789 ABNORMAL METABOLISM OF β mRNA IN β+-THALASSEMIA (β-THAL). Edward J. Benz, Jr., Richard A. Spritz, Alphonse L. Scarpa, Barry L. Tonkonow, Howard A. Pearson, A. Kim Ritchey, Sherman M. Weissman, Bernard G. Forget. Yale University School of Medicine, Departments of Pediatrics and Medicine, New Haven, Connecticut.

We have studied mechanisms causing reduced β mRNA accumulation in β-thal. One patient was studied by recombinant DNA cloning, nucleotide sequencing and in vitro functional analysis of the β-thal gene, and five others, by analysis of newly synthesized mRNA after pulse-chase labeling of erythroblasts with ^{3}H-uridine. The cloned β-thal gene exhibited normal transcription in a cell-free system. The only nucleotide sequence abnormality occurs within the small intervening sequence (intron) and creates a potential anomalous splicing site in the mRNA precursor, suggesting a structural basis for defective processing. In four additional patients, defective β mRNA processing was also observed. The initial β/α ^{3}H mRNA ratios of pulse-labeled RNA were normal, indicating normal transcription, but abnormally high accumulation of unprocessed β mRNA precursur sequences (introns) occurred in each case. A fifth patient exhibited normal ^{3}H β mRNA synthesis and processing in nuclear RNA, but β mRNA in cytoplasm declined to steady-state levels during a 20-hour "chase," indicating cytoplasmic instability. These studies identify at least two distinct post-transcriptional lesions in β mRNA metabolism in β-thal: inefficient processing of introns and cytoplasmic instability of mature β mRNA.

790 CARBOXYHEMOGLOBIN VALUES IN INFANTS WITH ELEVATED NEUTROPHIL COUNTS. E. M. Bifano, D. A. Clark, S. Landow and F. A. Oski, Dept. of Pediatrics, Upstate Medical Center, Dept. of Medicine, V. A. Hospital, Syracuse, NY.

Infants with jaundice have been reported to have elevated poly morphonuclear cell (PMN) counts, thus reducing the utility of white blood cell counts in detecting infants with sepsis. We have evaluated the relationship of white cell count to the presence of hemolysis using carboxyhemoglobin as an index of red cell destruction.

Twenty-eight infants, 2-28 days of age, with elevated total PMN counts (as defined by the Manroe reference range, J. of Ped. 95:89 '79) had carboxyhemoglobin (HbCo%) determinations performed. All infants were screened for other causes of increased total PMN counts and none were septic or had a previously diagnosed hemolytic process.

Fourteen (50%) of these infants had evidence of hemolysis with an elevated HbCo%(>0.9%). The mean total PMN count in the group with hemolysis was 14,670±7,000 and in the group without hemolysis it was 8,900±4,600. All infants with total PMN counts greater than 15,000 had elevated HbCo% values. Repeat HbCo% determinations were done in the hemolytic group when the total PMN counts returned to normal. All repeat HbCo% values were within the normal range.

Increased total PMN counts can be used to identify some infants with previously undiagnosed hemolytic disease. Infants with jaundice and elevated white cell count should be suspected of having an associated hemolytic anemia particularly when the total PMN count is greater than 15,000/mm^{3}.

791 THE INCIDENCE OF CHLORAMPHENICOL-INDUCED NEUTROPENIA John P. Blank, Kala A. Sukerkar, Shrinivas H. Naidu (Spon. by Celia Kaye, Lutheran General Hospital, Dept. of Pediatrics, Park Ridge, IL)

The emergence of resistant organisms has increased the use of chloramphenicol. The hematologic toxicity of chloramphenicol is of two types: idiopathic and dose-related. In the dose-related toxicity, recent reviews emphasized the red cell changes and commented that neutropenia is rare. In order to assess clinically significant marrow toxicity, we reviewed charts of 56 patients treated with chloramphenicol between August 1975 and August 1980. These patients were treated for life-threatening infections, either meningitis or epiglotitis, believed to be caused by Hemophilus influenzae. All patients were treated with chloramphenicol at a dose of 100 mg/kg/d. There were 17 patients with epiglotitis, the mean age being 45 months (range 12-96 mos.). One patient in this group developed neutropenia (ANC<1500/mm^{3}). Thirty-nine patients had meningitis, the mean age being 18.9 months (range 4-84 mos.). Of the 39, eight developed neutropenia. Of patients treated with chloramphenicol, 14 were less than 12 months of age and 42 were older than 12 months of age. The mean onset of neutropenia was 9 days into therapy. The incidence of neutropenia was 2/42 for those patients greater than one year old and 7/14 for those less than one year of age. We believe our data indicates that neutropenia is a clinically significant side effect, particularly in patients less than 12 months of age. Such findings emphasize the need to measure chloramphenicol levels in the child less than one year old.

792 FACTOR VIII INHIBITOR RESPONSE TO CYCLOPHOSPHAMIDE (C) John D. Bouhasin, (intr. by Thomas Aceto), St. Louis University, Cardinal Glennon Memorial Hospital, Department of Pediatrics/Adolescent Medicine, St. Louis, MO.

Immunosuppressive therapy for Factor VIII inhibitors has been studied by different investigators with variable results. Over a 6 year period, 68 patients with Factor VIII inhibitors were randomized into 3 separate protocols:

ANTIBODY RESPONSE TO FACTOR VIII	ORAL(C)	ORAL&IV(C)	IV(C)	CONTROL
High Anamnestic Responders	5	8	7	4
Low Anamnestic Responders	9	8	10	17

The high responder group showed 3 separate patterns: 1) reduction of inhibitor & prevention of anamnestic response as long as cyclophosphamide therapy was maintained; 2) disappearance of inhibitor & no recurrence when exposed to Factor VIII after cyclophosphamide therapy termination; 3) persistence of inhibitor but in low responder range without anamnestic response to Factor VIII infusions after cyclophosphamide therapy termination. Controls developed an anamnestic inhibitor response each time they were exposed to Factor VIII. The low responder group showed 2 patterns: 1) disappearance of inhibitor without recurrence when exposed to Factor VIII; 2) persistence of inhibitor in low responder range. The low responders reacted the same during immunosuppression & after termination of the immunosuppression therapy. Controls showed the same results as the protocol group. It is evident from these results that immunosuppression was apparently unnecessary for the low responders but gave beneficial results with the high responders.

793 DISSOCIATION OF THE NEUTROPHIL FUNCTION OF CHEMOTAXIS FROM DEGRANULATION AND AGGREGATION: Grace J. Boxer, John M. Allen, Robert L. Baehner, Laurence A. Boxer. Indiana University School of Medicine, Veterans Administration Medical Center and J.W.Riley Hospital, Departments of Medicine and Pediatrics, Indianapolis.

To establish whether degranulation is an important prerequisite for granulocyte (PMN) aggregation, we employed the chemoattractant synthetic peptides Gly-His-Gly (GHG) and n-formyl-Met-Leu-Phe (FMLP). As previously shown, GHG caused chemotaxis of PMN at 10 ug/ml. GHG at concentrations of 10, 15, and 50 ug/ml did not cause degranulation of cytochalasin (CB)-treated PMN beyond that observed for control as measured by the percent total release of the granular constituent lysozyme into supernate (GHG 8.4%; control 8.7%). GHG also did not cause release of the cytoplasmic constituent LDH (GHG 5.9% of total; control 4.0%). GHG (range 12.5 to 100 ug/ml) also did not induce aggregation of CB-treated PMN as measured by light scattering. In contrast, $2x10^{-7}$M FMLP provoked the release of lysozyme (33% of total) from granules, but not release of LDH (5.3% of total), and caused aggregation of CB-treated PMN. When CB-treated PMN were incubated with GHG (15 ug/ml) followed by FMLP, there was no inhibition of lysozyme release (29.2% of total) nor of PMN aggregation. These studies suggest that GHG and FMLP interact with the PMN at distinct loci. Although GHG is chemotactic for PMN it failes to induce PMN aggregation, probably through its inability to provoke degranulation.

794 LACTOFERRIN: MEDIATOR OF GRANULOCYTE STICKINESS IN VIVO Laurence A. Boxer, Bengt Bjorksten, Jacob Bjork, Hsin Yang, John M. Allen, Robert L. Baehner. Department of Pediatrics, Indiana University School of Medicine and Pharmacia Research, Indianapolis, and Uppsala, Sweden.

Following exposure of granulocytes (PMN) to chemotactic factors in vitro, the cells will degranulate and aggregate and upon infusion of chemotactic factors in vivo animals will be rendered neutropenic. In order to assess whether the specific granule product lactoferrin (LF) could mediate PMN stickiness in vitro and in vivo, PMN were exposed to human LF in vitro and animals were infused with LF in vivo. LF at concentrations as low as 6 ug/ml aggregated human PMN and 12 ug/ml LF promoted their attachment to human umbilical endothelial (44 + 4% control compared to 55 + 4% LF-treated). Infusion of 1 mg/kg LF into rabbits caused a 40% fall in PMN count by 2 min; whereas 2 mg/kg of transferrin or granule lysozyme lacked any effect. In order to visually assess the effect of LF, the hampster cheek pouch was prepared for in vivo microcirculation observation by displaying a venule on a TV-monitor. The number of PMN passing a segment of the venule was then determined. Within 20-30 seconds after administration of 70 to 280 ug rabbit LF/100g to hampsters the PMN rolling frequency markedly decreased. PMN stickiness was then sustained for 3-5 min. In contrast intravenous administration of albumin or transferrin lacked effect. These studies indicate that the PMN LF can sustain PMN autostickiness and serve to mediate the effect of chemotactic factor in rendering animals neutropenic.