (10,900-20,900)	9,725 (4,150-12,300)

The premature and SGA infants have significantly lowered WBC counts and this must be considered if this test is to be a valuable indication of clinical conditions in the newborn.

PLATELET INJURY BY LIGHT. Harold M. Maurer, Joyce C. Haggins, W.J.S. Still, Depts. of Ped. and Path., Medical College of Virginia, Richmond, Virginia.

Although phototherapy with blue or cool white fluorescent light is widely employed for neonatal hyperbilirubinemia, its effect on substances other than bilirubin is largely unknown. The function of human platelets (platelet rich plasma) exposed in vitro for up to 170 minutes to blue or cool white fluorescent light (8 20 wt lamps) was studied. Within 110 minutes, blue light ($1,908 \text{ uwt/cm}^2$) inhibited platelet aggregation by ADP and connective tissue suspension, and resulted in platelet loss of ADP, ATP, and glycogen. Electron micrographs revealed these platelets to be less dense than normal, depleted of glycogen granules and organelles, and to have ill-defined membranes. Platelet injury could be enhanced by adding a photosensitizing agent, hematoporphyrin, to platelet samples before exposure. In contrast to blue light, cool white light ($2,670 \text{ uwt/cm}^2$) had no effect on platelet aggregation unless platelet samples contained hematoporphyrin. Platelets kept in the dark for 170 minutes maintained their integrity.

These results indicate that platelets are damaged in vitro when exposed to amounts of blue light used in phototherapy. Results suggest that cool white light may be safer than blue for newborns.