ERYTHROCYTES


The clinical and hematological features of 39 patients are reviewed. Anemia and jaundice are characteristic; less than a quarter of the patients are sufficiently anemic to need repeated transfusions. The spleen is usually moderately to markedly enlarged and the liver is often slightly enlarged. The bone marrow is strikingly abnormal, with many erythroblasts containing two or more nuclei. The circulating red cells show moderate morphological abnormalities: unlike normal cells, they are strongly agglutinated by anti-i, and lysed by anti-i and anti-I. Moreover, the red cells are agglutinated and lysed by an alloantibody present in many normal sera, but they are not lysed in the patient's own serum. It seems possible that an abnormality of the cell membrane may be the cause of the defective cell division, the failure of the multinucleated erythroblasts to expel their nuclei, and the characteristic serological reactions. - K.P.


A new specific and simple method for the determination of ALA-synthetase activity in human bone marrow cells has been developed. Decrease of ALA-synthetase activity was found invariably in erythroblasts of patients with primary sideroblastic anemia. - K.F.

**Chronic Severe Hemolytic Anemia Due to G-6-PD Charleston: A New Deficient Variant.** E.
ABSTRACTS


A boy with deficient G-6-PD activity and a new enzyme variant, required exchange transfusion for neonatal jaundice, then was apparently well except for scleral jaundice until 8 yr of age when he developed an acute hemolytic episode after which he continued to demonstrate evidence of chronic severe hemolyosis. Interestingly, his mother was heterozygous for G-6-PD A- and G-6-PD Charleston. She had evidence of a mild hemolytic process, and only the A-enzyme could be detected in her red cells.—J.B.S.


Electron microscopic study of the red pulp of spleen and liver from a patient with GPI deficiency hereditary nonspherocytic hemolytic anemia showed phagocytosis of reticulocytes by the splenic macrophages. Erythropagocytosis at the reticulocyte stage, together with a rise in reticulocyte count and a more shortened red cell life span following splenectomy, suggests heterogeneity in GPI content of newly formed reticulocytes. Selective destruction of severely impaired reticulocytes with markedly low GPI levels by the reticuloendothelial system appears to be a cause of hemolyosis.—K.F.

Folic Acid in Blood Serum of Patients With Ulcerative Colitis. W. Tkaczewski, N. Niedzielska, E. Malafiej, and M. Draminski. Department of Infectious Diseases, Faculty of Microbiology and Faculty of General and Physiological Chemistry, Military School of Medicine, Lódz, Poland. Pol Arch Med Wewn 49:129-132, 1972.

The level of folic acid was determined in the blood serum of 20 patients with ulcerative colitis and of ten healthy subjects. The microbiological method using Lactobacillus casei was applied. It was found that the serum level of folic acid depends on the clinical course of ulcerative colitis. Significantly decreased values were observed in subacute forms of the disease (mean 5.93 μg/ml) while there was no difference between the results obtained in patients with the chronic form of ulcerative colitis and in controls. The respective values were 10.38 and 10.9 μg/ml.—M.K.


Bone marrow was aspirated from 28 children with iron deficiency anemia and incubated in autologous serum containing 1 μCi radioactive ferric chloride (59Fe) per ml. Marrow smears were made before and 24 hr after incubation at 37°C and the erythropoietic elements compared. Within 24 hr a rapid differentiation of normoblasts had occurred, oxyphilic forms increasing on the average from 24.2% to 70.5%, while sideroblastic forms were unchanged. Specimens incubated without iron showed little or no effect. Such changes in the rate of normoblastic differentiation, in vitro, can permit the diagnosis of iron deficiency anemia and differentiate other similar anemias in which iron therapy would be ineffective.—J.V.

A two-pool model is suggested for food iron absorption—one heme iron and one nonheme iron pool. The absorption from these two pools can be measured as they can be separately labeled using two radioactive iron isotopes given as hemoglobin and as an iron salt. The new two-pool concept is based on the earlier observation that heme iron is absorbed in a different way than iron salts and on new observations indicating a complete isotopic exchange between an iron salt and the non-heme iron compounds in a number of foods. The present paper reports an almost identical absorption from two radioiron tracers—one biosynthetically incorporated in various foods and the other added as an inorganic salt during preparation of the food. Studies were made on wheat white flour, wheat bran, soybeans, and eggs. Similarly radioiron labeled hemoglobin was absorbed to about the same extent as radioiron labeled veal meat when administered together. Preliminary results are reported on the application of the method to study iron absorption from a homogenized composite meal in eight subjects.


Phosphate compounds of the so-called acid soluble fraction were examined in erythrocytes from patients with virus hepatitis and compared with control values obtained in healthy people. A significant decrease of AMP, NAD, and NADP concentration was detected in erythrocytes of patients with hepatitis indicating a disturbance of erythrocyte metabolism in this disease.


Survival of 51Cr erythrocytes was examined in 15 patients with Wilson’s disease of long duration. Significant shortening of erythrocyte survival was found in nine cases and was accompanied by neither anemia nor overt signs of hemolysis. No correlation was observed between the erythrocyte survival and ceruloplasmin in plasma, copper level in serum, and the 24-hr urinary Cu excretion. The authors consider liver cirrhosis as the main pathogenic factor responsible for increased hemolysis in Wilson’s disease.


This is a case report of a very rare association, which had not yet been described in subjects of European extraction. The propositus is a 17-yr-old man, with a moderate hemolytic syndrome due in part to thalassemia and with prevalent clinical and hematologic signs of hereditary spherocytosis. The trait exists with a different degree of expression in both parents of the patient.


A fascinating family in which four otherwise normal males of the same father and two unrelated mothers had congenital hypoplastic anemia. Two daughters born to the first marriage were unaffected. The father had a history suggesting anemia in childhood. He and the two boys born of his second marriage had elevated levels of fetal hemoglobin. All four boys responded well to steroid therapy. It would appear that in this family, hypoplastic anemia is transmitted by an autosomal dominant gene.


A 15-yr-old boy with a 4½-yr history of hypochromic microcytic anemia unresponsive to iron despite extremely low serum iron levels, erythroid hyperplasia, and absent bone marrow iron, was found to have thrombocytosis, bone marrow plasmacytosis and histiocytosis, and increased immunoglobulin levels. No evidence of blood loss or chronic infection was demonstrable. Physical examination revealed mild
spleenomegaly and GGE. At laparotomy a 3 x 3 x 5-cm mass was found in the presacral pelvis, which contained large quantities of iron and which had the architecture of a distorted lymph node containing angiolymphoid follicles composed of a central capillary with laminated endothelial hyperplasia, surrounded by concentric layers of lymphocytes. There were many plasma cells, as well as hemosiderin-laden macrophages. Within two weeks his hemogram and serum iron studies became normal and remained so, and shortly afterwards he had an appreciable growth spurt. This picture has been described in three of the 75 reported cases of angiomatous lymphoid hamartoma. —J.B.S.


Cord blood erythrocyte filtration deformability is decreased in comparison to the deformability of adult red cells, irrespective of MCV, but correlated with hypoxia and acidemia. The defect appears to be due to an intrinsic red cell abnormality, perhaps related to impaired ATP metabolism. —J.B.S.


A summary of current knowledge concerning the biochemical properties of acetylcholinesterase and the changes in its activity in red cells as they age, as the individual ages, and as seen in diverse disease states. —J.B.S.

LEUKOCYTES


In 72 sibships, each containing a child with ALL, the leukemic children had significantly greater birth weights than expected. It is not clear what significance this phenomenon has, but it would seem that predisposition to malignancy is associated with increased intrauterine growth. —J.B.S.


Two patients suffering from acute leukemia with known hypersensitivity to asparaginase and whose disease was refractory to usual chemotherapeutic agents were desensitized as follows: Asparaginase was injected intravenously at half hr intervals on day 1 (total dose 2500 U) and day 2 (total 27,150 U). From day 3 to 24, the daily dose was about 60,000 U, and fractionated doses were administered at intervals of 1-2 hr (day 3), 3 hr (day 4), 6 hr (day 5), 8 hr (day 6), and 12 hr (days 9-24). Various hypersensitivity reactions occurred transiently in one case who nevertheless had a prolonged remission. No reaction was noted in the second case (but therapeutic effectiveness was not indicated). —J.M.P.


Cyclic variations of the white cell count were recorded for 12 mo in an untreated patient suffering from Ph1 positive, chronic myelogenous leukemia. The 67-74 day cycle was similar to that recorded in similar cases by other authors. In contrast, normal subjects and patients with cyclic neutropenia are known to have much shorter cycles (14-28 days). The authors explain this difference by a prolonged recruitment of stem cells, rather than by an increased marrow transit time of precursors. —J.M.P.


A brief summary of the experience at St. Jude’s in the treatment of childhood ALL from 1962 to 1971. The ineffectiveness of cranial irradiation when compared to craniospinal irradiation with 2400 r, or to cranial irradiation plus intrathecal MTX is described. Which program of “consolidation” therapy or of “continuation” therapy will prove to be best remains unclear. However, half-dose mul-

The activity of lactate and malate dehydrogenase (LDH and MDH) isoenzymes in the leukocytes of 45 children with acute leukemia and of 30 healthy children was measured. Five LDH fractions and four MDH fractions were determined. The activity of LDH3 and LDH4 was greater in the leukemic than in the healthy children. The activity of MDH4 was also greater in acute leukemia. Total LDH and MDH activity was lower in the leukocytes of leukemic children than in the leukocytes of healthy children. Thus, LDH and MDH spectra in leukocytes can be of differential diagnostic value in establishing the variant of acute leukemia. Isoenzymatic spectrum and total activity during complete remission showed almost no difference from the values during the acute period. – G.A.


Twenty-three patients suffering from malignant neoplasms or hemoblastoses with marked leukopenia induced by chemotherapeutic agents and/or irradiation were treated by transfusions of leukemic granulocytes from patients with chronic myeloid leukemia obtained by means of leuko-plasmapheresis. The fate of transfused leukocytes was traced in the recipient’s organism by means of the natural labels (Ph1 chromosome, sex chromosomes (XX,XY), and sex chromatin). In all cases a favorable therapeutic effect was obtained. – G.A.


The potential for proliferation of the “dormant” blast cells in acute leukemia was studied by in vitro culture, autoradiography, and cytochemistry. The percentage of leukemic cells in the S phase and their capacity for reduplication in cases of acute myeloblastic leukemia (AML) was demonstrated by thymidine-3H labeling in bone marrow cultures. These experiments, as well as the investigations of Clarkson et al. and Gavosto (1970), who used in vivo saturation of bone marrow with thymidine-3H, demonstrate that “dormant” blast cells are capable of reentering the mitotic cycle from the interphase (G0). – G.A.


A method for labeling leukocytes in vitro with 99mtechnetium was described. Organ distribution of 99mTc-labeled leukocytes in patients with various diseases was studied by means of gammascintillation camera. Leukocytes separated in ACD solution were incubated with 99mTc for 30 min at 37°C. 0.1 ml
of 100 µg/ml stannous chloride solution in ACD was added to the leukocytes as a reducing agent. After incubation for 10 min at room temperature, the leukocyte suspension was washed twice with physiologic saline, and 20 ml of resuspended labeled leukocytes were infused into the subject. The liver, spleen, and lungs were visualized as a significant concentration site of the marginal leukocyte pool. –K.F.


Electron microscopy of circulating leukocytes from a patient with the Hermansky-Pudlak syndrome revealed two different types of inclusions in a small percentage of monocytic cells. One of the inclusions closely resembled the large masses of lipopigment which fill the bone marrow macrophages in this disorder. The second type was unlike any inclusion previously reported in human leukocytes. –K.P.


Using their recently described (Nouv Rev Fr Hematol 11:799, 1971) phthalate-gradient method for obtaining 99% pure suspensions of neutrophile granulocytes, the authors have measured the activity of glycolytic enzymes, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase and peroxysdase, NADH and NADPH oxdases, and adenylate-kinase in normal granulocytes. All enzyme activities were measured on 3-ml blood samples, and most values were approximately the same as those previously obtained with more complex procedures. –J.M.P.

HEMOSTASIS

Absence per school year fell from a mean of 46 days to a mean of 20 days among 20 hemophiliacs who were on a home self-treatment program using cryoprecipitate, "on indication." The overall usage of Factor VIII in these patients was somewhat increased compared to the pre-home transfusion experience. –J.B.S.


Influence of dinitroortocresol, dinitrobutylphenol, and dinitroisopropylphenol on plasmin, trypsin, and thrombin activity was investigated. These compounds are contained in commercial preparations of herbicides. It was found that all examined dinitroalkyphenols inhibit cascinolyltic and fibrinolytic activity of plasmin. The inhibition of trypsin was less pronounced, and conversion of fibrinogen into fibrin by thrombin was not inhibited by dinitroalkylphenol derivatives. –M.K.

Soluble Fibrin, Fibrinolytic Activity and Fibrin Degradation Products in Experimental Hyperglycemia and Hypoglycemia in Rabbits. J. Kleniewski. Department of Biochemistry, Postgraduate School of Medicine, Warsaw, Poland. Pol Arch Med Wewn 49:337-343, 1972.

Hyperglycemia was induced in rabbits by infusion of 30% glucose, hypoglycemia by intravenous injection of insulin. It was demonstrated that hyperglycemia was associated with a pronounced decrease in plasma fibrinolytic activity, while in hypoglycemia the concentration of fibrin degradation products and of soluble fibrin in blood was found to be increased. –M.K.


A new method for quantitative determination
of soluble fibrin in plasma is reported. Using this method and a staphylococcal clumping test, soluble fibrin and FDP were investigated in the blood of patients with advanced, active RA. Increased concentration of soluble fibrin in plasma was found in approximately 50% of patients while titers of staphylococcal clumping test were higher than in controls in almost all rheumatic sera. No correlation could be detected between the concentration of soluble fibrin in plasma and FDP level, Rose-Waaler test's titer as well as erythrocyte sedimentation rate.—M.K.


Postadjuvant disease was induced in 36 Wistar rats by a single injection of complete Freund adjuvant. Fibrinogen level, staphylococcal clumping test, and semiquantitative determination of soluble fibrin in plasma were performed on 7th, 14th, and 21st days after adjuvant injection. As compared with the control animals in all experimental rats a rise was found in fibrinogen level, titer of staphylococcal clumping test, and in soluble fibrin concentration. The most pronounced changes in examined parameters were detected on the 21st day after adjuvant administration.—M.K.


Serum fibrin/fibrinogen degradation products (FDP) were measured in normal subjects and in 284 unselected patients within 24 hr after admission into an intensive care unit. The tanned red cell hemagglutination inhibition immunoassay was used. The upper limit of normals was 10 μg/ml. All patients with angina pectoris had normal FDP values. Serum levels equal to or higher than 20 μg/ml were found in 28% of the patients, including mainly acute myocardial infarction, cardiac arrhythmias not associated with infarction, acute respiratory failure in chronic obstructive lung disease, and sepsis. Nearly all patients with severe burns, sepsis, and pulmonary embolism had elevated FDP values. In the group of patients having elevated FDP levels, a mortality rate of 36% was found, as compared to 10% in the group with normal FDP values (p < 0.001). In myocardial infarction, elevated FDP levels were associated with a sevenfold increase in mortality, suggesting that the early determination of this parameter could have some prognostic value.—J.M.P.


Plasma kinins were examined in 22 patients with thromboangiitis obliterans and in a control group of 20 healthy subjects. A significant increase of kinin level was found in patients with thromboangiitis (mean 82.7 ng of bradykinine eq/ml) as compared with controls (mean 49.8 ng/ml). The highest values were observed in patients with active vascular process and coexistence of superficial thrombophlebitis. No significant correlations could be detected between the level of kinins in plasma and changes in blood coagulation and fibrinolysis.—M.K.


In vitro and in vivo studies of aggregation of rabbit platelets following exposure to adrenalin, heparin, and adrenalin-heparin complex (AHC) were made. In vitro studies showed that platelet aggregation was enhanced by adrenalin, inhibited by AHC, and unaffected by heparin alone. In vivo studies showed that injections of heparin and AHC both reduced platelet aggregation, this change being accompanied by increased fibrinolytic activity. Injections of adrenalin caused immediate transient increase in platelet aggregation with acceleration of coagulation and thrombin formation times; this was accompanied by a fall in platelets, thrombosis, and sometimes death. There was a second stage in which lessened platelet aggregation and intense fibrinolytic activity was noted.—J.V.

Platelet adhesiveness was found absent in defibrinated blood, but this property could be restored upon the addition of fibrinogen. Adhesiveness was increased in platelets obtained by clot lysis using cadaveric plasma, fibrinolysin, and also industrial fibrinolysin. The products of this lysis, when added to nonadhesive platelets obtained from defibrinated blood, restored adhesiveness to those platelets.—J.V.


Tracheobronchial lymph nodes, removed from cadavers, were homogenized in saline, and after centrifugation the supernatant fluid was used in various tests of blood of coagulation including thrombotest, prothrombin time, prothrombin consumption, recalcification time, heparin tolerance, and fibrinolytic activity. The extracts were found to exhibit high thromboplastic activity and also contained inhibitors of fibrinolysis.—J.V.


Thromboplastin generation tests, performed on the blood of 30 children with various forms of acute leukemia, showed deficiency of the various components not only during the height of the leukemic process but also in remission. This test, yielding information on the coagulation process and on the functional state of the liver, proved useful in assessing the completeness of remission in acute leukemia patients.—J.V.


Twenty patients with thromboangiitis obliterans were treated with intraarterial infusions of 100 mg heparin repeated at 12-24 hr intervals, during 19 to 83 days (on the average 42 days). The observed group comprised 18 men and two women, aged 26 to 36 yr. In 18 cases, the heparin infusions alleviated significantly pain at rest and during exercise and increased the rate of healing of ulcerations. In two patients with advanced secondary changes heparin administration was followed by no improvement.—M.K.


The anticoagulant activity of heparin administered to patients was studied with the diluted activated partial thromboplastin time (APTT) according to Marder. Such standard curves are practical, as we showed that there was no significant influence of interindividual variation. The reproducibility of the test was good with a variation coefficient of 4% both for a single determination and for day to day variation; much better than that of the whole blood clotting time (WBCT), with which it was compared. The APTT test was not influenced by the clinical picture or the type of dilution with normal plasma. The relation of the WBCT prolongation to the heparin activity measured was similar for different individuals. Prolongation of the WBCT by 14-28 min coincides with measured heparin levels of 0.3-0.6 IU/ml plasma. The mean in vivo yield of heparin administered intravenously was 70% of that expected. The biological half-life showed a considerable variation, with a tendency to a left-sided skewness of distribution.—K.P.

IMMUNOHEMATOLOGY


Evaluation of the effectiveness of antilymphocyte globulin was done in 18 patients with
kidney transplants. The antilymphocyte globulin was obtained by immunizing horses with human lymphocytes separated from thoracic duct lymph. Fifty-two other patients with kidney transplants were treated by local irradiation, corticosteroids, azathioprine, and, also, actinomycin D (Cosmegen), without the antilymphocyte globulin. These served as control group. There was no difference between the two groups with regard to number and intensity of organ rejection. The authors concluded that the dosage and type of antilymphocyte globulin employed did not show therapeutic effectiveness. The patients received daily 125 mg of ALG, intramuscularly, for a variable period of time, usually for 14 days. Also because of the severe side effects observed, the use of antilymphocyte globulin was temporarily discontinued at our Renal Transplant Unit. –M.J.


The ultrastructure of lymphocytes of healthy persons was studied before culture and after 1, 2, 3, and 6 days of short-term culture with phytohemagglutinin. Investigations showed that during blastic transformation of the lymphocytes there was a modification in the ultrastructure of nuclear chromatin, an increase in dimension and in the number of the nucleoli which appeared close to the nuclear membrane. In the cytoplasm there was an increase in the number of ribosomes, lysosomes, and lipid granules, and in the Golgi complex. The authors think that these submicroscopic changes are linked with the different growth and division of the lymphoid cells when cultured with phytohemagglutinin. –G.A.

MISCELLANEOUS


A family in which three children died in infancy with pathologic findings characteristic of erythrophagocytic lymphohistiocytosis was studied. Severe generalized hemorrhage with laboratory evidence suggesting DIC was seen in all patients as a terminal event. In the last of these siblings, hepatic uptake of 99mTc-sulfur was nil, and the authors postulate that the failure of hepatic reticuloendothelial function was secondary to the extensive histiocytic infiltration, and that this primed the children for the development of DIC. –J.B.S.


The effect of colchicine on cell division is well known (Eigsti and Dustin, 1955). There are however no studies on the effect of the drug on the frequency of aneuploid cells in the blood of patients taking it as a medication, except for two preliminary notes from our Laboratory (Cestari et al., 1965; Ferreira and Buoniconti, 1968) and a few subsequent ones (Hoefnagel, 1969; Hansteen, 1969; Timson, 1969; Walker, 1969; Ferreira and Frota-Pessoa, 1969). Cestari et al. (1965) reported an increase of tetraploid cells in lymphocyte cultures of peripheral blood of a patient who was under colchicine treatment at the time of conception of a child presenting “atypical Down’s syndrome.” This prompted a quantitative study on the induction of aneuploid cells by colchicine treatment. Lymphocyte cultures from three patients with gout treated with colchicine and from healthy controls of the same sex and age were studied. A significant increase of cells both with tetraploid and peridiploid numbers of chromosomes was found. The peridiploid cells could conceivably originate from nondisjunctions secondary to slight disturbances in the mitotic spindle induced by therapeutic doses of colchicine. The finding of an increased frequency of cells with 47 chromosomes in the blood of each of our three patients (significant in two) suggests that the drug enhances the risk of trisomic offsprings and makes it advisable to refrain from conceiving during colchicine treatment. –M.J.

Red cell indices were determined in rats fed through four generations for 2 yr with plant protein mixtures. Microhematocrit readings, red cell counts, and hemoglobin estimations were made in about 140 animals. Results of animals of two test groups were statistically analyzed to reveal variance between generations and between diets. The values obtained were similar, indicating that in this species the diets did not affect substantially the red cell indices.

–M.J.