913 PREVENTION OF CYCLOPHOSPHAMIDE (CY) INDUCED HEMORRHAGIC CYSTITIS: UPDATE ON THE USE OF ASCORBIC ACID (AA) TO REDUCE URINARY PH. P. Jean Henslee, Norman E. Sladek, Norma K.C. Ramsay, John H. Kersey, Phillip B. McGlave, Anne Goldman, and William Krivit, University of Minnesota, Minneapolis, MN 55455.

HC occurs in up to 50% of patients(pts.) receiving CY which results from bladder mucosal damage caused by acrolein, a degradation product of CY metabolism. Attempts to decrease HC which leads to severe morbidity have included urinary acidification with AA to diminish the formation of acrolein. In an earlier report (Proc. ASCO: 23, 1983), we evaluated 47 pts. receiving high-dose CY prior to bone marrow transplant(BMT) and found a low incidence of HC(10.6%) in pts. receiving AA. Subsequently, an additional 100 pts. undergoing BMT between 1982 and 1984 have been evaluated. Disease categories included: acute lymphocytic leukemia, 33; chronic myelogenous leukemia, 22; acute myelogenous leukemia, 14; aplastic anemia, 8; immunodeficiency, 8; neuroblastoma, 6; Ewing's sarcoma, 2; lymphoma, 2; myelodysplastic syndrome, 2; osteopetrosis, 2; and Hurler's syndrome, 1. The incidence of HC was 23%. The development of HC dependent upon various preparative regimens differed as follows: CY and fractionated total body irradiation(TBI), 17 of 64 (26.5%); CY, vinsristine, melphelan and TBI, 5 of 8(62.5%); CY and single dose TBI, 1 of 11 (9%); CY, procarbazine, antithymocyte globulin and TBI, 0 of 4 (0%); and in all other regimens not including TBI, 0 of 13 (0%). The pts. at highest risk were those with malignancy who had frequently been previously treated with CY. We conclude that AA alone is not sufficient to prevent HC, particularly in pts. at high risk. Further studies are needed to evaluate the efficacy of sulfhydryls such as 2-mercaptoethanol sodium or N-acetylcysteine with or without AA for the prevention of HC.

● 914 USE OF MONOCLONAL ANTIBODIES TO DIAGNOSE CENTRAL NERVOUS SYSTEM LEUKEMIA. Alan C. Homans, Barbara E. Barker, Edwin N. Forman. Brown University Program in Medicine, Rhode Island Hospital, Dept. of Pediatrics and Pathology, Providence, RI

The early identification of small numbers of leukemic cells in cerebrospinal fluid (CSF) presents a diagnostic problem in the treatment of children with acute lymphoblastic leukemia (ALL). We used a latex sphere rosetting technique to identify monoclonal markers on CSF lymphocytes in 201 samples from 112 children with ALL. Spinal fluid samples were incubated with mouse monoclonal antibodies against CALLA (J5), Ia, B1, or OKT 11. Presence of monoclonal antibodies on cells was determined by their ability to rosette 3 or more latex spheres bound with goat anti-mouse antibody. Cells were then cytocentrifuged and Wright stained for sumultaneous examination of cell morphology and immune markers.

In children without leukemic meningitis only 3% of CSF lymphoid cells were J5 (CALLA) positive, 8% were positive for Ia, 16% were positive for B1 and 69% were positive for OKT 11. Similar percentages were found in 15 adults having myelograms done. In contrast, in 5 children with CSF lymphoblasts seen, larger numbers of J5 or Ia positive cells were seen (24%-92%), sometimes with decreased numbers of OKT 11 positive cells (3-23%). In two children with small numbers of blast-like cells but with normal monoclonal markers subsequent CSF examinations were normal. We conclude that the use of this methodology provides valuable information complimentary to that obtained by examination of CSF samples for morphology alone.

● 915 SPONTANEOUS IN VITRO NEUTROPHIL DIFFERENTIATION IN SEVERE CONGENITAL NEUTROPENIA: EVIDENCE FOR A NORMAL MARROW MICROENVIRONMENT. Raymond Hutchinson, Michael Long, Jennifer Appleyard, John Curnutte, Laurence Boxer. Univ. of Michigan Medical School, Ann Arbor, MI

Marrow cells from 5 patients with severe congenital neutropenia (SCN) associated with maturation arrest at the promyelocyte stage were found to differentiate spontaneously into segmented neutrophils in long-term marrow culture (LTC). Previous studies have indicated that the granulocyte/macrophage progenitor cells (CFU-GM) in SCN are normal in number and in responsiveness to exogenous colony stimulating activity. This latter observation suggests that part of the defect in SCN may be extrinsic to the granulocytic lineage. To test this hypothesis, we have used autologous LTC to examine the ability of the patient's microenvironment to support granulopoiesis. By week 2 of culture, all 5 patients demonstrated terminal neutrophil differentiation with $2.9\pm3.6 \times 10^6$ (mean ± S.D.) band and segmented neutrophils/flask (12-45% of total cells), a value comparable to that seen in cultures from a normal control ($2.0 \pm 0.6 \times 10^6$/flask). Differentiation to mature neutrophils persisted through 6 weeks of culture in all but 1 patient. In that patient the addition of 13-cis-retinoic acid (RA) (10^{-6}M) to culture at week 3 produced a resurgence of neutrophil differentiation at week 6. We conclude that CFU-GM from patients with SCN are able to proliferate and differentiate on an autologous marrow adherent layer, suggesting the development of a normal in vitro microenvironment. Furthermore, the spontaneous differentiation which occurred in these cultures implies the presence of an in vivo suppressor of granulopoiesis. Finally, stimulation of differentiation by RA suggests a possible therapeutic role for this agent.

916 AGGREGATION OF PERMEABILIZED PLATELETS IN RESPONSE TO INOSITOL TRISPHOSPHATE (IP_3): Sara J. Israels, Jonathan M. Gerrard, Dept. of Pediatrics, University of Manitoba, Winnipeg, Manitoba, CANADA.

IP_3, a product of phospholipase C-induced phosphoinositide hydrolysis has been proposed as an intracellular messenger generated by receptor stimulation of cells. IP_3 has been shown to cause Ca^{++} release from intracellular stores in permeabilized pancreatic acinar cells and hepatocytes. The effect of IP_3 on platelet aggregation and ultrastructure was studied using washed human platelets permeabilized with saponin and suspended in a Ca^{++}-free buffer (120 mM KCl, 4.9 mM NaCl, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, 10 mM HEPES, 15 mM dextrose, 0.2% albumin, pH 7.4). Aggregation occurred with an IP_3 concentration of 0.5 µM, with maximum aggregation obtained at 10 µM. No aggregation occurred either with saponin alone or with IP_3 in non-permeabilized platelets. Examination of ultrastructure revealed aggregates with centralization of granules, typical of morphologic changes seen with increases in cytoplasmic Ca^{++}. These findings suggest that IP3 produced during platelet stimulation may act as a second messenger to promote Ca^{++} flux and may serve a similar function in other cells.

● 917 PHYSIOLOGIC CHARACTERISTICS OF SODIUM AND POTASSIUM FLUXES STIMULATED BY DEOXYGENATION OF SICKLE RED CELLS. Clinton H. Joiner (Spon by George Cassady). University of Alabama School of Medicine, Department of Pediatrics, Birmingham.

Dehydration of sickle red blood cells (SSRBC) is an important pathophysiologic determinant. We have recently shown that the increase in sodium (Na) and potassium (K) permeability which occurs on deoxygenation of SSRBC (but not normal RBC) can contribute to dehydration, despite the balanced ratio of this cation leak. We measured net cation movements in SSRBC incubated in phosphate buffered saline with 0.1 mM ouabain. The "deoxy" fluxes of Na and K were defined by the difference in ion movements in cells equilibrated with 100% N_2 vs. 21% O_2. The deoxy fluxes of both Na and K were maximal at pH 7.4 - 7.5, and the ratio of deoxy Na influx to deoxy K efflux remained one, over pH 7.0 - 7.9. The increase in ion permeability in SSRBC occurred less than 10 min. after deoxygenation. Similarly, SSRBC cation fluxes returned to baseline within 10 min. upon reoxygenation. Deoxy cation fluxes were activated at pO_2 levels of 30 to 45 torr., with both deoxy Na influx and deoxy K efflux showing similar pO_2 dependence. These data demonstrate that the increased cation permeability triggered by deoxygenation of SSRBC occurs at physiologic pH and pO_2, is rapid in onset and readily reversible. Futhermore, modulation of this transport system by pH and pO_2 leads to parallel changes in both Na and K permability. These results suggest that deoxy Na/K fluxes are activated in vivo, and support the involvement of this phenomenon in the process of cellular dehydration of SSRBC.

† 918 PULMONARY METASTASIS IN OSTEOSARCOMA: A REVIEW OF TREATMENT AND OUTCOME IN 88 PEDIATRIC PATIENTS. R.P. Kadota, G.S. Gilchrist, P.C. Pairolero, W.F. Taylor and R.L. Telander. Mayo Clinic, Rochester, MN 55905.

We analyzed the records of 88 patients <21 years of age who had the initial diagnosis of osteosarcoma established at Mayo between 1972 and 1981, and subsequently developed pulmonary metastases (PM). All had classic high grade extremity or girdle primary lesions with no evidence of PM at diagnosis based on imaging techniques in use at that time. All patients were considered for surgical resection if PM represented the sole site of active disease and the lesions appeared resectable on radiographic studies. Sixty nine of 88 children underwent 148 thoracotomy episodes (1 thoracotomy episode was defined as 1 or 2 thoracotomies performed for unilateral or simultaneously diagnosed bilateral PM). Twenty three children are currently alive, 21 disease free. All disease-free survivors (DFS) had successful surgical excision of their PM, median number of thoracotomy episodes= 2 (range= 1-6). Fifteen DFS had concurrent or sequential bilateral pulmonary involvement. Eleven DFS received no adjuvant therapy after their last thoracotomy. The median follow-up since the last PM for the 21 DFS is 77 months (range= 12-117), 19 being free of tumor >27 months. Of the children who underwent >1 thoracotomy, 23/69 (33%) are currently alive. None of the 19 patients not subjected to thoracotomy survived. Of children considered to be surgical candidates at the time of the most recent PM, 23/51 (45%) are alive. Overall, 23/88 (26%) patients survive. We conclude that surgical excision of PM plays a major role in the successful treatment of metastatic osteosarcoma. Supported in part by NCI grant #CA15083.