

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  $\frac{1}{4000}$  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 (Thomaïdis, 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 ( $65.6 \pm 12.1$  mg) was higher than that in healthy newborns ( $44.3 \pm 13.6$ ,  $P < 0.001$ ), in ABO set-up, or jaundice of unknown etiology. In the Rh isoimmunization group (IgG E-C was not increased  $46.3 \pm 11.7$ ). 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 post-mortem 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. HRTZIG, 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.