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 $1/1000$ 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.

The three propositi (two females, one male) had signs of hyperhemolysis from early infancy, one of them even needed two exchange-transfusions. In two of them on one or several occasions stomatocytosis was found, and in one of them high sodium and low potassium in the erythrocytes was observed. Family studies revealed several apparently healthy members with the same deficiency and presenting evidence of mild to moderate hyperhemolysis. Genetic transmission was dominant-autosomal. The phenotypic expression seems to differ greatly. In one case who presented with gallstones at the age of 19 years splenectomy beneficially influenced the intensity of the hemolytic process.

61. Pyruvate kinase deficiency, a family study. H. M. HØYERAAL, M. HJELM, and P. J. MOE. *Univ. of Bergen, Norway, and University Hosp., Uppsala, Sweden.*

A 3-year-old girl with pyruvate kinase deficiency has been investigated together with her family. Erythrocyte survival studies with ^{51}Cr-labeled donor cells in the patient's circulation showed a half life of only 17 days. Direct Coomb's test was negative and cold agglutinin titres normal. Cholecystography showed gallstones and no gallbladder excretion. The pyruvate kinase activity in erythrocytes at 25° and pH 7.5 was only 9.5 and 6 μM substrate turnover/min 10^{11}, respectively, on two occasions, whereas 2 SD of blood donors is 25.0–49.0. Corresponding activities for hexokinase, phosphofructokinase, phosphofructoaldolase, lactate dehydrogenase, glucose 6-phosphate dehydrogenase and glutathion reductase were either within or above normal range. In her erythrocytes adenosine triphosphate was low, adenosine diphosphate normal and 2,3-diphosphoglycerate high. Further studies seem to indicate that the pyruvate kinase in our patient has altered kinetic properties, an abnormal sequence of amino acids. The pyruvate kinase activity in erythrocytes from the patient's mother was low. Similar activities in erythrocytes from the patient's father and brother were at the lower limit of normal. One brother of the maternal grandmother had hemolytic anemia, thrombocytopenia and cholelithiasis, another brother had cholelithiasis and a sister had anemia. The result of further family studies will be reported.

62. Adenosine triphosphatase (ATPase) deficiency in a family with nonspherocytic hemolytic anemia. J. COHN and K. H. HANFL. *Copenhagen Univ. Hosp., and Bispebjerg Hosp., Copenhagen, Denmark.*

A 2-year-old boy was referred as an outpatient to the University Hospital because of anemia. He was found to have a congenital hemolytic anemia. Several relatives had the same disease. Biochemical studies revealed an intraerythrocytic enzymic defect consisting in lowered activity of potassium-sodium-magnesium-sensitive ATPase (S-ATPase). Because of inhibition by digoxin this enzyme could be separated from the insensitive ATPase (I-ATPase). The ATP level in the erythrocytes was normal. Autohemolysis in the patients was increased. Urea inhibition of ATPase in patients as well as in normal subjects showed similar values, suggesting that the deficiency in S-ATPase is due to reduced synthesis of the enzyme. Preliminary genetic studies have indicated that the defect is a sex-linked, recessive disorder.

63. Stability of red blood cell acid phosphatases and association between their phenotypes and clinical favism. E. BOTTINI. *Univ. of Rome, Italy.*

In previous experiments we have observed that the incubation *in vitro* with GSSG or APH can modify the electrophoretic pattern of RBC acid phosphatases (SH-dependent enzyme) and can reduce their total activity; we have also demonstrated that the isoenzyme fractions present a differential liability towards the treatment with these substances. The present investigation suggests that in enzymopenic subjects for G-6-PD, acid phosphatase phenotypes show a differential liability toward the hemolytic effect *in vivo* of *Vicia faba*. A total of 84 male children who had had severe episodes of hemolytic favism have been studied. Acid phosphatase phenotype was determined according to Hopkinson, Spencer, and Harris. The results have shown that 58.33% of the children who had had favism are carriers of P^a allele either in single or double dose; the same phenotypes in the general population have a frequency of 44.59% ($P < 0.02$). P^a gene frequency is 0.357 and 0.258, respectively ($P < 0.01$). This observation seems to indicate that an allele (P^a) of a gene polymorphic in all human populations affects in special conditions (genotypic and environmental) the fitness of the involved phenotypes. From a practical point of view it would be important to identify those subjects which present a higher risk of hemolytic episodes induced by *V. faba*.

64. Pathogenesis of anemia in acute leukemia. R. ZAIZOV and Y. MATOTH. *Tel Aviv Univ. Med. Sch., Israel.*

The pathogenesis of anemia was studied in eight cases of untreated acute childhood leukemia. Varying degrees of erythroid hypoplasia, as judged by ferrokinetic studies and/or studies of marrow cellularity, were present in all cases. Urinary erythropoietin excretion (UEP) was assayed in exhypoxic polycythemic mice. An increase in UEP within the range commonly found in aplastic anemia was seen in two cases. In three other cases, however, the UEP was very low for the degree of anemia. In one such case, presenting with a Hb concentration of 7 g/100 ml, massive leukemic infiltration and scarce erythroid elements in the marrow, the UEP was 0.01 24 hr. There were, on the other hand, two patients who had only mild anemia and yet their UEP was excessively high. One such patient, whose Hb concentration was 10.3 g/100 ml, excreted 60 units/24 hr. An inhibitor of erythropoiesis has been looked for in the plasmas of our patients but none was found so far. These preliminary observations suggest that some interference with the regulatory mechanism of erythropoiesis is present in acute leukemia.

65. Blastogenesis of lymphocytes from different sources in acute lymphoblastic leukemia (ALL) of children. L. MASSIMO, F. DAGNA-BRICARELLI, P. G. MORI, A. FOSSATI-GUGLIELMONI, and G. ASTALDI. *Univ. of Genoa, and Blood Res. Found. Ctr., Tortona, Italy.*

In 1966 we showed high extents of blastogenesis in the 3-day PHA culture of peripheral blood lymphocytes from ALL (Lancet, *i*: 1265, 1966). The results of the PHA-responsiveness of lymphocytes obtained in ALL from different sources besides peripheral blood, such as bone marrow, lymphonodes, leukemic pleural effusion and leukemic spinal fluid, are now discussed. Concerning the peripheral blood lymphocytes, all but 2 of a total of 19 untreated patients examined at the time of diagnosis showed extents of blastogenesis from 50 to 90%. The lymphocytes from quite enlarged lymphonodes of 2 children showed normal high percentages of blasts. On the other hand, the bone marrow PHA cultures of 11 of the above mentioned 19 patients gave high extents of blastogenesis (ranging from 58 to 88%) only in 4 of them. In fact, the bone marrow of 3 patients showed blast per-