649 THE RELATIONSHIP OF ASCORBIC ACID (C) AND DEFEROXAMINE (DF) TO EDTA-CHELATABLE IRON ("free Fe") IN THALASSEMIA MAJOR (Thal). Richard D. Propper and Susan K. Marino (Spon. David G. Nathan). Division of Hematology and Oncology of the Children's Hospital Medical Center and Sidney Farber Cancer Institute, Department of Pediatrics, Harvard Medical School, Boston, MA.

Patients with Fe overload often demonstrate saturated Fe binding capacities and low white cell C levels when measured by standard techniques. It has been shown that serum Fe in excess of the Fe binding capacity ("free Fe") can be measured in patients with Fe overload by EDTA binding and ultrafiltration. We found "free Fe" of 8.2±2.0 µg% in 15 previously unchelated patients with thal, compared to 0.1±0.1 µg% in normal controls. Since "free Fe" might be a particularly potent oxidant and therefore responsible for some of the pathology in thal, and since C is thought to increase the rate of transfer of Fe from one pool to another, we examined 5 of these patients to determine the effect of C and DF on "free Fe". All 5 thals had low initial white cell C levels. Each received alternating 12-hour continuous I.V. regimens of C alone, saline, and C + DF with samples for "free Fe" drawn every 4 hours. Results showed that "free Fe" was 9.8±2.3 µg% during saline infusions and that the level was unchanged when C was added. When DF was present, "free Fe" was eliminated. These results show that thals with Fe overload have measurable amounts of "free Fe" which are not affected by C. Continuous chelation with DF is effective in eliminating this potentially toxic pool.

650 TOXICITY IN THE INTERGROUP RHABDOMYOSARCOMA STUDY(IRS): A PRELIMINARY REPORT, Abdelsalam H. Ragab, Milton Donaldson, Thomas E. Moon, William Newton, Jr., Frederick Ruymann and Melvin Tefft for the IRS Committee of CALGB, CCSG and SWOG, Emory University School of Medicine, Atlanta. To date a total of 640 patients have been registered in the Intergroup Rhabdomyosarcoma Study. Twelve early deaths have been reported. All occurred within 34 days of the start of therapy. Five had pharyngeal primaries and 2 had primaries in the maxillary sinus - all seven had Group III disease (gross residual disease). Most fatalities were associated with granulocytopenia and thrombocytopenia. Four fatalities were associated with membranous colitis at autopsy.

Forty-seven of 417 evaluable patients (11%) developed hematuria. The hematuria was moderate or severe in 80% of cases. It occurred with equal frequency throughout treatment and occurred for the first time in some patients after therapy was stopped. Severe neutropenia ($<500/mm^3$) was observed in 67% of patients in Regimen E and 72% of patients in Regimen F (Clinical Groups III and IV). Adriamycin cardiomyopathy has not been observed in this study.

Since most early deaths occurred in patients with head and neck primaries, the use of less intensive initial chemotherapeutic regimens may be advisable for these patients.

651 THE EFFECT OF SERUM AND BLAST CELLS FROM CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA ON IN VITRO GRANULOPOIESIS, Abdelsalam H. Ragab, Victor K. Lui, Harry Findley and M. Susan Kennedy, Emory University School of Medicine, Department of Pediatrics, Atlanta.

The purpose of this study was to investigate the cause of granulocytopenia in children with acute lymphocytic leukemia (ALL). The double layer agar technique for myeloid colony formation was utilized in all studies. Various concentrations of sera from 16 children with ALL at the time of diagnosis were added to bone marrow cells in culture. Only 2 sera were observed to produce inhibition of colony formation. Various concentrations of initial bone marrow cells from children with ALL were mixed with normal bone marrow cells and cultured. No inhibition of colony formation was observed in agar. To circumvent any effect of HL-A antigens, initial bone marrow cells from children with ALL were frozen in liquid nitrogen. After a remission bone marrow was obtained, the initial and remission bone marrow cells were mixed and cultured together. No inhibition of colony formation was observed.

It may be concluded that the neutropenia observed in children with ALL at diagnosis is not due to serum factors or inhibition by leukemic cells.

652 ADVANCED CHILDHOOD RHABDOMYOSARCOMA: TOXICITY AND RESPONSE TO INTENSIVE CHEMOTHERAPY. R. Beverly Raney, Milton H. Donaldson, Harold M. Maurer, William A. Newton, Abdelsalam H. Ragab, Frederick B. Ruymann, Wataru W. Sutow, for the Intergroup Rhabdomyosarcoma Study (IRS) Committee of the CALGB, CCSG, and SWOG. Children's Hospital of Philadelphia, Philadelphia, Pa. 19104

Twenty-six children with gross residual (18) or metastatic (8) rhabdomyosarcoma were treated with pulse-VAC (Vincristine weekly x 11 plus actinomycin D and cyclophosphamide simultaneously for 5 days) and radiotherapy. Toxicity during the 12-week induction period included 22/26 patients (85%) with an absolute neutrophil count (ANC) under $500/mm^3$; 15/26 (58%) received intravenous antibiotics. Three developed Gram-negative sepsis and 2 of them died. In 25 patients followed 12 weeks, there were 4 complete responses (CR) and 5 partial responses (PR), a total of 36%. At 12 weeks 6 patients randomly received intermittent pulse-VAC (Regimen H) and 9 received pulse-VAC alternating with Adriamycin (Reg. I). After this pulse, 7/15 (47%) had a low ANC; none had severe infection and only 3 received antibiotics. Severe toxicity disappeared with subsequent dose reduction. To date, CR rate averages 48%, 36% on Reg. H and 58% on Reg. I; the overall partial response rate is 26%. However 5/7 CR patients on Reg. I had an orbital primary. Despite increased toxicity, these regimens are no better than current IRS schedules for advanced disease. Introducing adriamycin before 12 weeks may deserve trial in attempting to improve the response rate.

653 NEUROPSYCHOLOGICAL LATE SEQUELAE OF HISTIOCYTOSIS X. J. Laurence Ransom, Morris Powazek, John R. Goff, H.R. Anderson, and Sharon B. Murphy. St. Jude Children's Research Hospital, Memphis, Tn. 38101. (Spon. by Alvin M. Mauer).

Prompted by clinical observations in several patients, a cross-sectional study of 18 long-term survivors of disseminated histiocytosis X was made to correlate clinical course, disease status, and extent and severity of neuropsychological disability. Evaluation is complete in 15 patients, 8 boys and 7 girls, ranging in age at diagnosis from 2 months to 12 years. Lahey scores at diagnosis ranged from 1-7 (median 3). No cases of solitary eosinophilic granuloma are included. Diabetes insipidus is present in 7 of 15 patients. Treatment consisted of steroids in 3 patients and multiple drugs sequentially or in combination in 12 patients; cranial irradiation (range 500-1500 rad) was given to 5 patients concomitantly. Current survival from diagnosis is 2-18 years (median 5 years). Thirteen of 15 patients are off therapy for periods of 1 month to 14 years (median 3½ years). Intellectual function was below average (I.Q. < 89) in 7 of 15 patients (.47) using the Wechsler Adult Intelligence Scale or Wechsler Intelligence Scale for Children. Results of the Wide Range Achievement Test revealed below average results in spelling and arithmetic, and average results in word recognition. In the 10 oldest children, results of either Halsted Children's or Reitan Battery indicated cortical dysfunction in 4, equivocal findings in 2, and no abnormality in 4 patients. Emotional difficulties, poor school performance, and delayed developmental milestones were common findings. Two children, surviving 2-4 years with Letterer-Siwe disease have evidence of extrahypothalamic CNS disease: truncal ataxia, pyramidal tract signs, and behavioral changes. We conclude that long-term survivors of histiocytosis X are at high risk for neuropsychological disability. Formal evaluation of the status and developmental progress of each patient should be a part of the longitudinal treatment plan. (Supported by NCI grants CA08480, CA21765, and ALSAC)

654 TEMPORAL RELATIONSHIP BETWEEN ACCUMULATION OF METHOTREXATE POLYGLUTAMATES AND PROLONGED INHIBITION OF DNA SYNTHESIS. D.S. Rosenblatt, V.M. Whitehead, A. Pottier, N. Vera, M. Dupont and M.-J. Vuchich. (Spon. by C.R. Scriver) MRC Genetics Group and Hematology Service, McGill Univ.-Montreal Children's Hosp. Res. Inst., Montreal, Quebec.

Human diploid fibroblasts grown in the presence of methotrexate (MTX) accumulated the drug and metabolized it to poly-γ-glutamyl metabolites (polyglutamates). After 15 min incubation in 1 µM MTX, the non-diffusable component of MTX in these cells was 58 pg MTX equivalent/mg cell protein, none of which consisted of polyglutamates. Accumulation of polyglutamates was linear with time so that after 6 h the cells contained 285 pg/mg of which 45 pg/mg was MTX, 162 pg/mg was MTX monoglutamate and 78 pg/mg was MTX diglutamate. It has been suggested that the continued presence of a component of intracellular MTX in excess of that bound to intracellular binders is required for the inhibition by MTX of deoxyuridine (dU) incorporation into DNA. When fibroblasts were preincubated for 1 h in 1 µM MTX-containing medium and then transferred into MTX-free medium, there was complete recovery of their ability to incorporate dU into DNA. However when the pre-incubation was increased to 4 and 6 h, the 24 h incorporation of dU decreased from 65.5 nM dU/gm cell protein to 17.5 and 4.9 nM/gm respectively. Thus inhibition by MTX of dU incorporation into DNA after the longer incubations was not dependent on the continued presence of MTX in the culture medium and was associated with a rise both in total intracellular MTX derivatives and most particularly in MTX polyglutamates.