ERYTHROCYTES

On the Molecular Basis of Pyruvate Kinase Deficiency. II. Role of Thiol Groups in Pyruvate Kinase from Pyruvate Kinase-deficient Patients. T. J. C. van Berkel, G. E. J. Stoel, J. F. Koster, and J. G. Nijssen. Department of Biochemistry I, Faculty of Medicine, Erasmus University Rotterdam, Rotterdam, The Netherlands, and Hematological Department, State University Hospital, Utrecht, The Netherlands. Biochim Biophys Acta 334:361-367, 1974

Human erythrocyte pyruvate kinase from the class of pyruvate kinase-deficient patients, characterized by an increased affinity towards PEP and a loss of cooperative interaction towards this substrate, shows less affinity for the allosteric inhibitor ATP. Incubation of pyruvate kinase, obtained from this class of pyruvate kinase-deficient patients with mercaptoethanol changes the abnormal kinetics into normal kinetics, as can be concluded from the change in PEP dependency and ATP inhibition. The effect of mercaptoethanol on the kinetics of pyruvate kinase suggests that the alteration in the enzyme is a consequence of a modification of the -SH groups. It is suggested that pyruvate kinase deficiency is a secondary defect and that the process which causes the change in the -SH groups of pyruvate kinase, may also be responsible for the increased rate of hemolysis, found in these patients. — K. P.


Sterile inflammation was produced in Sprague Dawley rats by intraperitoneal injection of turpentine oil and early (24-48 hr) fall in plasma iron and iron-binding capacity observed. Transferrin synthesis, measured as 3H-lysine uptake of immunoprecipitated transferrin in rat liver slices, was normal and unrelated to the serum iron. Human splenic macrophages
and rabbit pulmonary macrophages maintained in culture for up to 1 wk showed an ability to take up protein-bound iron and labeled plasma proteins into the cytoplasm. The implication of this work is that the reticuloendothelial (RE) cell may take up iron from the plasma thus resulting in a low serum iron concentration. This hypothesis is an attractive alternative to the previously held view that iron accumulates in the RE cell as a result of impaired release, the so-called RE block.—J.A.W.


Five members of an English family with a new high oxygen affinity variant of Hb A are described. Four family members had mild polycythemia and were asymptomatic, the propositus having clinical features of polycythemia. The hemoglobin variant (Hb Heathrow) had the same electrophoretic mobility as Hb A. Of the II other known Hb variants associated with familial polycythemia only one (Hb Olympia, Stamatoyannopoulos et al., 1973) could not be electrophoretically separated from Hb A. The authors emphasize the need for measuring the O2 affinity of Hb in patients with a high hematocrit when features of other forms of polycythemia are absent.—J.A.W.


CDA type II (also called HEMPAS) is characterized by varying degrees of anemia, splenomegaly, and jaundice, associated with multinucleated erythroblasts, a positive acidified serum test, and red cell susceptibility to agglutination and lysis by anti-i sera. Two children with this disease are described, and the authors review their laboratory findings as well as those from the literature. The precise mechanism for this congenital disorder, which appears to be transmitted by an autosomal recessive gene, is unclear, but markedly ineffective erythropoiesis is apparent, with varying degrees of superimposed hemolysis, possibly more the result of hypersplenism than of an intrinsic red cell defect.—J.B.S.


Bernstein’s method and Tötz and Betke’s modification of Motulsky’s method were used for qualitative determination of G-6-PD in 1167 men in the population of seven districts in Transcaucasus. In Azerbaijan, the male population of Lenkoran lowlands revealed 9% positives, the male population of Kurinsk lowlands showed 6%, positives, and in Georgia, the male population of Kolkhida lowlands showed less than 0.2%, positive cases with G-6-PD deficiency. Ethnic differences were statistically significant.—G.A.


A study of the activity of G-6-PD and glutathione reductase (GR) in the erythrocytes was made in 20 premature children with early anemia. No changes in the activity of G-6-PD was noted; the activity of GR doubled in early anemia. Causes promoting increase in activity of GR in the erythrocytes are discussed.—G.A.


Bernstein’s express method was used for qualitative determination of the activity of G-6-PD in the erythrocytes precipitate among the Russian population of the Arkhangelsk region. Among 642 people (279 men and 363 women) there were 2.14% of hemizygotic men. This result correlated well with the general European incidence of G-6-PD deficiency.—G.A.

Distribution of Hereditary Deficiency of Glucose-6-phosphate Dehydrogenase Activity of Erythrocytes in Azerbaijan. A. Y. Lyseko, R. G.
The rapid Bernstein's method was used for qualitative determination of the activity of glucose-6-phosphate dehydrogenase in erythrocyte precipitate of 22,040 persons in 70 populated localities of Azerbaijan, SSR. The percentage of men carrying the anomalous gene varied in different villages from 3.6% to 37.3%. Detection of many homozygotic females affected with G-6-PD deficiency is explained by numerous marriages between siblings.—G.A.

Genogeography of Thalassemia in the Soviet Republics of Transcaucasus. A. A. Voronov, Institute of Ethnography, Moscow, USSR. Probl Hematol and Blood Transfusion No. 11:32-36, 1973

A total of 1488 people were examined for β-thalassemia: in Azerbaijan (878), in Georgia (440), and in Armenia (180). Quantitative determination of Hb A₂ and the alcaline denaturation of Hb (Singer's method) were done; morphology and osmotic resistance of erythrocytes were also studied. A considerable concentration (from 1.3% to 9.4%) of heterozygotic carriers of β-thalassemia was revealed in the lowlands of Azerbaijan. In the lowlands of Georgia the incidence of heterozygotic β-thalassemia was low (from 0.2% to 1.2%). In one district of Armenia the incidence of β-thalassemia was 1.7%. Ethnic differences in the incidence of heterozygotic β-thalassemia were proven to be statistically significant.—G.A.


A total of 1286 people were examined for the purpose of studying β-thalassemia distribution. Among the Georgian population the greatest incidence of β-thalassemia (average 6.6% in 814 persons) was revealed in the lowlands and the valley regions. Thalassemia gene was either absent or had low incidence in the mountainous regions (average 0.6% in 472 persons). The ethnic relationship was revealed by comparing the data with the incidence of β-thalassemia in various other ethnic groups living in similar geographic conditions.—G.A.

Concerning Criteria and Difficulties in the Diagnosis of Erythromyelosis. G. B. Berliner. Faculty of Medicine, Petrozavodsk, USSR. Probl Gematol Pereliv Krovi No. 1:38-39, 1974

Difficulties in the diagnosis of erythromyelosis emerge in those cases when the proliferation of erythroblasts appears either in the initial or in the final stage of the disease. In the first variant a quick transformation of erythroblastosis into myeloblastosis is observed. In the second variant, characterized usually by the appearance of megaloblastosis, the latter must be considered as the manifestation of antifolic effect of methotrexate and other cytostatics (D. Kundel and B. Nies, 1965; G. Alexeieff, 1971). Differential diagnosis with pernicious anemia presents no peculiar difficulties. The “spectacular” effect of treatment by vitamin B₁₂ easily settles all doubts. Peculiar difficulties emerge in the differentiation of erythromyelosis with the refractory sideroblastic anemia, as the “preleukemic” stage of the disease. The authors accept Heilmeyer’s and Dameshek’s concept which considers erythromyelosis and refractory sideroblastic anemia to be the same disease.—G.A.


According to the author’s data, the erythrocytes of 20 patients with Marchiafava-Micheli disease (paroxysmal nocturnal hemoglobinuria, PNH) and erythrocytes of 20 patients with autoimmune hemolytic anemia were destroyed much more intensively by hydrogen peroxide, than the erythrocytes of healthy persons. But the normal erythrocytes treated with proteolytic enzymes and reduced glutathione acquired the properties of complement sensitivity. In the erythrocytes incubated with hydrogen peroxide, a considerable amount of malonic dialdehyde was formed. But a marked increase in polyunsaturated fatty acids, particularly of eicosadienoic acid, was revealed only in the erythrocyte membranes of patients with PNH. Following erythrocyte treatment of healthy persons by reduced glutathione, a marked reduc-
tion of the amount of polyunsaturated fatty acids was observed (these changes were the opposite of those occurring in Marchiafava-Bicheli disease). The authors advance the hypothesis that the somatic mutation in PNH leads to the appearance of a pathologic clone of erythroblasts characterized by a disturbance in the synthesis of enzymes that are necessary for normal formation of fatty acids with a great number of carbon atoms. As a result, in the membrane of the erythrocytes the functionally defective isomeric forms of fatty acids appear: these are the cause of complement sensitivity of the erythrocytes and of their lysis.—G.A.


Red cell ghosts were prepared from blood of three patients with PNH and from normal red cells treated with AET. The membrane pellets were subjected to sodium dodecyl sulfate electrophoresis in polyacrylamide gels. The only difference between control membranes and naturally occurring or artificially produced PNH membranes was the presence in normal cells of a band with a molecular weight of 82,000 and in abnormal cells the consistent disappearance of this band with appearance of a band of molecular weight of 77,100. Both the normal and abnormal bands were glycoproteins.—A.A.M.


Eight adults with hereditary spherocytosis (HS) were studied 1–21 yr after splenectomy by means of iron absorption tests (whole body counting after oral $^{59}$Fe). Mean $^{59}$Fe absorption was 28.3%, compared with 13.5% in controls, a significant difference, mainly due to relatively large increases in three of the eight patients. There was no Fe deficiency to explain the increased absorption. The increased absorption may account for the hemochromatosis which has been occasionally reported in post-splenectomy patients with HS.—F.W.G.

LEUKOCYTES


Leukocyte chemotaxis was assessed by a modified Boyden procedure involving migration of leukocytes through a Millipore filter in response to a chemotactic stimulus derived from a culture filtrate of Escherichia coli. Phorbal myristate acetate, acetylcholine, and carbamylcholine are substances believed to increase intracellular concentrations of cyclic GMP and addition of these substances and 8-bromo cyclic GMP to the system significantly increased the degree of chemotaxis.—A.A.M.


Mononuclear cells were collected by density-gradient centrifugation and then separated into adherent and nonadherent fractions. These were tested singly and in combination for ability to proliferate following inoculation with EB virus. Neither adherent nor nonadherent populations showed sustained proliferation similar in degree to that observed with the original unfractionated population but recombining the fractions restored the proliferative response. The findings suggested some type of cooperation between adherent and nonadherent cells, a phenomenon similar to that observed with some antibody responses.—A.A.M.


A new technique for measurement of phagocytosis stimulated glucose oxidation in granulocytes is reported. The technique described is simple and efficient and has the advantages of being both instantaneous and continuous—J.A.W.

Acute blast cell crisis occurred in two patients with chronic lymphocytic leukemia one of whom had not received any treatment. Using a direct immunofluorescent technique, IgM monoclonal globulins were demonstrated on the surface of blast cells in one patient and in both lymphocytes and blast cells in the second patient. Blast cells were treated with trypsin to remove immunoglobulins attached to the cell membrane, and the cells were then cultured in vitro and shown to synthesize IgM with anti-IgG antibody activity. Lymphocytes from the second patient did not yield synthesized IgM when similarly cultured. These findings suggest that the blast cells are related to B lymphocytes and support the view that lymphoid cells in the acute and chronic phases of the disease originate from the same clone.—**J. A. W.**


Evidence is presented which suggests that the characteristic "hairy" cell seen in leukemic reticuloendotheliosis is a B-lymphocyte. "Hairy" cells were less active than monocytes in adhesion to glass, transformation to macrophages and phagocytosis of candida albicans. "Hairy" cell surface bound immunoglobulins did not form rosettes with sheep's red cells; the "hairy" cells did not respond to phytohemagglutinin in three of four patients studied or to pokeweed mitogen.—**J. V.**


The diagnostic features of six patients with leukemic reticuloendotheliosis (LRE) are discussed. Patients presented with a history of chronic illness of insidious onset, had large spleens without prominent lymphadenopathy and were often misdiagnosed as having chronic lymphatic leukemia or lymphosarcoma. Marked pancytopenia, variable numbers of abnormal "hairy" mononuclear cells, and occasional nucleated red cells were seen in the blood while the bone marrow contained 50%–80% "hairy" mononuclear cells. Cytochemically, LRE cells were acid phosphatase and PAS positive. Electron microscopy of the cells showed irregular cytoplasmic edges and large "ribosome-lamellae" complexes in 25%–30%.

Prednisone was of transient benefit in treatment, but the authors recommend splenectomy, their three splenectomized patients surviving for 3+, 7, and 10+ yr while three patients who did not have a splenectomy all died within 2 yr. It is suggested that LRE should be classed with the lymphoproliferative diseases.—**J. A. W.**


This is a postmortem study of 163 cases of acute leukemia in which separation of the leukemic cell types was made on the basis of azurocin and oxidase stains. Myelocytic-promyelocytic leukemia was distinguished from myeloblastic leukemia by the presence, in the blast cells, of abundant oxidase-positive granules and was associated with a fulminant course with severe, usually fatal, hemorrhages. The author suggests the designation immature cell (blastic) or malignant be used for acute leukemia and mature cell (benign) for chronic leukemia.—**J. V.**


In initial bone marrow smears of 216 new cases of lymphocytic leukemia in children two patterns of lymphoblast morphology were observed. Some cells had compact nuclear chromatin with inconspicuous nucleoli and weakly stained cytoplasm while the other type had reticular nuclear chromatin, more distinct nucleoli, and intensely stained cytoplasm. In most patients undergoing relapse, reticular lymphoblasts were prevalent; reticular lymphoblasts also showed a higher mitotic index and lower glycogen content than the others.—**J. V.**

Peripheral blood cells of 22 children with acute leukemia and of three with infectious mononucleosis were studied using fluorescent anti-IgA, anti-IgG and anti-IgM sera. In the cells of lymphoblastic and plasmablastic acute leukemia, immunoglobulin synthesis was detected, the number of positive cells and the intensity of fluorescence being related to the levels of the immunoglobulins in the serum; there was one exception in which the cells reacted only to anti-IgD and anti-IgE. In the myeloid variants, cells showed only nonspecific reactions while in monoblastic and myelomonoblastic cells, no reaction was seen. In infectious mononucleosis, atypical mononuclears gave no reaction, though weak anti-IgM fluorescence was noted in some typical lymphocytes.—J.V.


From a detailed study of 44 patients with acute leukemia in which hyperplasia of the erythroid series was a feature, with or without atypical morphology, the author differentiates, from other leukemias, two varieties. In the first, erythroblastic leukemia occurs as the preponderant proliferation of a cell type in the marrow and in the second it appears as part of a mixed cell proliferation, erythroblastic-myeloblastic leukemia. These two types also differ from other acute leukemias in clinical, cytologic, cytochemical, histologic, and ultrastructural details. The appearance of such mixed cell variants of acute myeloid leukemia supports the concept of a common progenitor cell for all myeloid tissue elements.—J.V.


Clinical, hematologic, and special studies including blast transformation and determination of surface immunoglobins were carried out in 19 patients with chronic lymphocytic leukemia. Data suggest that chronic leukemia can arise from T-lymphocytes, though much less frequently than the more commonly observed leukemias of B-lymphocyte origin.—J.V.


The results of whole-body gamma radiation therapy in 45 patients with chronic lymphocytic leukemia were summarized. The conclusion was drawn that the most rational total focal dose was 800–2000 rads on the spleen and 1500–3000 rads on the lymph nodes. The best results were obtained in patients with the increase in size of the spleen and/or no more than two groups of lymph nodes. In patients with generalized lymphadenopathy the effect was of short duration. Simultaneously with the decrease in total lymphocyte count, an increase in the blast transformation index (under the influence of phytohemagglutinin) was observed. In the authors’ opinion, this testifies to a greater vulnerability of the leukemic lymphocytes to gamma radiation. Gamma radiation therapy did not exert any depressing influence on erythro- and thrombopoiesis. In case of infectious complications it was necessary to use large doses of wide-spectrum antibiotics, transfusions of blood, plasma or infusions of antistaphylococcal immune globulins.—G.A.

HEMOSTASIS


Of 1837 patients treated at U.K. Hemophilia Centres in the period 1969–71 inclusive, 88.5% had hemophilia and 11.5% Christmas disease. Jaundice occurred in 3.48% of these patients, the average annual incidence being 1.83%. Episodes of jaundice seem just as likely to follow the use of cryoprecipitate or freeze-dried plasma. Antibodies directed against Factor VIII occurred in 6%–7% of patients but showed neither a familial tendency nor a tendency to increase in proportion with subsequent treatment.—J.A.W.

Alternate-pathway Complement Activation by IgA in Schoenlein-Henoch’s Syndrome. E. H. Baart de la Faille-Kuyper,* J. B. van der Meer,*
ABSTRACTS

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Biopsy specimens from purpuric and clinically uninvolved skin of 13 patients with Schönlein-Henoch's syndrome were examined for the presence of IgG, IgA, IgM, fibrinogen, Clq, C4, C3b, proactivator, and IgA-secretory piece. Finely granular deposits of IgA, C3c, C3d, and C5 were found in the walls of superficial capillaries in all specimens. IgM, IgG, C4, and C3b could be located here less frequently, whereas Clq, C3-proactivator, and IgA-secretory piece were not detectable. The concomitant occurrence of IgA, fragments of C3 and C5 in the granular deposits is suggestive of alternate-pathway complement activation. Finally, it is concluded that the immunofluorescence study of skin biopsy specimens can be considered as a useful aid in the diagnosis of Schönlein-Henoch's purpura. —K.P.


Contact product (CP), i.e., the product of the reaction between factor XII and XI, was injected through the saphena vein in virgin female rats, with or without previous intraperitoneal administration of epsilon amino caproic acid (EACA). The 70 rats were divided into four groups: Group 1, 25 rats administered EACA intraperitoneally prior to CP injection; Group 2, 24 rats injected CP only; Group 3, 13 rats injected 0.15 M NaCl and EACA intraperitoneally; and Group 4, eight rats injected 0.15 M NaCl only. At 5, 15, 30, 45, 60, and 90 min after the injection, liver, lung, spleen, kidney, adrenal, and heart were removed and processed for light microscopy. Routine buffered 10% formalin fixed sections were stained with phosphotungstic acid—hematoxylin. Fibrin thrombi were present 5 min after the injection in Groups 1 and 2. The same findings were observed in rats 90 min after the injections. Lung, kidney, and liver were affected in some degree, but we have never seen glomerular deposits of fibrin to an extent comparable to that of the generalized Shwartzman reaction, as in the other organs. Numerous lung capillaries showed aggregation of platelets in their lumen in close contact with the endothelial wall. In control Groups 3 and 4, fibrin thrombi were not found. The findings described above support our previous contention that the injection of CP is capable of eliciting a generalized Shwartzman reaction. —M.J.


Chemotherapeutic agents, particularly 5FU and TIC-mustard in high concentration, appear to inhibit formation of normal fibrin and/or to inactivate Factor XIII. The authors speculate that by doing so these agents may interfere with implantation of metastases. —J.B.S.


Home transfusion for apparent bleeding was initiated in 17 hemophiliacs, using cryoprecipitate, 1 pack/kg of body weight. Fourteen of the patients increased their usage over the previous year by between 12 and 287 packs. The mean increase was 2.2 packs/kg/yr. —J.B.S.


Coagulation profiles were performed on healthy 1-day-old infants delivered at varying gestational ages between 27 and 42 wk. Platelet counts and Factor V levels were in the normal range for all gestational age groups. Factor VIII levels increased with gestational age and were significantly above normal in infants delivered after 37 wk gestation. Factor II levels also increased with gestational age, but at term was still significantly below the mean for normal children and adults. —J.B.S.

IMMUNOHematology

Immunologic functions (PHA response, skin responses to Candida, mumps and streptococcal antigens, immunoglobulins plus complement estimations) were assessed in 11 patients with marrow failure of varying etiology. Scattered deficiencies were found but no correlations existed except between blood monocyte levels and numbers of positive skin tests. The authors believe that some of the infections occurring in patients with marrow failure may be caused by immunologic deficiencies.—F. W. G.


Unlike the patients with leukemia and various malignant neoplasms, in which a depression of tuberculin allergy appears only in the terminal stage of the disease, in the patients with lymphogranulomatosis the suppression of skin tuberculin reaction is observed at the early stage of the disease. The decrease of immunologic reactivity of delayed type in the patients with lymphogranulomatosis is connected with the defective system of immunocompetent lymphocytes. In the patients studied, the tuberculin anergy was independent of the total lymphocyte count. —G. A.

Alteration of Natural Immunity in the Course of Acute Leukemia. T. V. Golosova, V. A. Martyanova, L. G. Kovaleva, E. M. Abakumov, V. V. Alperovich, and G. I. Kozinetz. Central Institute of Hematology and Blood Transfusion, Moscow, USSR. Probl Gematol Pereliv Krovi No. 3:15-20, 1974

Signs of natural immunity were studied in 80 patients with acute leukemia at various stages of the disease. The investigations demonstrated that cellular factors (phagocytic defense and β-lysin) were altered because of the morphologic and functional disturbances of the granulocytes and platelets. On the other hand, humoral antibody formation (in the bactericidal test, agglutination and in the study of lymphocytes in short-term cultures with phytohemagglutinin) was not altered. In the authors' opinion, the use of biologic stimulants which increase the nonspecific resistance of the organism is useful in the complex therapy of acute leukemia. —G. A.


In 54 patients with lymphogranulomatosis (Hodgkin's disease) the total lymphocyte count and the number of immunocompetent lymphocytes (blastic transformation with phytohemagglutinin) were determined. The authors demonstrated that the decrease in number of immunocompetent lymphocytes is already observed in localized forms. This finding may serve as an early diagnostic symptom of the disease. During the second and third stage of the disease a reverse relationship existed between the total number of lymphocytes that decreased and the blastic transformation that was at times normal. In the fourth stage (with dissemination of the disease) both indices were decreased. The impression was gained that variations in the total lymphocyte count in patients with lymphogranulomatosis takes place mainly at the expense of the pathologic nonimmunocompetent lymphocytes. A conclusion was drawn that lymphogranulomatosis represents a malignant process of the thymus-dependent lymphoid system with quantitative and qualitative disturbances of the immunocompetent lymphocytes. —G. A.

Trypsin as a Potent Activator of Immune Phagocytosis by Macrophages. A. O. Lima, M. O. Javierre, W. Dias da Silva, and H. K. Massuda. Division of Immunopathology, Department of Medicine, Federal University of Rio de Janeiro, Medical School and Department of Biochemistry and Immunology, Federal University of Minas Gerais, Medical School, Brazil. Rev Bras Pesquisas Med Biol 6(6):387-392, 1973

Trypsin greatly increases both attachment and engulfment of sensitized sheep erythrocytes by mouse peritoneal macrophages. This enzyme not only increases the number of cells ingested but also the number of macrophages containing engulfed erythrocytes. Several hypotheses were formulated to explain the mechanism by which trypsin exerts these effects.—M. J.

MISCELLANEOUS


Polycythemic hyperviscosity in neonates often mimics cyanotic congenital heart disease.
and frequently leads to seizures and other evidence of CNS disturbance. Eighteen infants with hematocrits ranging between 63% and 77% are reported. In addition to the plethora and cyanosis accompanied by CNS and cardiorespiratory symptoms, many of the infants had mild to moderate thrombopenia, hyperbilirubinemia, hypoglycemia, and abnormal blood smears showing burr cells, fragmented RBC, and normoblastosis. Viscosity values were over 2 standard deviations above the mean for normal infants. Erythrocyte deformability and filterability were abnormal in four of the babies. Twelve infants were treated by removal of 15-22 ml/kg whole blood, replaced with fresh plasma. Postexchange hematocrit values dropped by an average of 11%, and in ten of the 12, whole blood viscosity decreased to the normal range. Of the 14 infants reexamined between ages 7 and 23 mo, two of the 12 who were treated and two of the six who were not demonstrated significant motor and/or mental retardation.—J. B. S.


The authors describe a method for purification of glucose-6-phosphate dehydrogenase from erythrocytes and leukocytes. The advantage of this method is the elution of glucose-6-phosphate dehydrogenase from a CM-Sephadex column by the coenzyme NADP. The method permits an overall yield of 55% and the enzyme preparation is homogeneous from an immunologic point of view. The utilization of leukocytes from patients with chronic myeloid leukemia permits the purification of a quantity of enzymatic material sufficient for physicochemical and structural studies from a single donor. This may be important in studies of certain unstable variants.—K. P.