643 THE PERIODIC ACID-SCHIFF (PAS) REACTION AND PROGNOSIS IN CHILDHOOD ACUTE LYMPHOCYTIC LEUKEMIA (ALL). (Spon. by John Parks). Beverly Raney, Robert Festa, and David Manson. Univ. of Pa. School of Medicine, Children's Hosp. of Phila. Dept. of Pediatrics, Philadelphia, PA

The degree of PAS positivity was determined by counting 200 blast cells in the initial bone marrow specimen of 38 children with ALL. Each cell was scored on a 0 to 5 scale according to the number of PAS-positive granules per cell (0-4), or the presence of 1 or more large blocks per cell (5). The patients were stratified into 3 groups according to their mean PAS score. Fourteen children had a low score (mean 65, range 3-150), 12 were intermediate (mean 315, range 153-445), and 12 were high (mean 692, range 465-860). The mean white blood count (WBC) at diagnosis was higher in the low-score group compared to the high-score group, but the difference was not significant ($p>0.05$); 5/14 low-score patients had an initial WBC below 20,000/mm^3, and 3/12 high-score patients were above 20,000/mm^3. There were no significant differences among the groups with respect to age, hemoglobin and platelet levels at diagnosis or presence of a mediastinal or abdominal mass. However, 13 of 14 low-score patients were male, compared to 4/12 and 6/12 in the intermediate and high groups ($p<0.05$). In addition, 7 of 13 low-score patients were in relapse by 6 months from diagnosis (3/7 had an initial WBC below 20,000/mm^3), compared to none of the intermediate and only 1 of the high patients ($p<0.05$). These results suggest that a low PAS score is related to a poor prognosis in childhood ALL, regardless of the level of the initial WBC. Females tend to have higher PAS scores than males, and also seem to have a better prognosis.

644 Childhood Lymphoblastic Malignancy: Subgroups Defined by Nonhuman Primate Antisera. R.B. Raney, T. Mohanakumar, R.S. Metzgar, H. Hann, and M.H. Donaldson. Children's Hospital, Philadelphia, PA, Duke University Medical Center, Durham, NC, and Fox Chase Cancer Center, Philadelphia, PA.

Lymphoblasts from 36 children with acute lymphocytic leukemia (ALL) and 12 with lymphoblastic lymphoma (LBL) were studied by complement-dependent microcytotoxicity with 2 nonhuman primate antisera defining leukemia- and lymphoma-associated antigens. Twenty-seven patients with ALL and one with LBL had cells reacting only with anti-chronic lymphatic leukemia (CLL) antiserum (Group I). These children were usually females with pancytopenia and no localized mass; their cells were usually periodic acid-Schiff (PAS) positive and E-rosette (ER) negative. Cells from 8 boys with LBL and 1 with ALL reacted only with anti-lymphosarcoma (LS) antiserum (Group II). All but one had a mediastinal (6) or abdominal (2) mass; their hemoglobin and platelet levels at diagnosis were higher ($p < 0.05$) than those of Group I patients, and their cells were usually PAS negative and ER positive. Eleven patients had cells reacting with both antisera (Group III), and showed features of both Group I and II patients.

These antisera appear able to distinguish two forms of childhood lymphoblastic malignancy with differing prognosis. Patients whose lymphoblasts reacted with CLL antiserum had PAS-positive ALL and no mass or ER positivity. Those whose lymphoblasts reacted with LS antiserum had bad prognostic features: localized mass, ER positivity, and PAS negativity.

645 THE USEFULNESS OF BONE SCANS IN DIFFERENTIATING OSTEOMYELITIS FROM THROMBOTIC CRISIS IN CHILDREN WITH SICKLE CELL DISEASE. S.P. Rao, Anthony Tavormina, A.N. Rao, Nathan A.Solomon, Audrey K. Brown. SUNY-Downstate Medical Center, Department of Pediatrics and Nuclear Medicine,Brooklyn, New York.

While the usefulness of a bone scan showing increased uptake of 99m Technitium diphosphonate in the diagnosis of osteomyelitis is well documented, its efficacy in differentiating thrombotic crisis from osteomyelitis in Sickle Cell Disease is not known. In an effort to differentiate these two clinical entities, we have performed bone scans in 15 children with Sickle Cell Disease who presented with bone pain. Decreased or normal isotope uptake was found in 8 patients; all of them had negative cultures and had thrombotic crises; none had osteomyelitis. Increased isotope uptake was found in 7 of the children; two of these proved to have osteomyelitis. These findings suggest that even in Sickle Cell Disease, a normal (or decreased) uptake of isotope during a bone scan is a useful diagnostic test indicating the absence of osteomyelitis.

646 PRIMARY EWING'S SARCOMA: 75% DISEASE-FREE 5 YEAR SURVIVAL WITH T-2 CHEMOTHERAPY -- CONSIDERATIONS FOR FUTURE THERAPEUTIC TRIALS. Gerald Rosen, Manuel Gutierrez, Chrisanta Mosende, Beryl Chabora and Ralph Marcove. Depts. of Pediatrics, Radiation Therapy and Surgery, Memorial Sloan-Kettering Cancer Center, New York, N.Y.

In 1973, we reported initial results of 4-drug chemotherapy, namely dactinomycin, adriamycin, cyclophosphamide and vincristine (T-2) in children with Ewing's Sarcoma(ES). Twenty previously untreated children with primary ES and 8 with metastatic disease were treated with surgery or radiation therapy(RT)(6000-7000 rads) and T-2 chemotherapy. Of the 20 children with primary ES, 15 have no evidence of disease for from 25+-76+ months(median 41+ months)from the start of treatment. The actuarial disease-free 5 year survival rate for this group of patients is 75%. Of the 8 patients presenting with metastatic disease, all had a complete response to T-2, but 7/8 had tumor recurrence. Examination of the T-2 treatment failures revealed that all relapses occurred at the end of the second year of T-2 or after therapy was stopped. In addition, of 23 patients receiving "curative" RT to their primary, 5 had local recurrences(22%) and 6(26%) had severe functional debility secondary to combined RT and T-2 chemotherapy. The conclusions drawn from this experience have led us to consider a new approach to the treatment of ES, including more aggressive initial chemotherapy and the use of surgery alone or in combination with moderate doses of RT in those patients in whom we can predict a high frequency of local recurrence or of "functional failures". Supported by NCI Grant#CA-08748.

647 E-ROSETTE POSITIVE EOSINOPHILS AND BASOPHILS IN A LYMPHOMYELOPROLIFERATIVE DISORDER. Ronald C. Rosen, John J. Hutter, Jr. and James J. Corrigan, Jr. Department of Pediatrics, University of Arizona Health Sciences Center, Tucson, Arizona.

Chronic myelogenous leukemia (CML) is a clonal disease of a pluripotent stem cell compartment, and lymphoblastic transformation in CML has been noted. Conversely, malignant diseases that were primarily lymphoid at onset have been documented to evolve into myeloproliferative disorders with and without the presence of a Philadelphia chromosome. We have observed a 3 ½ year old girl who presented with a diffuse lymphoblastic non-Hodgkin's lymphoma involving liver, spleen, bone marrow, and an inguinal lymph node. Therapy with vincristine, prednisone, and cyclophosphamide resulted in complete remission for over 18 months. Subsequently she developed features of a myeloproliferative disorder characterized by basophilic meningitis, splenomegaly, and hypereosinophilia (WBC=142,000 with 63% eosinophils) plus hyperbasophilia, markedly elevated serum B_{12} and absent neutrophil alkaline phosphatase. Peripheral blood eosinophils and basophils were noted to form spontaneous rosettes with sheep RBC (E rosettes), a feature not present in normal eosinophils. The initial presentation of this patient as a lymphoma along with the presence of T-lymphocyte surface markers on eosinophils as the myeloproliferative phase of this disease evolved is further evidence for a lymphoid differentiation in certain myeloproliferative diseases such as CML.

648 SYNTHESIS OF METHOTREXATE POLYGLUTAMATES IN CULTURED HUMAN CELLS. D.S. Rosenblatt, V.M. Whitehead, M.M. Dupont, M-J.Vuchich and N. Vera (Spon. by C.R. Scriver). MRC Genetics Group, McGill Univ.-Montreal Children's Hosp., Res. Inst. Montreal General Hospital, and Depts of Pediatrics and Medicine, McGill Univ. Montreal, Quebec, Canada.

The pteroylglutamate analog methotrexate (MTX) is a potent inhibitor of the enzyme dihydrofolate reductase and an important antineoplastic agent. Recently synthesis of poly-γ-glutamyl metabolites of MTX has been reported in both mice and rats. Cultured human fibroblasts were incubated with purified tritium-labeled MTX and Sephadex G-15 gel chromatography was used to study the formation of these MTX polyglutamates. Synthesis of polyglutamates occurred sequentially and was dependent on the concentration of MTX in the culture medium, duration of incubation, and stage of the culture cycle. After incubation with 0.1 μM MTX the cells accumulated methotrexate monoglutamate [MTX(G_1)] and diglutamate [MTX(G_2)] so that by 24 hours they represented the majority of labeled MTX in the fibroblasts. After 4 hours MTX level remained relatively stable and further increase in labeling was due to accumulation of polyglutamates. When tritiated MTX was removed from the culture medium at 24 hours, no change in the proportion of MTX and MTX polyglutamates occurred over the next 24 hours. However when triated MTX was replaced after 24 hours with equimolar cold MTX, rapid loss of labeled MTX occurred with sequential formation and loss of methotrexate polyglutamates. These results suggest that like the folate polyglutamates, MTX polyglutamates may represent major active compounds.