steadily during the first 3 to 4 months, and by 5 months of age approximates 2 to 3, which is similar to the ratio of the Hb-F from normal adults. These observations suggest that the switch from γ chain to β (and δ) chain production after birth apparently involves an unequal repression of the multiple γ structural genes at the time the β and δ structural genes are activated.

- 124 *Hemoglobin-Oxygen Affinity and Erythrocyte 2,3-Diphosphoglycerate (DPG) Content in Hyaline Membrane Disease (HMD) and Cardiac Malformations (CM).* GABRIEL V. DUC and KNUD ENGEL, Dept. of Ped., Coll. of Physicians and Surgeons, Columbia Univ., New York (introduced by John C. Sinclair).

The effect of DPG on the affinity of hemoglobin for oxygen, expressed as P^{50} (the PO_2 at which Hb is 50% saturated with O_2), is well known. In the present investigation whole blood from newborns with hyaline membrane disease (HMD) and cardiac malformations (CM) revealed a statistically significant difference between the two groups in both P^{50} and DPG content (HMD: P^{50} 24.6 mm Hg $\pm$ 3.5 S.D., DPG 0.78 mol/mol Hb $\pm$ 0.16; CM: P^{50} 29.1 mm Hg $\pm$ 3.4, DPG 1.31 mol/mol Hb $\pm$ 0.30). Patients with CM were more mature with higher birth weights and had lower arterial PO_2 and O_2 content (10.2 vol% O_2 $\pm$ 4.0 vs. 16.7 $\pm$ 4.9). BE, pH, PCO_2 , Hb and HbF were comparable. The effect of the observed difference in P^{50} between babies with HMD and CM can possibly explain the greater tolerance to hypoxemia of infants with CM since blood with P^{50} of 29.1 mm Hg can unload 15% more O_2 to the tissues than blood with P^{50} of 24.1 assuming the same PvO_2 , CaO_2 and cardiac output. Such differences in P^{50} have almost no effect on the amount of O_2 loaded in the lungs. Further observations showed that DPG concentration varied directly with pH in normoxemic patients (>90% saturation, >15 vol% O_2). Low pH could cause a decrease in DPG by inhibition of enzymes responsible for the synthesis, but also low DPG could produce a decrease in pH by impairment of unloading of O_2 to the tissues. The hypoxemic patients showed a higher DPG concentration than predicted from their Hp, again suggesting that hypoxemia causes an increase in DPG concentration.

- 125 *Molecular Interactions of Normal and Abnormal Hemoglobin in Cell-free Systems.* JAMES G. WHITE and BEATRICE HEAGAN, Univ. of Minnesota Sch. of Med., Minneapolis.

Increased viscosity, fragility and distortion of erythrocytes from patients with inherited defects of hemoglobin (Hb) have been related to sol-gel and sol-crystal transformation. A method has been developed in this laboratory which permits definition of the nature of molecular assembly underlying abnormal hemoglobin interactions. Cell free solutions of HbAA, HbAS, HbSS, HbSC, HbCC, and HbF combined with phosphate buffer, or phosphate buffer containing sodium metabisulfite, formed solid gels, crystals or amorphous precipitates. Gels of reduced HbSS, HbAS and HbSC consisted of masses of 170 Å rods identical to those observed in intact sickled erythrocytes. Gels of oxy HbSS, HbAS, HbAA and reduced HbAA contained 240 Å hollow tubular polymers similar to microtubules. Fresh samples of oxy HbSC and HbCC rapidly formed crystals, but a gel stage could be demonstrated by stirring solutions during transformation. Solutions of oxy and reduced HbF developed amorphous precipi-

tates, but did not gel or form crystals. Inability of HbF to form polymers may relate to its inhibitory effect on the assembly of HbS into rods. The capacity of oxy or reduced HbAA to transform into microtubules, on the other hand, reflects its ability to combine with HbS in rod-like polymers which distort cells containing HbAS. Molecular interactions of HbC favor crystallization, but as in all other examples, the gel state appears to precede crystallization. These findings indicate that hemoglobin molecules in intact normal and abnormal erythrocytes are in a high degree of molecular association or pre-gel state. Slight alterations in hemoglobin molecular structure can produce rapid and profound changes in the organization of hemoglobin into gels or crystals.

- 126 *Chronic Myelogenous Leukemia: Cyclic Leukocytosis and Identical Twin Discordance.* RICHARD A. GATTI, AMOS S. DEINARD, MARK E. NESBIT, WILLIAM A. ROBINSON and ROBERT A. GOOD, Univ. of Minnesota, Dept. of Ped., Minneapolis; Univ. of Colorado, Dept. of Med., Denver.

A cyclic leukocytosis with cycles of approximately 72 days and counts ranging from 4,000 to 145,000 cells/mm³ has been followed for 15 months with no treatment, in a 13-year-old white female with chronic myelogenous leukemia (Ph¹ positive). An identical twin has no clinical, morphologic or cytogenetic evidence of leukemia. Differential counts of per. bl. and marrow remain unchanged during the patient's cycles although marrow cellularity is increased during peaks. Studies which were normal include: Rebuck skin windows, phagocytic and bactericidal functions of neutrophils, glutathione peroxidase levels, fetal hemoglobin and immunologic responses. Epinephrine stimulation produced a total release of marginal pool cells at the high point with only partial release of cells at a low point. Endotoxin and steroid stimulation studies indicate that mechanisms for delivery of cells from marrow are intact and of similar magnitude at both high and low points. Ph¹ positive cells were seen in 81% of mitoses from per. bl. cells taken at a peak while in per. bl. specimens taken at a low point such Ph¹ positive cells were absent. At both high and low points over 90% of mitoses of bone marrow cells were Ph¹ positive. Mixed leukocyte cultures showed a non-reciprocal response of the patient's leukocytes to cells of her identical twin, suggesting partial loss of transplantation antigens in some of the patient's cells. Leukokinetics with DFP³²-labeled cells, alkaline phosphatase and granulopoietin levels and marrow proliferation studies with H₃T were measured at high and low points. These studies would be compatible with the view that controls of leukocyte production and release as well as in immunogenetic characteristics of cells are involved in this leukemic process.

- 127 *An Unusual Neutrophil Cycle in Down's Syndrome and Acute Leukemia.* RENATO MASTRANGELO, DOUGLAS E. COX, WOLF W. ZUELZER and JAMIL KHEDEH, Child Res. Center of Mich. and Dept. of Ped., Wayne State Univ. Sch. of Med., Detroit, and Plymouth State Home and Tr. Sch., Northville, MI.

A short-term cyclic fluctuation (14 to 24 days) of the neutrophil count in normal adults was shown by MORLEY [Lancet ii: 1220, 1966]. In contrast, a longer duration cyclic fluctuation (30 to 120 days) was seen by MORLEY, BAIKIE and GALTON in 4 patients with

chronic granulocytic leukemia [Lancet *ii*: 1320, 1967]. We have observed similar long-term cycles in 8 children during remission of acute leukemia (myeloblastic or monomyelogenous) in the absence of infections or changes of any chemotherapeutic agents. Likewise, a child with trisomic Down's syndrome and an unusual aplastic anemia showed long-term cycles for 18 months prior to his demise.

A prospective study of neutrophil cycles in 6 prepuberal males was undertaken under controlled conditions for 3 ½ months. Results of this study are as follows:

Type of patient and cycle	Period of cycle	Mean ampl.
Nonmongoloid short cycles	8.6– 15.5 days	1280–2250
Mongoloid short cycles	11.3– 19.0 days	2240–3460
Mongoloid long cycles	59.5–101.5 days	2265–3438
	Max. ampl.	Min. ampl.
Nonmongoloid short cycles	2700–3900	600–1500
Mongoloid short cycles	4000–6900	1000–1200
Mongoloid long cycles	2850–3550	1100–2600

Graphic display of neutrophil counts done twice weekly in 3 nonmongoloid controls shows only short-term cycles. However, the 3 patients with trisomy 21 reveal long-term cycles as well, upon which the short-term cycles are superimposed.

The short-term cycle appears to be an oscillatory feature of normal granulopoiesis, whereas the long-term cycle may denote abnormality of granulopoiesis.

- 128 *The Contribution of Relative Renal Mass to Erythropoietin Production.* J. A. SOLER, W. D. NOYES, H. L. CROMROY and G. A. RICHARD, Univ. of Florida, Sch. of Med. (introduced by Elia M. Ayoub).

Androgenic hormones have been reported to increase erythropoiesis by direct stimulation of erythroid marrow. Because androgens also possess a renotropic effect, the contribution of renal mass to erythropoietin production was investigated.

Rats were injected for 6 weeks with depot testosterone (in cotton seed oil), prior to and after nephrectomy. Controls received cotton seed oil or were sham-operated. Endogenous erythropoietin production was suppressed during the experiment by transfusion with packed red blood cells. Erythropoietin production was stimulated by subjecting the animals to hypoxia for 18 h. Kidneys were then removed and blood obtained for erythropoietin bioassay.

Hypoxia resulted in variable increases in erythropoietin levels in all animals. This variation in erythropoietin production was found to correlate closely with the relative renal mass (renal weight:body weight ratio), with erythropoietin levels increasing proportionately to renal mass in animals receiving either testosterone or in controls. Animals not subjected to hypoxia did not show any increase in erythropoietin levels, despite administration of androgens and resultant increase in renal mass.

These studies show that the enhanced production of erythropoietin by androgens during hypoxia is due not only to its direct action on bone marrow, but is also related to the renotropic effect of the steroid.

- 129 *Primary Erythrocytosis, Increased Erythropoietin Excretion and an Intrarenal Perfusion Abnormality.* C. THOMAS KISKER and LEONARD I. KLEINMAN, Dept. of Ped. Univ. of Cincinnati Coll. of Med. (introduced by Alvin M. Mauer).

A unique etiology for erythrocytosis was found in a 6-year-old boy. He was normotensive with increased erythropoietin and renin levels. His hemoglobin was 24 g% and hematocrit 73%. The red cell mass by ⁵¹Cr tagging was greater than 2 standard deviations above expected values. WBC, platelet count, hemoglobin electrophoresis and serum electrolyte values were normal. No tumor was demonstrated and he increased normally his urinary erythropoietin excretion after phlebotomy. Arterial oxygen saturation was 98% and his hemoglobin did not have increased oxygen affinity. Despite normal creatinine clearance, urine concentration to 1,260 milliosmoles/l, and normal renal biopsy, intrarenal narrowing of distal arteries was found on selective renal arteriography. Increased levels of renin (0.105 and 0.133 au/ml) and erythropoietin (2.6 and 2.5% ⁵⁹Fe incorporation) were found in renal vein blood. During simultaneous steady state infusion with inulin and PAH, renal plasma flow (282 ml/min) and glomerular filtration rate (85 ml/min) were normal. However, inulin extraction was high normal (30%) while PAH extraction was abnormally low (80%). These values are best explained by intrarenal shunting of blood, establishing areas of ischemia resulting in increased erythropoietin and renin excretion and thus causing the clinical and laboratory characteristics of primary erythrocytosis.

- 130 *Bone Marrow Transplantation for Aplastic Anemia Following Hepatitis.* MARGARET W. HILGARTNER, PHILIP LANZKOWSKY, RALPH L. NACHMAN and MARK B. WEKSLER, The New York Hospital-Cornell Univ. Med. Center, New York, N.Y. (introduced by Carl H. Smith).

The fatal outcome of aplastic anemia following hepatitis is well documented. Therefore bone marrow (BM) transplantation was performed in a 12-year-old girl who developed pancytopenia following mild hepatitis. Repeated BM aspirations revealed absent megakaryocytes, and erythroid and myeloid precursors. Despite prednisolone and oxymethyline therapy the pancytopenia progressed (hg 6.4 g%, WBC 100/mm³, platelets < 10,000/mm³). Staphylococcal sepsis following a finger infection and gram-negative sepsis following a perirectal abscess were treated with multiple antibiotics. Thawed frozen red cell and platelet transfusions were given as required. Because the clinical course was one of progressive deterioration without evidence of BM regeneration, BM transplantation was performed. The patient was pretreated with transfused donor buffy coat cells followed by cyclophosphamide 60 mg/kg for 3 days. 505 ml of HLA matched BM from an uncle were given. On the 8–10th day following transplantation reticulocytes were seen and the WBC rose to 2,200/mm³. BM revealed megakaryocytes and clusters of myeloid and erythroid cells. Chromosomal analysis revealed XY pattern. Concurrently the patient developed periorbital edema and desquamation, erythematous rash, purpura, abdominal pain, gross bleeding from the bowel and low factors II, V, VII and VIII with elevated factor I suggesting a consumption coagulopathy. She died 10 days following BM transplantation. Postmortem revealed regenerating marrow and extensive plasmacytoid cell proliferation