ABSTRACTS

Mario Baldini, M.D., Editor

Klaus Betke, M.D., Munich, Germany
Arthur Bloom, M.D., Cardiff, Wales
Thomas H. Bothwell, M.D., Johannesburg, S. Africa
John M. Bridges, M.D., Belfast, Northern Ireland
Jacques Caen, M.D., Paris, France
Pietro de Nicola, M.D., Pavia, Italy
Ludvik Donner, M.D., Prague, Czechoslovakia
Patricia Farnes, M.D., Providence, R.I.
Katsuhiko Fukutake, M.D., Tokyo, Japan
Frederick W. Gunz, M.D., Sydney, Australia
Susan R. Hollán, M.D., Budapest, Hungary
Michel Jamra, M.D., São Paulo, Brazil
Hans-Jürg Huser, M.D., Berne, Switzerland
Sven-Age Killmann, M.D., Copenhagen, Denmark
Frederick A. Klipstein, M.D., San Juan, Puerto Rico
Maria Kopec, M.D., Warsaw, Poland
Guido Lucarelli, M.D., Pesaro, Italy
Georges Mathé, M.D., Villejuif, France
Alexander A. Morley, M.D., Adelaide, Australia
Thomas F. Necheles, M.D., Boston, Mass.
Bracha Ramot, M.D., Tel Hashomer, Israel
Peter G. Reizenstein, M.D., Stockholm, Sweden
Zoran Rolovic, M.D., Belgrade, Yugoslavia
Edgardo S. Sack, M.D., Buenos Aires, Argentina
Julian B. Schorr, M.D., New York City
Manfred Steiner, M.D., Ph.D., Pavtucket, R.I.
John E. Ultmann, M.D., Chicago, Ill.
John Vaughn, M.D., Savannah, Ga.
Martin C. Verloop, M.D., Utrecht, The Netherlands
Ralph O. Wallerstein, M.D., San Francisco, Calif.
Harvey J. Weiss, New York, N.Y.

ERYTHROCYTES


The influence of pH on the contributions of the Embden-Meyerhof and of the oxidative pentose phosphate pathways in the utilization of glucose by human erythrocytes in the presence and absence of methylene blue were investigated. In the absence of methylene blue, NADP+ and phosphofructokinase are controlling factors. At pH 7.0, the oxidative pentose phosphate pathway contribution is nearly one-half of the metabolic flux. This contribution decreases with increasing pH. In the presence of methylene blue, the limitation by the NADP+ concentration disappears. At pH values of 7.4 and below, the phosphofructokinase is inhibited. The limitation of the glucose influx resides in the hexokinase. At pH 8.2, both the Embden-Meyerhof and the oxidative pentose phosphate pathway participate in the metabolic flux, since the inhibition of the phosphofructokinase is released.—M.C.V.


A mild hemolytic anemia characterized by vitamin E deficiency, an abnormally elevated in vitro red cell H2O2 fragility, and reticulocytosis may occur in the second month of life in premature infants. One role of vitamin E is thought to be that of antiperoxidant at the cellular level. The oral administration of ferrous sulfate aggravated the anemia and reticulocytosis. The simultaneous administration of vitamin E reduced the reticulocytosis and anemia. Valent iron may act as a catalyst in the nonenzymatic autoxidation of unsaturated fatty acid of the red cell membrane. Oral
iron supplement in prematures should probably be deferred until the third month of life. One wonders if iron dextran, which remains in its complexed state while in circulation, might be free of this hemolysis aggravating effect of valent iron.—R.O.W.


The differential diagnosis of a patient with jaundice and fulminating hemolytic anemia is discussed. E. coli sepsis was present probably caused by cholangitis. Adequate control of the sepsis with antibiotics improved but did not arrest the hemolytic process. Thereupon, laparotomy was performed, and the bile ducts were drained. A liver biopsy showed signs of cholangitis and extrahepatic cholestasis. The patient made a complete recovery after the operation.—M.C.V.


In a cooperative study from six cities in the USA, liver samples were obtained from 259 individuals who died suddenly from trauma or disease. Analyses for nonheme iron results were expressed in mg/100 g of liver tissue (wet weight). Mean value in men over 20 yr of age was 23 mg/100 g, representing approximately 300 mg in the liver. Abstractor's comment: Stainable iron implies at least 10 mg/100 g. In women and younger men the mean value was 12-16 mg/100 g. Evidence is presented that levels of 5 mg or less indicate iron deficiency. In males, values of less than 5 mg occurred in fewer than 10%; in females of menstruating age the incidence was 40%.—R.O.W.

Hemoglobin was labeled with Na$_{51}$CrO$_4$ in red blood cells or in hemolysates. Labeled hemoglobin was dissociated into chains with p-chloromercuribenzoate. Chains were separated by electrophoresis on starch gel (Tris-EDTA-borate buffer, pH 8.6). Both in hemoglobin from the labeled red blood cells and from labeled hemolysates about 90% of $^{51}$Cr recovered was bound to the $\beta$-chains. Labeled $\beta$-chains, like labeled hemoglobin A, have greater electrophoretic mobility than unlabeled $\beta$-chains and hemoglobin A. —M.K.

LEUKOCYTES


Ultrastructural analysis of the formation of cytoplasmic granules in neutrophils of normal mink and of mink with a homologue of the Chediak-Higashi syndrome (C-HS) has revealed that the abnormal granules present in the neutrophils of mink with C-HS originate through an aberration in the genesis of primary (azurophil) granules. During the early stages of cell maturation in the bone marrow, the primary granules in neutrophils of mink with C-HS undergo an abnormal process of fusion that results in the formation of a relatively small number of enlarged atypical granules. Investigation of the other cells in the bone marrow and peripheral blood of normal mink and mink with C-HS has demonstrated that the genesis of abnormal granules is similar to that observed in neutrophils.—J.E.U.

Human Leukemic Cells: In Vitro Growth of Colonies Containing the Philadelphia (Ph$^1$) Chromosome. P. Chervenick, L. Ellis, S. Pan, and A. Lawson. University of
Human leukemic cells with a marker (Philadelphia; Ph1) chromosome gave rise to granulocytic and mononuclear cell colonies when grown in vitro. All metaphases from a single colony were either Ph1 positive or Ph1 negative. No colonies contained a mixed cell population. This suggests that leukemic and normal cells exist simultaneously and that in vitro colonies are clonal in origin.—J.E.U.


The report describes the fine structure of crystalline inclusions detected in myeloma cells and renal tubular cells of a patient with multiple myeloma. (1) Crystalline inclusions in the myeloma cells: The examination of bone marrow smears revealed in the cytoplasm of the myeloma cells numerous stick-shaped inclusions with hyaline appearance that were heavily stained with May-Giemsa stain. In the electron microscopic picture, the cross-sections of these “sticks” were found to be hexagonal in shape and variable in length. It was interesting to note that the sticks were always enclosed by a limiting membrane without ribosomes. With higher magnification, fine structure of the inclusions showed the parallel line or honeycomb-latticed network appearance, which consisted of the dense and light lines with periodicity of about 60Å-90Å (average 75Å). (2) Crystalline inclusions in the proximal tubules of the kidney: It was revealed from electron microscopic studies of biopsied kidney specimens that tubules contained numerous granular, hexagonal, rhomboid or rodlike electron-opaque inclusions but were scant of microvilli and basal intussusceptions. Large amounts of reaction products showing acid phosphatase activity were found around and in each inclusion. Two types of inclusions with or without a limiting membrane were detected. On higher magnification, the inclusions showed similarity in the fine structure with those in myeloma cells. From the findings noted above, it was postulated that crystalline inclusions in myeloma cells might be coacervates of light chains secreted from Golgi areas, and the inclusions in tubular cells might be coacervates of Bence Jones proteins that were reabsorbed from the proximal tubules. The inclusions in tubular cells might become changed in shape by the enzymatic action of lysosomes.—K.F.


Leukocyte kinetic studies in patients with chronic myelocytic leukemia (CML) were investigated using the DF32P in vitro and in vivo labeling methods and liquid scintillation counting by the 3H-thymidine (3H-TdR) in vivo labeling method. The results were compared with values for normal patients. The life span of granulocytes labeled in vitro with DF32P was obtained in 16 studies of 13 patients with CML. In relapse, granulocyte specific activity disappeared slowly. In remission, it returned to the normal range. Pool-size indices (TBGP, CGP, and MGP) and GTR increased remarkably in relapse, but these parameters returned to normal with suitable treatment. Granulocyte specific activity, labeled with 3H-TdR in vivo in patients with CML, was characterized by an initial high level followed by the highest peak at the 36th hr and the second major peak on the seventh day. The blood from patients with CML in relapse labeled with DF32P in vitro was transfused into a hematologically normal patient with carcinoma. GTR t½ value was slightly prolonged. Normal blood, on the other hand, labeled with DF32P was transfused into the patient with CML in relapse, and the life span was normal. Leukokinetik, by labeling with 3H-DFP in vitro was carried out by radioautography for each morphologic type of granulocyte and by liquid scintillation counting for all leukocytes. Myelocytes disappeared most slowly from the peripheral blood, and segmented neutrophils disappeared rapidly. The reason for
the markedly prolonged survival of granulocytes in patients with CML was discussed. It would be suspected that environmental factors in CML in relapse, as well as the presence of immature granulocytes in the peripheral blood and the increased granulocyte pools, played an important role.—K.F.


A patient with chronic myeloid leukemia is reported in which a reciprocal translocation between the long arms of two G chromosomes is the most likely explanation for the origin of the Philadelphia chromosome. In all metaphases analysed, the enlargement of one G chromosome (G/G) was impressive enough to draw prompt attention. The area measurement confirmed this visual observation. The most probable explanation for the origin of this chromosome, occurring together with a Ph1, is a translocation between the long arms of two small acrocentrics. This assumption is strengthened by the small differences between the values for c+G and G/G+Ph1 chromosomes. This observation represents evidence that a reciprocal translocation can originate the Ph1 chromosome and that it can be detected when involving at least two chromosomes of group G. This mechanism obviously could involve a G21 chromosome and another chromosome of the group.—M.J.


Alloantiserum was administered to seven patients with lymphoproliferative disorders to assess its in vivo lymphocytotoxic potency and possible side effects. Six patients responded with drops in lymphocyte and platelet counts averaging 48.8% and 25.5% of pretreatment values. Hemoglobin rose an average of 16.8%. Effects were maximal by 45 min and lasted less than 1 day. Two of five patients had shrinkage of peripheral lymphatic tissue, lasting 1–3 days. Liver and renal function and uric acid generally remained unchanged. Four patients had minor side effects including fever, chills, nausea and vomiting, and back pain. The two patients with the highest initial white blood counts also suffered short-lived respiratory distress; one developed frank pulmonary edema and temporary renal decompensation.—J.E.U.


This study evaluates the cyclic use of a combination of four drugs, cyclophosphamide, vincristine, procarbazine, and prednisone, in 12 patients with generalized lymphosarcoma (LSA) and in 11 patients with reticulum cell sarcoma (RCS). Seven patients with LSA achieved a category I response, and of these, four are clinically free of disease. In the RCS group, only one achieved a category I response. Myelosuppression was severe in four patients (17%) and may have contributed to their deaths from infectious complications. A high incidence of extranodal involvement in this group of patients appeared to correlate with the low incidence of useful therapeutic responses.—J.E.U.


Two patients with a chronic hypereosinophilic syndrome of 11 and 17 yr duration are described. In both, there was evidence of multisystem involvement leading to pulmonary insufficiency and hepatosplenomegaly. One patient apparently has significant cardiac involvement. No identifiable etiology has been found, and there has been no significant response to a variety of therapies. These cases do not fall into a readily identifiable syndrome. It is believed that they represent a chronic, relatively benign form of syndrome characterized by peripheral eosinophilia and multisystem eosinophilic
infiltration. The exact role of the eosinophil in this syndrome and its cause or effect relationship are unknown.—A brief report with a good discussion.—J.E.U.


Two patients with systemic mastocytosis are described. One patient showed generalized osteoporosis. The bone marrow of both patients contained slight mast cell proliferation. After removal of an enlarged spleen with massive mastocytosis, there was evident diminishing of complaints in one of these patients.—M.C.V.


ASAC activity was examined by a histochemical method in lymphocytes cultured in vitro. On the third day of culture, 33.8 ± 15.1% of blastlike cells showed high ASAC activity, 55.5 ± 19.5% moderate activity, and 10.7 ± 6.9% showed no activity.—M.K.


Results obtained in almost 1000 cases of acute lymphatic leukemia in children are reviewed. The rate of remission was about 50%. Chronic lymphatic leukemia did not respond at all. The authors assume that cases with immunocompetent cells are the only ones that may respond to the treatment and that L-asparaginase should be considered as a simple immunodepressing agent. Abstractor's comment: Short review, with ample bibliography and clear evaluation of the problem.—P.d.N.


It was previously shown that heparin causes an increase in viscosity of suspensions of nucleated cells whose membrane has been injured. In the present work, it is demonstrated that heparin affects the lymphoid cell even if the cell membrane has not been injured before the experiment. Thus, it causes morphologic changes in the cells and liberates DNA into the medium. The liberation of protein-bound radioactive chromium has not been observed.—M.K.


Case report of a 44-yr-old male with Hodgkin’s disease and hypergammaglobulinemia with a myelomallike pattern (45.09%) and with bone marrow invasion by abnormal cells is reviewed. Immunologic analysis revealed the presence of a gamma-G, k type. The case should be considered because of its rare occurrence in Hodgkin’s disease.—P.d.N.

HEMOSTASIS


Lyophilized ellagic acid in vitro caused an increase of blood coagulability in normal samples, as shown by the thromboelastographic examination, and was able to normalize, completely or in part, the thromboelastographic patterns of patients with coagulation defects. These modifications were similar, though less marked, to those observed with ellagic acid dissolved in phosphate buffer. In dogs given a $10^{-4}M$ solution of lyophilized ellagic acid, an increase of blood coagulability took place. Similar find-
ings were observed in normal subjects given 7 ml/kg of such a solution and in hemophiliacs in whom the effect of a plasma transfusion was considerably enhanced. No side effects were evidenced. Abstractor’s comment: These investigations represent an interesting approach in the treatment of coagulation defects, as well as a further development in the clinical applications of ellagic acid. —P.d.N.


Ten patients with idiopathic thrombocytopenia and five with acquired hemolytic anemia were examined. The activity of 5'-nucleotidase and ATPase of megakaryocytes with undivided and divided nuclei in bone marrow smears was determined using cytoenzymatic methods. Slight enzymatic activity in the former and strong activity in the latter type of megakaryocytes was found. This was connected probably with the maturation of cells and the process of “shedding off” the platelets. No difference was found between two examined groups of patients. —M.K.


Influence of some vasoactive drugs (papaverine, ephyloline, Complamin, nicotinic acid) in vitro on platelet aggregation produced by adenosine diphosphate (ADP) was investigated. Papaverine and ephyloline were found to inhibit platelet aggregation. Nicotinic acid increased platelet aggregation, and Complamin had no significant influence on this process. —M.K.

Role of the Lymphocyte in Antihemophilic Globulin Production: A Rise in Antihe-

The best technical conditions for producing large and well-delineated precipitation rings are given on the basis of comparative investigations on the immunodiffusion technique and of the caseinolytic assay: 4% antiserum concentration in agar, 2 µl of plasma applied to the strip, and 24-36 hr diffusion time. A good correlation between the two methods was found in normal persons. In some cases, the values obtained by immunodiffusion were higher.

-P.d.N.


A study of 51 cases is reported. With the decrease of the prothrombin complex, a more marked deficiency of factor VII than of factor V was found. Prothrombin consumption and thromboplastin formation (TGT) were impaired, and the bleeding time was prolonged. No significant modifications of clotting time, recalcification time, clot retraction, and platelet count were found. Some correlation between the degree of liver cirrhosis and the coagulation defect was found, while there was no correlation between the degree of the coagulation defect and the severity of the bleeding tendency.

-P.d.N.


Five of 25 patients with hemophilia B had a grossly prolonged one-stage prothrombin time with ox brain thromboplastin. It was shown that this was due to the inhibitory activity of plasma from patients with hemophilia B+ and not to prothrombin complex deficiency. Some data are presented indicating that this inhibitor has competitive character and probably interferes with the reaction involving factor VII and ox brain thromboplastin.-M.K.


Previous observations (Vanacore, C.: Progr. Med. 22:418, 1966) had shown that in relapsing, idiopathic ocular hemorrhage (Eales' disease) abnormalities of hemostasis are present. At the time of the hemorrhagic episode, a diminution of prothrombin consumption and of capillary resistance was evidenced. Thirty-seven cases were investigated in this study, including also a few patients with diabetes and with hypertension. Inhibitors of the early stages of thromboplastin formation (on the basis of indirect criteria: no assays of individual factors were carried out) were described.

-P.d.N.


Forty patients with acute, nonmyeloblastic leukemia were studied by the method of inhibition of tanned human red cells agglutination. In 12% of cases with no signs of increased fibrinolysis, there was a definitely abnormal increase of fibrinogen degradation products. Abstractor's comment: This study shows that such phenomenon is relatively rare in acute, nonmyeloblastic leukemias, in contrast to acute myeloblastic leukemias in which it is almost constant.

-P.d.N.


This paper presents an extensive evaluation of hemostasis in five family members...
who developed severe bleeding during an outbreak of epidemic hemorrhagic fever. Studies of hemostasis were performed immediately after the beginning of the hemorrhagic manifestations and were then followed during the course of the disease. The evidence presented shows that the syndrome of disseminated intravascular coagulation was the cause of the hemostatic defect in the patients. The biphasic changes of some coagulation tests were also noted, i.e., an early depression was followed by an activation, and vice versa. This observation has been postulated to be also a reflection of disseminated intravascular coagulation.

—Z.R.


A life-long bleeding disorder in two brothers characterized by abundant epistaxes and bruises was identified as a Glanzmann type of thrombasthenia. The diagnostic criteria included: normal platelet count, prolonged bleeding time, impaired clot retraction, and failure of the platelets to adhere to glass and to aggregate in the presence of ADP, thrombin, adrenalin, and collagen. On the other hand, their thrombo-plastic activity was normal. No abnormality of hemostasis was observed in the parents, except for a decrease in capillary resistance. A bleeding tendency was not recorded in any of the relatives. The pedigree of the parents revealed consanguinity with a common ancestor in the fifth generation. This finding was consistent with an autosomal recessive mode of inheritance of the platelet defect in Glanzmann’s thrombasthenia. Abstractor’s comment: This is the first completely documented case of Glanzmann’s thrombasthenia so far reported in Yugoslavia.—Z.R.


Five activated and three nonactivated partial thromboplastin time methods were studied on a pool of normal plasmas and on lyophilized control plasmas. The activated partial thromboplastin method gave the best results. The optimal incubation time was 5–6 min. Pools of normal plasmas were better than lyophilized commercial plasmas, in which factors V and VIII may be decreased. No significant variations were observed between citrated and oxalated plasma. Reagents proved to be stable at +4°C after reconstitution, as indicated by the manufacturers.—P.D.N.


A case report in a 10-yr-old girl with neither adhesiveness nor aggregation of platelets is presented. A slight reduction of platelet factor 3 and normal activity of other platelet factors were observed. There are only a few cases of this type in the literature.—P.D.N.

IMMUNOHEMATOLOGY


Three hundred and four Bantu-speaking black people (presumably Zulu because they lived in Natal) were studied for four serum protein groups. No variation was found in the case of albumin, confirming the belief that albumin variants are rare in black populations. There seem to be more alleles at the ceruloplasmin locus in black people than in white people, and the $Cp^b$ gene had a frequency of 0.035 in this study, somewhat lower than in U.S. black people. Three individuals heterozygous for $Cp^b$ and $Cp^{NH}$ were encountered. Gene frequencies at the transferrin and haptoglobin loci were similar to those found in previous studies: $Tf^{D1}$, 0.040 and $Hp^1$, 0.514. Anhaptoglobinemia was found in 3% of subjects, and 24 showed
the Hp 2-1 modified phenotype. Haptoglobin subtyping was carried out on 112 Hp 2-1 samples giving an Hp1S frequency of 0.365, over twice as high as Hp1F; in white populations the ratio is reversed and in Mongoloids Hp1F is virtually nonexistent.—T.H.B.


Four horses immunized with 26.8–95.3 × 10⁹ human lymphocytes given in between three and eight doses produced marked increases in the titers of antihuman red cell activity as judged by various test procedures. Absorption of this antired cell activity by human red cells resulted in the removal of lymphocyte antibodies as well, confirming the fact that red cells and lymphocytes share common antigenic determinants. The in vivo effects of ALG on the red cells of a baboon were shown to be due to potent antired cell antibodies, because the “generalized anemia and potentially dangerous thrombopenia” were not produced when the same ALG had been repeatedly absorbed with red cells prior to administration. Serum complement levels were lowered in the animal receiving toxic preparations of ALG; this was thought to be due to complement utilization by antigen-antibody interaction. It is suggested that subcellular components of the lymphocyte should be used as the antigenic stimulus for producing a nontoxic immunosuppressive agent.—T.H.B.


The influence of actinomycin C on production of 19S hemolysin-producing cells (plaque-forming cells, PFC) in mice was found to be dependent mainly on the time of injection of actinomycin C and its total dose. The strongest inhibitory effect was obtained when actinomycin C was injected before the antigen.—M.K.


Blastic transformation of lymphocytes stimulated by PHA in vitro cultures was examined in 22 patients with isolated enlargement of mediastinal lymph nodes. The percentage of blastic and mitotic forms was calculated in 3-day-old cultures. The lowest values were observed in malignant lymphoproliferative diseases (eight cases of Hodgkin’s disease, three cases of lympho- or reticulosarcoma) and the highest in tuberculosis (three patients) and sarcoidosis (three patients). The blastic transformation was impaired in five patients with cancer metastases to mediastinal lymph nodes, but the values obtained were higher than in lymphoma.—M.K.


The authors studied 16 infants with the hemolytic-uremic syndrome (HUS). Coagulation and platelet studies did not suggest the presence of disseminated intravascular coagulation. The authors suggest that the HUS is an antigen-antibody mediated reaction with the kidney as the target organ. The pathogenetic mechanism may be similar to that which occurs with hyperacute renal allograft rejection.—H.J.W.

Sixteen cases of autoimmune hemolytic anemia were classified into five serologic types depending on the globulins found on the erythrocytes (IgM + C, IgG, IgG + IgM + C, C). The fifth type included cases in which the globulins on the erythrocytes could be detected only with human antisera. No correlations were observed between the individual types and the course of the disease. Determining the survival type of isologous erythrocytes, it was found that the above serologic investigations may be helpful in the choice of appropriate blood for transfusion.—M.K.


Lymphocytes of peripheral blood were examined in 38 control healthy people, 63 patients with chronic lymphatic leukemia (CLL), 35 with Hodgkin’s disease (HD), and 28 with aplastic anemia (AA). Three methods for blood smear staining were used, the panoptic method with methylene blue, staining with acridine orange, and staining for nucleoli examination according to Stockinger and Kellner. Blastic transformation of lymphocytes in in vitro culture with PHA was investigated, and percentages of blast cells and mitotic forms were calculated. No correlation could be demonstrated between morphologic changes observed in blood smears, particularly in nucleoli, and the impairment of blastic transformation. It is stressed that in CLL, when the number of leukocytes containing nucleoli and showing homogenous fluorescence was increased, the blastic transformation in vitro was markedly diminished. In active stages of HD, the blastic transformation of lymphocytes was impaired, and the structure of nuclear chromatin was blurred.—M.K.


Determination of plasma cholesterol, phospholipids, and glycerides in blood stored in ACD was performed and compared with the respective values of blood containing dimethyl sulfoxide, stored at various temperatures. A significant rise of plasma cholesterol, phospholipids, and glycerides of blood stored at +4°C with dimethyl sulfoxide was observed when compared to blood stored at +4°C in ACD medium. Storage of blood in the frozen state at −196°C, after short incubation with the preserving solution containing dimethyl sulfoxide, significantly decreased liberation of lipids from the red cells. The hypothetical mechanism of action of dimethyl sulfoxide on the blood cells is discussed.—M.K.