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ERNST R. JAFFÉ, M.D., Editor

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LEUKOCYTES


Pseudohyperkalemia was described in 2 patients with chronic granulocytic leukemia. The elevated serum potassium developed in vitro during coagulation and did not reflect the plasma potassium concentration existing in vivo. In contrast to all previously reported cases, the white cells and not the platelets or red cells were shown to be the probable source of the excess potassium in the patient’s sera.—T. E. B.


Blood samples were obtained at different time intervals after the administration of uridine-H3. High resolution autoradiography was employed. RNA labeling of the blast population appeared to be very stable. This finding was considered to be due to true metabolic stability, to continuous intracellular resynthesis of the metabolites of the labeled macromolecules or to reutilization of the extracellular degradation products. Early nuclear and possible nucleolar synthesis of RNA were postulated.—P. d. N.


Polymorphonuclear leukocytic granules were investigated by examining with the electron microscope developing granulocytes from normal rabbit bone marrow which had been fixed in glutaraldehyde and postfixed in OsO4. Two distinct types of granules, azurophil and specific, were distinguished. Azurophil granules were larger and more dense, were formed only during the promyelocyte stage and seemed to arise from the concave face of the Golgi complex. The specific granules were smaller and less dense, were formed during the myelocyte stage and seemed to arise from the convex face of the Golgi complex. Polarity of the Golgi complex appears to have been demonstrated. Only azurophil granules were found in promyelocytes, but in mature cells about 85 per cent of the granules were specific and only 15 per cent were azurophilic. No evidence was obtained to support past suggestions that azurophil granules
degenerate, are discharged from the cell or are transformed into specific granules. The azurophil and specific granules seemed to represent two separate lines, distinct morphologically from the time of their formation. The authors suggested that the number of azurophil granules per cell decreases simply because of mitotic division occurring after the production of azurophil granules has ceased.—T. E. B.


A reference is always needed in comparative investigations of the PAS reaction. The microdroplet model, involving both microinterferometric x-ray absorption and microspectrophotometric measurements, is relatively complicated and time-consuming and is used mainly for estimating the absolute amount of PAS-reactive material in neutrophils. As information about the relative amount of PAS-reactive material is equally valuable, it has been suggested that human neutrophils might be suitable as references for the PAS reaction.—O. P. J.


Lysozyme activity of the various formed elements of the peripheral blood and bone marrow was evaluated by an indirect histochemical technic utilizing the capsular swelling of Micrococcus lysodeikticus. In peripheral blood, segmented and band neutrophils and monocytes consistently demonstrated the greatest amount of lysozyme activity. There was some evidence that lysozyme plays an important role in the intracellular destruction of certain phagocytized bacteria. The importance of this enzyme in overall bacterial defense mechanisms, however, remains unclear.—O. P. J.


Morphologic evidence strongly suggests a dual origin or source of the lymphocytes in the developing appendix and tonsil iliaca of the rabbit: a population derived originally from reticular cells and a population derived extra-appendiceally, possibly of thymic (epithelial) origin.—O. P. J.


Starch gel zymograms were used to chart the effect of standard test meals on the non-specific esterase isozymes of rat intestinal lymph. Using a-naphthyl butyrate as substrate. The serum was studied in a similar manner and the results were compared. A marked increase in esterase activity, largely confined to two molecular fractions, occurred in the intestinal lymph and later in the serum, following a meal of corn oil. The fat meal had no effect on the esterase activity of bile, although the presence of bile was essential for increased esterase activity to occur in the intestinal lymph.—O. P. J.


Reports on the ultrastructure of circulating monocytes and macrophages from various sources have been published, but not studies of the transformations of these cells. This paper presents the results of an electron microscope study of the changes observed in blood monocytes in tissue culture and it deals with changes in cytoplasmic organelles, phagocytosis and the formation of multinucleated giant cells.—O. P. J.


A study of the number of leukocytes and their osmotic and mechanical resistance after storage in solutions of glucose-citrate, glucose-citrate with addition of the antihistamine, analergin sulfate, and in glucose-saccharose was carried out over a period of 22 days. The properties studied were practically uninfluenced by analergin sulfate. Addition of saccharose significantly retarded dis-
integration of leukocytes and improved their mechanical resistance.—L. D.

ERYTHROCYTES


Erythropoiesis was megaloblastic in 13 of 18 patients with overt tropical sprue and in 3 of 11 asymptomatic subjects. This abnormality was secondary to deficiency of vitamin B₁₂ in every instance and was associated with folate deficiency in 6 patients. Every person studied had malabsorption of vitamin B₁₂, but folate absorption was usually normal. The factors responsible for the surprisingly low incidence of malabsorption and deficiency of folate in patients with tropical sprue in this country remain unexplained.—F. A. K.


Assay of serum folate levels in 225 patients who had either acute or chronic leukemia, lymphoma or multiple myeloma showed subnormal values in many patients with active disease and normal values in the majority of cases in partial or complete remission. No such correlation between the status of disease and serum vitamin B₁₂ levels was found—F. A. K.


Twenty-seven patients with malignant blood diseases in remission were studied. In chronic myeloid leukemia and in Hodgkin’s disease, a significant reduction in the mean values for basal serum folate activity and for serum folate activity and urinary folate output after folic acid loading was found. In chronic lymphatic leukemia, only after folic acid loading was urinary folate output significantly lower than normal. Urinary excretion of histidine derivatives after histidine loading was higher than normal in Hodgkin’s disease and in chronic lymphatic leukemia. Folate deficiency was observed in 16 patients. Various types of deficiency were postulated (tetrahydrofolate, primary folate, folate metabolic abnormalities). Folate deficiency was not correlated with other parameters of disease.—P. d. N.


The plasma clearance of folic acid was abnormally rapid in 23 and the urinary excretion of FICLu excessive in 10 of 25 patients with neoplastic disease. Treatment with folic acid reduced FICLu excretion, but did not normalize the clearance rate of folic acid. The authors attributed these abnormal findings to an increased demand for folic acid in neoplastic disease.—F. A. K.


Maximal vitamin B₁₂ absorption of from 76 to 89 per cent occurred following ingestion, by healthy volunteers, of a meal containing 3 μg. of Co⁶⁰B₁₂ incorporated into ewe meat. Previous intake of the vitamin within 4 to 6 hours did not interfere with subsequent absorption.—F. A. K.


In the method presented, serum was added to a complex of intrinsic factor-radioactive vitamin B₁₂ and the resultant antigen-antibody complex was precipitated with ammonium sulfate. The radioactivity in the precipitate was found to be proportional to the amount of antibody present in the serum.—F. A. K.


The staining characteristics of cytoplasmic lactic dehydrogenase were similar in normoblastic and megaloblastic bone marrow preparations. The authors construed these data to support the hy-

Despite a high incidence of siderosis in the Bantu, evidence of iron deficiency was present in only 4 per cent of a group of 50 pregnant Bantu females who were anemic. This finding reflected the fact that, whereas siderosis is common in males, it is rare in young females in this population. Thirty-six per cent of the patients studied showed evidence of folate deficiency, but vitamin B₁₂ deficiency was present in only 4 per cent.—F. A. K.


Siderocytes, prepared by treatment of swine with pyridoxine-deficient diets or by inducing rapid blood regeneration with phlebotomies, were different. Pyridoxine-deficient siderocytes were not reticulocytes, were “pitted” by the spleen in vivo. They were increased markedly in the peripheral blood of splenectomized animals and were not altered by incubation in vitro. In contrast, after phlebotomy, siderotic granules were present within reticulocytes, disappeared equally well in vivo in the presence or absence of the spleen and disappeared upon incubation in vitro. It was proposed that with defective heme synthesis (pyridoxine deficiency), iron unavailable for incorporation into heme was stored in a nonmetabolizable form, possibly in mitochondria, and was removed or dissipated by the spleen. With increased erythropoiesis, terminal maturation divisions of narrow normoblasts may be eliminated and reticulocytes whose hemoglobin synthesis is incomplete may be released. Iron granules may be utilized for heme synthesis while the cell circulates and siderotic granules may disappear rapidly, irrespective of the spleen.—H. S. J.


The morphologic aspects of the iron cycle in the spleen of the newt were studied with the electron microscope. Reticular cells from the spleen were found to have, simultaneously, siderosomes with iron in the form of ferritin and others with iron in the form of irregular micelles (true hemosiderin). Siderosomes, containing some “true” hemosiderin in which the iron no longer appeared to be organized in the form of ferritin, seemed to constitute an obligatory step in the iron cycle. In this manner, the iron, after denaturation of apoferritin, gradually may be liberated and can be utilized through the circulatory system.—O. F. J.


Red cell membranes, prepared either in the presence or absence of Mg⁺ + and then disrupted by sonic vibration or lipid-active agents, have been analyzed for glyceroldehyde-3-phosphate dehydrogenase (GAPD) and phosphoglcerate kinase (PGK) activities. Membrane disruption in the absence of Mg⁺ + stimulates PGK, but not GAPD, activity. Both activities are stimulated by the presence of Mg⁺⁺. The author suggests that these enzymes are oriented oppositely in the membrane, GAPD toward the interior and PGK towards the lipid core. These two enzymes generate ATP and it is postulated that their spacial orientation might function to provide ATP for ATPase which also is a resident of red cell membranes and is responsible for cation pumping.—H. S. J.


A new electrophoretically abnormal variant of G-6-PD was described in a Scotch-Welsh family. Some of whose members had red cells deficient in G-6-PD activity. No tendency toward hemolytic anemia was noted, even in one patient given nitrofurantoin. Glutathione stability tests were only mildly abnormal. The absence of symptoms, despite significant reductions in overall enzyme content, was unexplained, although it may have been related to the abnormally low Michaelis constant.
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and, therefore, increased reactivity of the abnormal enzyme.—H. S. J.


Agglutination of normal washed red cells by a saline extract of fava beans was inhibited by several simple sugars in high concentration (glucose, maltose and fructose). Previous work from this laboratory (J. Lab. Clin. Med. 56:695, 1960) had demonstrated that serum, except from one patient with favism, inhibited hemagglutination in vitro by fava bean extract. Since a concentration of 600 mg. per cent of d-glucose inhibited, the authors suggested that induced hyperglycemia or plasma transfusions may have a therapeutic effect in human favism.—H. S. J.


A mass survey of unrelated subjects revealed that 0.3 per cent of American Negroes and 0.7 per cent of Caucasians have erythrocytic 6-PGD activities in the range of 42 to 65 per cent of normal. The mode of inheritance appeared to be as an autosomal dominant characteristic with complete penetrance. Current screening tests for hexose monophosphate shunt activity, utilizing TPN reduction, were not sensitive enough to detect partial 6-PGD deficiency. None of the affected individuals had physical or hematologic abnormalities and primary administration probably had no significant hemolytic effect.—H. S. J.


Of 912 white individuals, 98.6 per cent had hemoglobin A. The other hemoglobins were: AS 0.2, AC 0.2, CC 0.2, AD 3.3, A+a group G 0.5, thalassemia/hemoglobin A 0.2 and increased hemoglobin A₂ 0.75 per cent. Of 398 negroes, 90.4 per cent had hemoglobin A. The others were: AS 6.5, SS 0.9, SC 0.6, AD 0.4, A+a group G 0.2 and increased hemoglobin A₂ 2.9 per cent. The incidence of hemoglobin S was 8.2 per cent in 440 negroes. The incidence of abnormal hemoglobins was higher in negroes and mulatoes (9.6 per cent) than in whites (1.4) and was higher in out-patients (6.9) than in the extra-hospital group (4.1). Homozygotes were more numerous in the hospitalized group (10 of 427) than in the extra-hospital patients studied (1 of 928). The hypothesis of an Indian source for hemoglobin D in Brazil was not well supported. The most probable source was the Iberic Peninsula. The incidence of hemoglobin S in Brazil among the negroes was lower than that in African people in their own countries (25 per cent among Bantus, Vandepitte, 1959). The authors concluded that hemoglobin S in whites in São Paulo was derived from four sources: Italian, Spanish, Greek and Arabian. An increase in hemoglobin A₂ in patients with thalassemia/hemoglobin S was observed.—M. J.


A Negro family and an Italian family were described in which there was good evidence that homozygosity for beta-thalassemia genes led only to mild anemia and normal patient survival. All biochemical and morphologic studies of the Italian father of a homozygous child were normal, except for an increased number of cells resistant to hemoglobin elution in the Betke slide test. The findings supported the concept of heterogeneity of thalassemia genes and their phenotypic expression and emphasized the possibility of homozygosity in "thalassemia intermedia." The authors suggested that, in extremely mild trait patients, the slide elution test for fetal hemoglobin may be the most sensitive technic for documenting the diagnosis.—H. S. J.


Two allelic adult hemoglobins, A and B, are found in sheep, as well as fetal hemoglobin. When sheep with hemoglobin A are made anemic by bleeding, the proportion of hemoglobin A falls
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Distribution curves of red cell volumes made in automatic cell counters are skewed, the left hand side being relatively steep and the right hand side flat. This has been shown to be due to the presence in normal blood of young erythrocytes which are larger than mature ones. As cell counters are sensitive to cell volumes as well as diameter, curves made with their aid are more accurate than Price-Jones curves which take only cell diameters into account.—F. W. G.

HEMOSTASIS


A serrated teflon clip for occluding partially the inferior vena cava to prevent pulmonary embolism was used in the management of thrombo-embolic disease in 42 patients. The indications for use were pulmonary embolization in patients previously treated with anticoagulants and thrombophlebitis in patients with contraindications to anticoagulant therapy. Testing in dogs indicated that clips with 4 to 5 mm. apertures trapped all emboli 5 mm. in diameter or larger without significantly raising the venous pressure. There was no operative mortality from insertion of the clips nor postoperative pulmonary embolization. Pulmonary angiography preoperatively was used in 8 patients to confirm the clinical impression of embolism. Four patients who apparently had no further embolus formation after clipping were studied by postoperative venograms of the inferior vena cava and passage of a cardiac catheter through the clip site. The cava was patent in each. In one patient who died 3 months following application of the clip from disseminated broncho-

Homogenates, prepared from platelets isolated from freshly drawn blood, were subjected to continuous sucrose gradient ultracentrifugation. Two main particulate bands were obtained; one composed primarily of membrane fragments and the other primarily of granules and mitochondria, as determined by electron microscopy. Cytchrome c oxidase, an enzyme found only in mitochondria, was localized in the granular layer. Three acid hydrolytic enzymes (lysosomal), acid phosphatase, beta-glucuronidase and cathepsin, were found in association with the granular layer. The activity could be increased by treatment with Triton X-100, but not by physical disruption of granules. In contrast to acid hydrolases, only small amounts of catalase activity were recovered in the granule and membrane fractions and 98 per cent of the lactic dehydrogenase activity was in the soluble portion. The granules and membranes were tested in the thromboplastin generation. Stypven and metabolic activity and the platelet membranes were tested and the differential was abolished. The authors concluded that the platelet membranes were more efficient as clot promoting agents than were the granules. When the lipids were extracted from the granules and membranes and were tested, the differential was abolished: clot-promoting activity of granule and membrane lipid was the same. The authors concluded that the lipoproteins of platelet membranes were more "available" for interaction with coagulation proteins than were the lipoproteins from the granules and they suggested that the platelet granules were concerned primarily with lysosomal and metabolic activity and the platelet membranes furnished a catalytic surface for the formation of intrinsic prothrombin activator.—R. G.

STUDIES ON PLATELET DYNAMICS IN PATIENTS WITH SPLENOMEGALY AND IN SPLENECTOMIZED PATIENTS. P. Boccaccio, L. Morra and A. Ponassi. From the University, Genova, Italy. Minerva Med. 57:328--342. 1966.

Fifteen normal subjects, 20 patients with different forms of splenomegaly and 12 patients splenectomized for various reasons were studied. Platelets were labeled with DFP. Studies were carried out in vitro and in vivo. In normal and splenectomized subjects, no significant differences were observed. Platelet survival following splenectomy should be modified, after the initial phase of postoperative thrombocytosis. After several years, platelet dynamics appeared to be normal. In congestive splenomegaly secondary to cirrhosis, platelet survival and thrombopoiesis were decreased. Various findings were obtained in cases of splenomegaly due to hyperhemolysis or systemic blood diseases. In chronic thrombocytopenia, complete normalization of platelet dynamics after splenectomy did not occur.—P. d. N.


The authors determined platelet thromboplastic function (PTF) levels (Platelet Factor 3) in the blood of patients with primary and secondary polycythemia and with thrombocythemia and thrombocytosis by means of a technic described previously (Brit. J. Haemat. 7:512). In untreated polycythemia vera (PV), PTF was reduced in 71 per cent of patients, or 100 per cent of those with elevated platelet counts; in treated PV, PTF often rose. Only 12 per cent of secondary and stress polycythemias had reduced PTF. All patients with primary thrombocythemia and 47 per cent of those with secondary thrombocytosis had a reduced PTF. The authors concluded that the determination of PTF is useful in the diagnosis of myeloproliferative disorders and that bleeding in states with high platelet counts may be caused by a qualitative platelet defect.—F. W. G.


Platelet-rich plasma was obtained from carefully taken venous blood to which citrate had been added at various times after venesection. Optical density was determined before and after addition of ADP. Low doses of ADP caused reversible reduction of optical density, due to "platelet aggregation." Higher doses caused irreversible reduction, preceded by a temporary increase. This change was "platelet activation." Normal
blood kept in glass up to 6 minutes without citrate or up to 10 minutes in siliconized containers showed no activation of platelets on subsequent addition of citrate. Centrifugation and treatment of plasma with ADP. Aggregation and activation were closely associated with clotting and platelet changes which occurred during the process probably were caused by thrombin.—F. W. G.


A diminution in polysaccharides of platelets and megakaryocytes and a deficiency in platelet factor 3 (thromboplastic activity) were observed. The latter finding could be corrected by incubating platelets in distilled water. Antiheparin activity of platelets was normal.—P. d. N.


In a case of malignant megakaryoblastoma in a girl of 25 who presented with splenomegaly and anemia, the abnormal cells (different from megakaryocytes) were observed in the blood, marrow and spleen. Many platelets, abnormal in form and often giants, were noted. After a course of 13 months, she died after a partial marrow and spleen. Man’s platelets, abnormal in form and often giants, were noted. After a course of 13 months, she died after a partial

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The rate of loss of Factor XI was followed in 10 units of whole blood during 21 days of storage at 4 C. A rapid loss of 80 per cent of the total Factor XI occurred during the first week of storage and correlated with the appearance of activated Factor XI. The survival in vivo of transfused Factor XI was followed in two patients with a congenital deficiency of this factor who were given single infusions of 10 ml. of fresh frozen plasma per kilo. A circulating half-life of 10 hours was found. Fresh whole blood or fresh frozen plasma should be used for replacement therapy of Factor XI.—R. G.


In 28 of 45 cases (62 per cent), an increase in fibrinogen was observed. A certain degree of correlation was found between hyperfibrinogenemia and the clinical state.—P. d. N.


The effect of normal serum and calcium on retraction and firmness of the fibrin clot was studied. Serum had a favorable effect on clot retraction and increased the maximal amplitude in the thromboelastogram. Serum lost this property after dialysis. After absorption on barium sulfate, serum lost the capacity to increase the maximum amplitude, but clot retraction was not affected. Clot retraction and its firmness, therefore, are supposed to be two different properties of the clot.—L. D.
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MISCELLANEOUS


A total of 651 units of blood have been frozen with ECA, stored at -85 C for up to 1 year and administered to 93 patients (average: 7 units per patient). Dimethylsulfoxide was the ECA used for the first 133 units. propylene glycol for 3 and glycerol for the most recent 515. The extent of hemolysis at different stages of processing was shown. When frozen cells were administered as packed cells, the recipient received 10 to 70 mg of free hemoglobin per unit. Recovery of erythrocytes after in vitro processing averaged 90 ± 5 per cent. Radioactive chromium studies showed 87 to 95 per cent of the labeled erythrocytes to be present in the recipient's circulation 24 hours after transfusion with a normal slope of decline of radioactivity thereafter. No detectable deterioration was seen in cells stored for more than 1 year. The 80 per cent overall efficiency of the process (in vitro recovery/in vitro survival X 100) compared favorably with the 70 per cent 24-hour survival of cells in ACD blood stored 21 days at 4 C. The biochemical status of the resuspended erythrocytes was reported. Of particular clinical importance was the low level of potassium and the virtual elimination of protein, blood group isagglutinins and viable white cells.—K. F.


A purified 7-S gamma G globulin of high anti-Rh activity has been prepared and administered to several nonimmune Rh-negative mothers 72 hours after delivery of Rh-positive babies. Previous findings that passive immunization to an antigen suppressed antibody synthesis prompted this trial in the hope that these mothers would not generate anti-Rh antibodies and, therefore, would not endanger future pregnancies. None of 48 treated mothers have become immunized when followed for 6 to 18 months, whereas 7 of 52 nontreated mothers harbored significant titers of anti-Rh antibody. Although final proof of the efficacy of this treatment must await results of subsequent Rh-positive pregnancies, these preliminary data have raised the hope that gamma G immunoglobulin to Rh factor might become a preventative of Rh hemolytic disease of the newborn.—H. S. J.


Total blood volume was measured with RIHSA-1151 in 44 patients. In pulmonary stenosis patients (group I), decreased total blood, plasma and red cell volume were found. The group with intercavitary communications (II) had hypervolemia and patients with mitral valvular insufficiency and interatrial septal defects had the most marked deviations (III). In the cyanotic group (IV), total blood and red cell hypervolemia with plasma hypovolemia were noted. In cyanotic congenital cardiopathies, hypoxemia may be the cause of the plasma hypovolemia.—M. J.


Electron microscope studies of the forms in erythrocytes, including gametocytes and asexual schizonts, have revealed a "cytostome," a specialized organelle of the pellicular membrane which is active in the ingestion of host cell cytoplasm. The cytostome in exoerythrocytic stages of P. falciparum has been observed only in merozoites and does not seem to play the same role in the feeding mechanism.—O. P. J.


Thirty patients received intravenous infusions of 250–500 ml of modified beef serum. No changes in blood pressure, pulse, temperature or respiration were noticed. The preparation was well tolerated by all patients, except for slight allergic reactions in two. There was a rise in the leukocyte count, maximal 3 hours following the start of the infusion. A slight decrease in Factor V and prothrombin, as well as variants in fibrinogen values, were found. All these changes were temporary and no irreversible clinical, hematologic or biochemical changes were noted.—L. D.

Chloramphenicol was coupled covalently to a protein carrier (BGG) by diazotization and was injected into rabbits. Antibody production was detected by precipitation and complement fixation to chloramphenicol coupled to rabbit serum albumin. The specificity of these reactions was demonstrated by the classic method of hapten inhibition. The author pointed out that chloramphenicol-specific antibodies may prove to be very useful in the investigation of problems related to the toxicity of this material.—I. G.


The amino terminal sequences (variable portion) of 13 different light chains were obtained from mouse and human myeloma proteins and from normal pooled human γ globulin. All the proteins studied had the same amino acid at the second, fifth and sixth positions; at the first and third positions, there were two alternatives and at the fourth position there were three alternatives. These findings were evidence against the "hypermutable theory" of somatic differentiation, since a single nucleotide change in the triplet codon would be expected to generate from 5 to 7 different amino acid differences. The authors suggested that the variable amino terminal ends of the L chains were coded by a large number of different genes, only one of which becomes activated in an individual plasma cell. A single gene may code for the invariable carboxy terminal end. Two possible mechanisms were proposed which could integrate the products of these two different genes so as to allow the formation of the complete L chain.—I. G.


Young C3H mice were irradiated lethally and were injected with (C3H × T6)F1 spleen cells. Several weeks later, liver necrosis followed by regeneration was induced with CCl4. Metaphase spreads of the regenerating livers were prepared to identify the presence of the T6 marker chromosome. Marker chromosomes were present in a high percentage of regenerating liver cells. The authors interpreted their data to indicate that either chromosomal material was exchanged between the descendants of injected spleen cells and the hepatic parenchymal cells of the recipient or that transformation of spleen cells to hepatic parenchymal cells occurred. (Abstractor's Comments: Although the authors gave reasons why the T6 chromosome markers were not interpreted as being in nonhepatic parenchymal cell metaphases, e.g., Kupfer cells, their evidence is far from convincing.)—I.G.