alkaline phosphatase activity and phosphoethanolamine excretion, eight heterozygotes were found in the family. In these heterozygotes phosphate reabsorption was normal, alkaline phosphatase activity was significantly decreased in the urine, but normal or elevated in the granulocytes. When their marker features were examined, the small number of the hypophosphatasia gene-bearing patients allowed no statistical analysis, but it was conspicuous that these persons were Rh negative, were nontasters for phenylthiocarbamide (PTC), and had an excess of ulnar loops in finger dermatoglyphics.

51. Control of erythropoiesis in the fetal and neonatal rat. Y. Matoth and R. Zaizov. *Tel Aviv Univ. Med. Sch., Israel.*

Erythropoiesis in the fetal rat was studied by measuring the incorporation of Fe59 into red cells of fetuses following injection of the isotope into their mothers on the 18th day of gestation. Two days later the amount of radioactivity present in the fetuses was determined by whole body counting. From this value and from the radioactivity found in fetal blood, the percentage incorporation of Fe59 into red cells (PI) was calculated. About 60% of the injected dose was found in the fetuses. The PI in individual fetuses varied with fetal weight, showing a linear relationship. It was therefore concluded that the rate of fetal growth is an important determinant of the rate of erythropoiesis. When mothers were bled or subjected to hypoxia during the 3rd week of pregnancy, or given 75–100 units of erythropoietin, they showed, as expected, an increase in PI. The fetuses likewise showed a significant increase in PI, controlled for fetal weight. The PI was decreased in mothers made polycythemic or kept at 4 atm abs but not in their fetuses. It has therefore been shown that in the rat erythropoietin can pass through the placenta and that the fetus responds to erythropoietin, either endogenously produced or transferred from the mother. Newborn rats hypertransfused during the 1st and 2nd postnatal weeks showed a marked decrease in PI and a good response to exogenous erythropoietin. Since the rat is born relatively immature and follows a fetal pattern of erythropoiesis for the first 2 postnatal weeks, these observations provide further evidence that erythropoiesis in the fetus is regulated through the hypoxia-erythropoietin mechanism.

52. Erythropoietic inhibitors in plasma and urine. S. Halvorsen, R. Lindemann, and P. Skjaelaaen. *Rikshospitalet, Oslo, Norway.*

The existence of specific inhibitors of erythropoiesis (EIF) has been suggested by several authors. The demonstration of this inhibitor, suggests that both activators (ESF) and inhibitors (EIF) participate in the normal regulation of erythropoiesis. In this study the presence of EIF in urine and neonatal plasma has been investigated.

Urine from normal healthy persons, from patients with aplastic anemia, and from patients with severe anemia due to chronic renal failure was investigated. The urines were passed through a Sephadex column, and the different fractions were tested for stimulatory and inhibitory effect of erythropoiesis. Plasma from normal newborn babies was withdrawn on the first 4–6 days of life. Plasma from newborns with hyperbilirubinemia but normal hemoglobin levels was also used for the plasma studies. Erythropoiesis in exhypoxic polycythemic mice was either stimulated by erythropoietin injections or by a second hypoxic period. Saline and test material were given simultaneously with erythropoietin or before and after the second hypoxic period. The effect was measured as ^{59}Fe uptake in red blood cells.

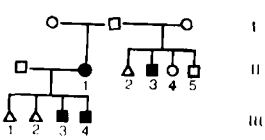
A marked inhibitory effect was found in the urine from normal healthy persons and from patients with aplastic anemia. In patients with chronic uremia a slight inhibitory effect and no active erythropoietin was found. The mice given neonatal plasma showed a marked reduction in iron uptake when the plasma was withdrawn between the 4th and 14th days of life.

The mode of action and the physiological role of EIF are unknown. EIF may be related to chalones. This is supported by the finding that EIF has the same molecular weight as reported for the chalones.

53. Dominant inheritance of congenital hypoplastic anemia. S. Garwicz and N. W. Svenningsen. *Univ. of Lund, Sweden.*

This paper is presenting the family history of a boy (*III-4*), who at the age of 2 months developed a typical picture of congenital hypoplastic anemia. His bone marrow showed 10.6% erythroblasts with predominance of young forms. Favorable response to corticosteroids was observed and at the age of 8 months the patient is doing well on small doses of prednisolone.

At the age of 2 months his older brother (*III-3*) showed a moderate anemia (lowest Hgb 7.9 g%), which subsided spontaneously at 7 months of age. Their mother (*II-1*) is herself the first case of Blackfan-Diamond anemia described in Europe 1939 by G. v. Sydow. She had been spenectomized at the age of 1½ year and later on showed a stationary course of the disease. During pregnancies her Hgb values were 7.8–8.7 g% without any blood transfusions. Her step-brother (*II-3*) developed a classical syndrome of congenital hypoplastic anemia at the age of 4 months. He was treated with blood transfusions and later on with corticosteroids. He died at the age of 12 and the autopsy showed generalized hemosiderosis. These step-siblings were described by Förare in 1963. According to available records the father of both step-siblings and his ancestors (mother, maternal uncle, and grandmother) had apparently had some kind of anemia. The family described indicates that dominant inheritance occurs in at least one type of hypoplastic anemia.



54. Binding capacity of human albumin for bilirubin. D. Bratlid, J. Fog, and S. O. Lie. *Rikshospitalet, Oslo, Norway.*

The binding between bilirubin and human serum albumin was studied spectrophotometrically and with Sephadex gel filtration. Spectral absorption curves of solutions of bilirubin in buffers containing decreasing amounts of albumin were registered. At a molar bilirubin to albumin ratio of 1:1 a change in these curves takes place, indicating that only one molecule of bilirubin is tightly bound to albumin. Sephadex gel filtration studies also showed that with solutions containing bilirubin and albumin in molar ratios above 1:1, bilirubin was retained on the column. A second molecule of bilirubin seemed, however, to be more loosely bound.

The toxic effect of bilirubin on human fibroblasts in tissue culture was also tested. When solutions containing bilirubin to albumin ratios above 1:1 were added to the growth medium, a toxic effect was seen on the fibroblast growth. With a bilirubin to albumin ratio of 2:1 rapid cell death was found with total bilirubin concentrations as low as 5 mg/100 ml. On the other hand, when the bilirubin to albumin ratio in the growth medium

was below 1:1, the cells tolerated bilirubin concentrations as high as 20 mg/100 ml, which was the highest tested. It therefore seems that only one molecule of bilirubin is tightly bound to each molecule of albumin, and that this molecule only is detoxified.

55. Kinetics of thymocytes. K. KOUVALAINEN and O. RUUSKANEN. *Univ. of Turku, Finland.*

The high content of alkaline phosphatase (AP) of the guinea pig thymocytes offers a simple endogenous label for these cells. AP can be easily demonstrated in histological sections and smears of cells. The lymphocytes of the guinea pig blood are mostly AP-negative, only every $1/4000$ white cell being an AP-positive lymphocyte. When given intravenously, the thymocytes very rapidly disappear from blood. They are then seen in the spleen, lymph nodes, bone marrow, and liver. Most of the lymphocytes in these peripheral lymphoid organs are AP-negative under normal conditions. The results indicate that either relatively few thymocytes leave the thymus or, if they leave in large amounts, they are rapidly destroyed and only few reside longer in the peripheral lymphoid organs.

56. Erythrophilic IgG-globulin coat in severe neonatal jaundice. T. THOMAIDIS, H. VALASSI-ADAM, and N. MATSANIOTIS. *Athens Univ., Greece.*

The erythrophilic IgG-globulin coat (IgG E-C) circulates in plasma; it coats red blood cells at low ionic strength medium (Thomaidis, N.: Biochemistry, *6:* 3369 and 3378, 1967). A method for quantitating IgG E-C, devised in our laboratory and consisting of (*a*) elution of IgG E-C and (*b*) determination of IgG in the eluate by circular immunodiffusion was applied to 83 newborns with severe jaundice. The mean value of IgG E-C in the ABO isoimmunization group (65.6 ± 12.1 mg) was higher than that in healthy newborns (44.3 ± 13.6, $P < 0.001$), in ABO set-up, or jaundice of unknown etiology. In the Rh isoimmunization group (IgG E-C was not increased 46.3 ± 11.7). It is concluded that this increase in the ABO isoimmunization group probably represents univalent and incomplete immune isohemagglutinins which are easily eluted, leaving the red cells naked and producing a negative Coomb's test. Investigations aiming at elucidating the pathogenesis of ABO isoimmunization should be diverted to the IgG E-C of neonatal erythrocytes rather than the maternal serum. Unfortunately, it cannot be used as an absolute diagnostic test in ABO isoimmunization because of overlapping of values.

57. Significance of the free anti-D antibody in the course of the hemolytic disease of the newborn due to Rh-isoimmunization. L. PATAKI. *University Med. Sch., Szeged, Hungary.*

The investigation of free anti-D antibodies in serum was performed in 65 mature Coombs-positive newborn. We found that the gravity of the disease depended in the first line on the presence or absence of free anti-D antibodies in the serum of the infants. No free anti-D antibodies were found in the serum of 27 Coombs-positive infants. In these cases the course of the disease was milder. In 14 infants the serum bilirubin did not rise to 20 mg/100 ml, so the exchange transfusion was not indicated. In 13 cases, a more marked rise of the bilirubin level called for exchange transfusion. Under such conditions if there are no circulating free anti-D antibodies, Rh-positive blood can also be used for the exchange transfusion. After the exchange transfusion mild bilirubin rebound occurred, no more blood exchange was necessary. Free anti-D antibody was found in the serum of 38 infants. The course of the disease was serious, exchange transfusion was indicated in every case. Nineteen infants were treated, in the usual way, with Rh-negative blood. In 9 cases the exchange transfusion had to be repeated; 19 babies were treated with "combined exchange transfusion", a method first applied by us. (The transfusion was begun with Rh-positive blood and completed by Rh-negative blood.) In this way free anti-D antibody could be removed efficiently, the exchange had to be repeated only in 4 cases.

58. Hemostatic failure in Rhesus isoimmunization. J. M. CHESSELLS and J. S. WIGGLESWORTH. *Hammersmith Hosp., London, England.*

Laboratory studies prior to exchange transfusion in a group of babies with Rhesus isoimmunization showed evidence of hemostatic failure in 6 out of 30. Findings in these infants included thrombocytopenia, low plasma fibrinogen, and abnormalities of the intrinsic coagulation system. Five babies had a clinically recognizable bleeding tendency. Fibrin degradation products were found in 11 infants including babies who had been treated by intra-uterine intraperitoneal transfusion, in addition to those with evidence of hemostatic failure. Eight babies in this group of 30 died, and at necropsy 4 out of 7 had subarachnoid and intracerebral hemorrhage. Three of the 4 had intravascular fibrin thrombi on microscopy of tissue sections. An additional postmortem study on babies with Rhesus isoimmunization who died prior to the main investigation revealed massive intracranial hemorrhage associated with the presence of intravascular fibrin thrombi in 5 cases out of 10. Babies at most risk of hemorrhagic complications are those with a cord Hb below 7 g/100 ml. It is concluded that disseminated intravascular coagulation is a major contributory cause of hemostatic failure in Rhesus isoimmunization although hepatic dysfunction may play a part in some infants.

59. Studies on the isoenzyme pattern of fetal and adult red cells. H. BARTELS. *Kinderklinik der Medizinischen Akademie, Lübeck, Germany.*

Quantitative differences in the enzyme activities of fetal and adult red cells are well known, but few data on differences in the isoenzyme patterns between these cells have been published. In this study isoenzymes of phosphopyruvate hydratase (EC.4.2.1.11) and pyruvate kinase (EC.2.7.1.40) were investigated in hemolysates of isolated fetal and adult erythrocytes. Electrophoresis was performed on cellulose acetate foils. Sites of enzyme activity were detected utilizing the fluorescence of nicotinamide adenine dinucleotide by illuminating the foils with ultraviolet light after incubation with specific identification-reaction mixtures. For both phosphopyruvate hydratase and pyruvate kinase differences in number, intensity, and/or electrophoretic mobility of isoenzyme bands between fetal and adult erythrocytes could be demonstrated. These findings suggest further evidence for the biochemical distinction of fetal and adult red cells.

60. Hemolytic anemia associated with reduced glutathione deficiency. S. S. LO, W. H. HITZIG, and H. R. MARTI. *Univ. of Zürich, and Kantonsspital, Aarau, Switzerland.*

Glutathione is present in high concentration in erythrocytes. The main part is kept in the reduced form. It is well known that a decrease of reduced glutathione may be linked with hyperhemolysis, but the exact mechanism is unknown. We have investigated three families with nonspherocytic hemolytic anemia in which diminished reduced glutathione was a constant finding.