

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. HØYERAAI., 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, phosphofruktaldolase, 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. CONN and K. H. HANFEL. *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. Auto-hemolysis 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. BORTINI. *Univ. of Rome, Italy.*

In previous experiments we have observed that the incubation *in vitro* with GSSG or APH can modify the electrophoretic pat-

tern 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. ZAIKOV and Y. MATOHE. *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-BRIGARELLI, 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-

centages of only 1.1, 11, and 14%, whereas the remaining 4 children had no lymphocyte transformation at all. Again, the lymphocytes obtained from the spinal fluid in 3 evaluable patients of 7 examined, all affected by meningeal leukemia, and from the pleural effusion in 1 patient did not undergo any blastic transformation. It appears that a quite different behavior concerning PHA blastogenesis of lymphocytes in ALL can be observed according to the source the lymphocytes come from.

66. *In vitro* sensitivity to vincristine of acute lymphocytic leukemia cells—a tentative to correlate *in vivo* and *in vitro* results. L. PICENI SERENI, G. MASERA, V. CARNELLI, and F. SERENI. *Univ. of Milan, Italy*.

Data were obtained on the influence of vincristine ($7 \times 10^{-5}M$) on the *in vitro* incorporation of both $6\text{-}^{14}C$ -orotate into RNA and of $1\text{-}^{14}C$ -leucine into proteins of leukemic cells from acute lymphocytic leukemia. Orotate and leucine incorporations were measured after 2 and 12 hr of incubation. A total of 17 patients were examined. Most of the *in vitro* studies were conducted on peripheral blast cells. In 4 instances also bone marrow leukemic cells were tested. In any case in which a good clinical response by vincristine administration was recorded, also a strong *in vitro* inhibition of both RNA and protein synthesis was observed. On the contrary, in the only 2 cases in which a definitive *in vivo* resistance to vincristine was observed, also an *in vitro* lack of response was found. Practical implications of these studies will be discussed.

67. Gluconeogenesis in human platelets. K. A. ZUPPINGER, N. NAKAMURA, J. P. COLOMBO, and P. WALTER. *Universitäts-Kinderklinik and Univ. of Bern, Switzerland*.

Quantitative evaluation of gluconeogenesis in humans is difficult since liver and kidney are not suitable for repeated investigation. Therefore, gluconeogenesis in easily available tissue such as platelets was studied during incubation in buffered solution. Upon addition of 20 mM pyruvate glucose formation in the incubation medium amounted to $6.99 \mu\text{moles/hr } 10^{11}$ platelets and glycogen utilization was inhibited to 28.8%. Incorporation of label from pyruvate- $2\text{-}^{14}C$ into carbohydrates revealed $2.22 \mu\text{moles pyruvate incorporated/hr } 10^{11}$ platelets. These results are interpreted as evidence for the existence of gluconeogenesis in human platelets. In two patients with glycogen storage disease type I incorporation of label from pyruvate- $2\text{-}^{14}C$ into carbohydrates was decreased to $0.95 \mu\text{mole/hr } 10^{11}$ platelets. Only 7% of the total radioactivity was found to be in glucose in these patients as compared to 81% in normals, indicating that most radioactivity was incorporated into nonglucose carbohydrates, probably glycogen. Incorporation of label from pyruvate- $2\text{-}^{14}C$ into carbohydrates by platelets of two patients with glycogen storage disease type III showed no increase in comparison to normals; however, in these patients 95% of the label was found in glucose. This finding indicates that in type III glycogenosis a greater relative part of pyruvate is incorporated into glucose than in normals. The conclusion is drawn from these studies that human platelets might prove to be a valuable material for studying gluconeogenesis under various conditions.

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