

A COMPARISON OF THE MEASUREMENT OF FREE ERYTHROCYTE PORPHYRIN (FEP) AND RED CELL AMINOLEVULINIC ACID DEHYDRASE ACTIVITY (ALAD) AS MEANS OF DETERMINING EXCESSIVE LEAD ABSORPTION. Frank A. Oski, Michael Weitzman, Barbara F. Oski and Theima R. Schneider. State University of New York, Upstate Medical Center, Syracuse, New York.

In 700 subjects, simultaneous measurements of FEP, ALAD and blood lead were performed. In 92 subjects, EDTA chelation tests were carried out in an attempt to assess the sensitivity of the two screening procedures and to determine how they reflected the chelatable body lead burden. Both the FEP and ALAD were abnormal in all subjects with lead values above 50  $\mu\text{g}\%$ . The FEP was abnormal in 86.4% of subjects with lead values between 41-50  $\mu\text{g}\%$ ; the ALAD in 81.3%. Of the subjects surveyed, 9.3% had abnormal ALAD while 32.4% had abnormal FEP, reflecting the fact that FEP is also elevated in iron deficiency. The mean urinary lead excretion with FEP values of less than 100 was 0.25  $\mu\text{g}\text{m}/\text{mg}$  EDTA administered and rose progressively with increasing FEP; subjects with FEP values above 300 excreted 0.83  $\mu\text{g}\text{m}/\text{mg}$  EDTA. The ALAD also reflected the body lead burden. With ALAD values above 25 units, lead excretion averaged 0.16  $\mu\text{g}\text{m}/\text{mg}$  EDTA while with ALAD values of less than 15 units the urinary excretion was 0.65  $\mu\text{g}\text{m}/\text{mg}$  EDTA. Both the FEP and ALAD are sensitive tests of the body lead burden and correlate with the EDTA mobilization test. The simplicity of the FEP makes it more useful despite its positivity with iron deficiency.

THE EFFECT OF PLASMA WITH COLONY STIMULATING ACTIVITY (CSA) IN CYCLIC NEUTROPENIA (CN). Lauren M. Pachman, Allen D. Schwartz, Roger Barron, Northwestern Univ. Med. Sch., Children's Mem. Hosp., Dept. Peds, Chicago and David W. Golde, UCLA Sch. Med., Dept. Med., Los Angeles.

A 2 year old M and a 10 year old F with 21-day cycles of neutropenia and aphthous stomatitis were studied. Bone marrow aspirates at the cycles' nadir showed a maturation arrest at the myelocyte stage. *In vitro* bone marrow culture in agar documented quantitatively normal colony formation with rare colonies of neutrophils (PMN). Serial liquid suspension cultures (Marbrook chamber) yielded cell counts which were in the normal range or higher than companion control cultures. CN PMN maturation was defective early in culture; some mature PMNs appeared after 7 days and persisted for up to 25 days. Plasma, obtained from normal donors and from those given typhoid vaccine and plasmaphoresed 1 hour later, was assayed for CSA, using normal and CN bone marrow. Only plasma which stimulated normal marrow promoted PMN maturation in CN marrow *in vitro*. *In vivo*, administration of plasma containing CSA increased PMN production and ameliorated the clinical symptoms of neutropenia. It is concluded that one type of CN involves a maturation defect of PMNs, which may be altered *in vitro* and *in vivo* by plasma containing CSA. Supported by Grant 5-M01-RR00199.

RADIOIMMUNOASSAY OF HUMAN CHORIONIC GONADOTROPIN (HCG) AND ALPHA FETOPROTEIN ( $\alpha\text{FP}$ ) IN THE MANAGEMENT OF HEPATOBLASTOMA. John S. Parks, K. Robert McIntire, Milton H. Donaldson, Louise Schnauffer and Alfred M. Bongiovanni. Univ. of Pa. Dept. of Pediat. Children's Hosp. of Phila. and Nat. Inst. of Health, Bethesda, Md.

Sensitive and specific radioimmunoassays for HCG and  $\alpha\text{FP}$  were used to monitor treatment of 3 boys with hepatoblastoma. In the first, chemotherapy and radiation produced an initial rise in HCG from 162 to 12,000 mIU/ml, followed by a decline to 70 mIU/ml. Testosterone values reflected the changes in HCG, but  $\alpha\text{FP}$  remained stable at 515,000 ng/ml. After surgery HCG fell to  $<2$  mIU/ml and  $\alpha\text{FP}$  was normal ( $<20$  ng/ml.) Tumor markers have not recurred in 2 yrs. A second boy had no virilization, no HCG and  $\alpha\text{FP}$  between 33 and 145 ng/ml during unsuccessful chemotherapy. The third patient had mild virilization, HCG of 15 mIU/ml and  $\alpha\text{FP}$  of 1,818,000 ng/ml at diagnosis. HCG disappeared post surgery, but  $\alpha\text{FP}$  has persisted at 20,000 ng/ml and metastases have appeared during 1 year of chemotherapy. These findings demonstrate a wide divergence between HCG and  $\alpha\text{FP}$  levels at diagnosis and during treatment. Transient increases during chemotherapy may indicate release of marker from damaged tumor cells and not treatment failure. Prompt decline and prolonged absence of both markers following surgery suggests a good prognosis.

SERUM LUTEINIZING HORMONE (LH) AND FOLLICLE STIMULATING HORMONE (FSH) CONCENTRATIONS IN SUBJECTS WITH SICKLE CELL ANEMIA (SCA). Robert Penny, N. Olatunji Olambiwonnu and S. Douglas Frasier. Univ. So. Calif. Sch. Med. and the Los Angeles County-USC Med. Ctr., Dept. Pediatrics, Los Angeles.

LH and FSH concentrations were determined in sera obtained from 40 subjects (20 males and 20 females; ages 5-16 years) with SCA (homozygous hemoglobin S). Mean LH concentrations were significantly increased above those of comparable age normal subjects in 5-8 year old males ( $5.3 \pm 0.6$  mIU/ml vs.  $3.4 \pm 0.6$  mIU/ml), 5-8 year old females ( $6.7 \pm 1.4$  vs.  $2.6 \pm 0.3$ ) and 9-10 year old females ( $6.8 \pm 1.9$  vs.  $4.0 \pm 1.3$ ). The mean FSH concentration was also significantly increased in 13-14 year old females ( $12.8 \pm 1.5$  mIU/ml vs.  $8.0 \pm 2.9$  mIU/ml). The LH concentration in 10 subjects (4 males and 4 females ages 5-8 and 2 females ages 9-10) and the FSH concentration in 7 subjects (all females ages 11-16) were  $> 2$  SD above the mean of normal subjects. The observed increase in LH concentration during the first decade of life is consistent with impaired gonadal function and correlates well with the period of greatest morbidity in SCA. While the observed increase in FSH concentration may represent racial variation in females (Clin. Res. 21:866, 1973), it is also consistent with impaired gonadal function. Transiently impaired gonadal function may, in part, account for the variations in sexual maturation seen in patients with SCA.

THE BINDING OF BILIRUBIN TO AGAR. Ronald L. Poland and Gerard B. Odell Wayne State Univ. and The Johns Hopkins Univ.

In animal experiments, variation was found between agar lots in their effect on fecal excretion of bile pigments and on lowering of serum bilirubin levels in the Gunn strain of rat. The binding of bilirubin to agar was studied *in vitro* to account for the variation. Suspensions of agar powder in bilirubin solutions (isotonic, 37°, pH 7.9) were employed to derive adsorption isotherms. From these, the association constants ( $K_a$ ) for the binding reaction was about  $2 \times 10^5$  for all products. The capacity for binding (n) varied between 25 and 56 moles of bilirubin/50,000 g. of agar. The variation in (n) was related to the calcium and sulfate content of the agar products. The calcium and sulfate content of the agar lots each varied from less than 1 to 11 moles/50,000 g. of agar. Calcium-depleted agar lost its bilirubin binding capacity and regained it upon readdition of calcium. Salicylate, sulfonamide, BSP and HABA dyes, and conjugated bilirubin were not bound by agar. Cellulose, phosphocellulose and DEAE-cellulose did not bind bilirubin as well as agar. Binding of bilirubin to agar decreased as pH, ionic strength, or urea concentration were independently increased. There was an increase in apparent  $K_a$  with a rise in temperature. Free energy change was about 2 kcal/mole. A model is proposed in which strands of carbohydrate are cross-linked with ionic calcium disulfate bridges to form relatively apolar interstices between chains and between bridges in which bilirubin is bound possibly by hydrophobic interaction or by hydrogen bonds or both.

THE REGULATION OF GRANULOPOIESIS IN CHILDHOOD NEUTROPENIC DISORDERS AND ACUTE LEUKEMIA. Abdel H. Ragab, Harold S. Zarkowsky, James P. Keating and Anthony Pagliara (Intr. by Philip R. Dodge). Washington University, St. Louis Children's Hospital, Department of Pediatrics, St. Louis, Mo.

The agar culture technic for the growth of human granulocytic colony forming cells (or CFC) from the bone marrow of patients may be used to study factors involved in the regulation of granulopoiesis. Bone marrow cells from 10 children with neutropenia were cultured in agar; the peripheral blood cells of these patients were also tested for their production of colony stimulating activity (CSA). It was observed that patients with aplastic anemia had decreased bone marrow CFC and decreased production of CSA. Other patients with neutropenia had normal CFC and increased CSA. This included 2 patients with "maturation arrest" neutropenia associated with type I(b) glycogen storage disease - a previously undescribed association. Leukemic children in relapse have decreased bone marrow CFC and peripheral blood CSA. However, when peripheral blood cells from certain neutropenic patients were used as feeder layers for bone marrow cells from leukemic children in relapse, a marked increase (up to fifty fold) in the number of granulocytic CFC was observed. It may be concluded that the defect in neutropenic conditions may be due to a decrease in CFC, or a decrease in CSA or "defective maturation". The defect in granulopoiesis in the acute leukemia patients that we have studied was due to decreased CSA production which was corrected by factors in the peripheral blood cells of certain neutropenic patients.