HEMOSTASIS


A hemorrhagic diathesis due to intermittent deficiency of Factors VII, IX, X and prothrombin responded quickly to vitamin K. Malabsorption, liver disease and known drug exposure were excluded, but Warfarin was demonstrated in the patient's serum. Solution: rat poison and la femme!—S.-A. K.


The clotting activity of amniotic fluid is probably due to the composite effect of antihemophilic globulin and platelet and thromboplastic material.—J. B. C.


Platelet counts were persistently below normal in 38 cases and in 3 were below 50,000. Atypical mononuclear cells (8–16 per cent) were frequently seen.—J. B. C.


In severe cases, thrombocytopenia and hypoprothrombinemia were present. Capillary damage also appeared to contribute to hemorrhagic episodes.—J. B. C.


In man, the number of megakaryocytes revealed by examination of pulmonary tissue obtained from persons dying accidentally appeared...
LEUKOCYTES

AUTOPSY STUDY OF LEUKEMIA IN HIROSHIMA.

In 157 cases of confirmed leukemia which were autopsied through 1962, nothing was found to distinguish a radiation-induced case from a spontaneous case. Proximally exposed cases (less than 1400 meters from epicenter) had a higher incidence of bone marrow fibrosis in chronic myelogenous leukemia, acute lymphatic leukemia and (1400-10,000 meters). The authors suggested that some may have been myelofibrosis originally. Proximally exposed cases of both acute and chronic myelogenous leukemia had shorter survivals from the time of first appearance of leukemic symptoms and at autopsy had much smaller spleens.—C. R. M.

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Leukemogens may affect DNA molecules directly. It was postulated that the normal base sequence of polynucleotide chains is altered and that this alteration is reflected in loss of leukopoietic control and formation of a new race of cells.—J. B. C.


In 11 cases of acute leukemia and 2 of erythro leukemia, previously untreated karyotypes in bone marrow cells were studied after colchicine block without culture. In the majority of cases of acute leukemia, normal karyotypes were observed, but in a few, abnormal numbers (45 and 47) and/or morphology of chromosomes were evident, sometimes with marker chromosomes. In both cases of erythroleukemia, abnormalities in group C chromosomes were observed.—P. d. N.


Two cases of fatal eosinophilic conditions are reported and the literature from 1956 to 1963 is reviewed. It is stated that a well-documented case of eosinophilic leukemia has never been described. Many reported cases have features of disseminated collagen disease.—S.-A. K.


Arylsulfatase, known to be present in the cytoplasm of human leukocytes, has not been studied in leukocyte nuclei. Using histochemical technics, leukocytes from normal individuals and from patients with acute and chronic myelocytous leukemia, acute lymphatic leukemia and acute stem cell leukemia, but not acute monocytic leukemia, were examined. The reaction was present in all lymphocytic cell nuclei, mature and immature. Since the reaction was present in all
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four cases of acute stem cell leukemia, the authors felt that these represented primitive cells of the lymphoid series. The reaction was not seen in nuclei of other cells. The observation is obviously of great interest, but one would wish to know the reaction of monocytic cells and plasma cells, in view of their relationship to lymphocytes.—C. R. M.

NORMAL FLORA AND LEUKOCYTE MOBILIZATION.

Comparing gnotobiotic and normal animals, the authors observed a larger leukocyte response to injection of sterile irritant in the normal animal. This difference was attributed to the presence of endotoxin or organisms elsewhere in the animal and was not dependent upon the presence of organisms at the site of injection. The process was apparently a general stimulation or improvement of the inflammatory response, rather than a local phenomenon.—C. R. M.


Removal of central lymphoid tissue (thymus, appendix or both) in neonatal rabbits often led to development of Coombs positive RBC, amyloid deposits in lymph nodes and plasmacytosis and reticulum cell hyperplasia of lymph nodes and spleen. Small lymphocytes in spleen and lymph nodes were decreased. Removal of central lymphoid tissue in adult animals followed by x-irradiation also produced Coombs positive RBC, amyloidosis and plasmacytosis. The authors suggested that, normally, central lymphoid tissue in some way modulates the autoaggressive tendencies of peripheral lymphoid tissue and they noted the high frequency of autoimmune processes in patients with immunologic deficiencies. (Abstracter’s note: It would be of interest to repeat these studies in germ-free animals.)—I. G.


Reports of 34 patients with M-type serum globulins who were followed for several years. The M-component never disappeared spontaneously. Benign, essential monoclonal hyperglobulinemia was characterized by a rather low M-protein concentration which remained constant. An M-protein level above 3 Gm. per 100 ml., a rapidly rising M-protein concentration, and anemia below 3 million RBC per mm.3 were highly suggestive of myeloma. The possibility that benign monoclonal hyperglobulinemia might be a state of "premyeloma" was considered, but only one patient has so far shown a possible transition from the benign process to myeloma.

—S.-A. K.


Turnover was studied in 22 patients after labeling with C14-glycine in vivo. In γG myeloma (18 patients), T1/2 was 0.9 to 15.6 days. In the γ1-2 variety (3 patients), the T1/2 was significantly shorter: 4.5 to 7.9 days. In a single case of γ1-2 myeloma, T1/2 was 5.6 days. In repeat studies, done in 3 patients at intervals of 19 to 30 months, the T1/2 was unchanged. The effect of Melphalan therapy was studied in 4 patients: the T1/2 remained unchanged in 2 and increased in 2.

—S.-A. K.

ERYTHROCYTES


An alpha chain abnormality found in a Negro patient had the approximate electrophoretic mobility of Hb S at pH 8.6, but differed on agar gel electrophoresis at pH 6.2. The substitution was found to be α2γGβ1γFα2—β2. A child heterozygous for both Hb G and Hb C had 4 hemoglobins (A. C. G Baltmore, and C/C) and in a second child the fetal form of the hemoglobin variant (α2γF) was observed.—H. M. R.
THE AMINO ACID COMPOSITION OF HEMOGLOBIN.
VI. SEPARATION OF THE TRYPIC PEPTIDES OF
HEMOGLOBIN knoxville, No. 1 on Dowex-1 X-2
AND SEPHADEX. A. I. Chernoff. From the Mem-
orial Research Center, Knoxville, Tenn. Bio-

HBKnoxville- No. 1, an α chain abnormality de-
tected in a Negro patient, was found to be identi-
cal to Hb Gphiladelphia \( (\alpha_2\) lys \( \beta_2 \) ). —H. M. R.

EFFECT OF IRON DEFICIENCY ON THE RELATIVE
RATES OF SYNTHESIS OF HEMOGLOBINS A AND
E AS STUDIED IN A HEMOGLOBIN E HETEROZY-
gote. S. Srivarup, S. K. Ghosh and J. B. Chatter-
jea. From the School of Tropical Medicine,

A boy, aged 6, developed severe iron deficiency
anemia due to hook worm infection. At the height
of anemia (Hb 4.0 Cm. per cent.), Hb E consti-
tuted only 20 per cent. As anemia improved with
iron, the relative proportion of Hb E gradually
increased to 39 per cent when total hemoglobin
was 11.9. Under the stress of iron deficiency, the
rate of synthesis of Hb E may be depressed more
than that of Hb A. —J. B. C.

FOLIC ACID STUDIES IN SICKLE-CELL ANEMIA. H.
A. Pearson and W. T. Cobb. From the Uni-
versity of Florida College of Medicine, Gains-

Serum L. casei folate concentrations were sub-
normal in 23 per cent and urinary FIGLU excre-
tions were elevated in 70 per cent of 25 patients
with sickle-cell anemia who did not have morpho-
logic evidence of folate deficiency; however, bone
marrow aspirations were not performed. Treatment
with folic acid (100 µg. to 2.5 mg. per day) in 11
patients who had excessive excretion of FIGLU
and 3 patients who had an aplastic crisis with
normal serum folate levels did not result in any
significant hematologic changes. —F. A. K.

PLASMA TETRAARYLE PORPHYRINE PIGMENTS IN SICKLE
CELL ANEMIA. LONG-TERM STUDIES AND THE EFFECTS
OF LOW TEMPERATURES. L. J. Stutman and G.
Y. Shinoura. From New York University
School of Medicine, New York. Amer. J. Clin.

The study of hemoglobin and hematin in plasma
of 3 men with SS anemia extended for at least a
year in each patient; specimens were obtained in
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peared to consist of two $\alpha_2\beta_2$ units linked by two disulfide bridges between $\beta$ chains.—H. M. R.


Adult and fetal hemoglobins had the same normal Bohr effect, but the reverse Bohr effect (below pH 6) was reduced in hemoglobin F.—H. M. R.


Erythrocyte diameters were measured on peripheral blood smears. Erythrocytes from infants with ABO disease had a mean diameter of 7.6 $\mu m$, compared with 8.15 $\mu m$ for normals and 8.0 $\mu m$ for infants with Rh erythroblastosis. The difference between the infants with ABO disease, and the two other groups was significant at the $P = < 0.01$ level. More than 15 per cent (mean = 25) of the red cells in infants with ABO disease were microcytic (<7.0 $\mu m$). None of the other infants demonstrated more than 15 per cent microcytes, the mean being 8 per cent.—J. B. S.


Three children who ingested PAS solution, prepared 2–3 weeks earlier and left to stand during hot weather, developed acute hemolytic anemia, hemoglobinuria and methemoglobinemia. The children complained that the medication had become bitter and the mothers noted a darkening in the color. These changes were presumed to result from decarboxylation of PAS and formation of phenolic polymers by the combination of aminosalicylic acid and m-aminophenol. Erythrocyte G-6-PD activities were normal. To forestall the formation of powerful oxidants, PAS solution should be stored at cool temperatures and used within a week of preparation. A stable resin form is also available.—J. B. S.


Serum folic acid activity, assayed with *L. casei*, was subnormal in 7 and borderline in 6 of 46 consecutively studied patients with chronic liver disease. Nine had megaloblastic bone marrows; serum folic acid concentrations were low in 2, borderline in 4, and normal in 3. Two patients with postnecrotic cirrhosis had megaloblastic anemia; both had normal serum folic acid levels. Reduced serum folic acid levels were found to correlate with anemia, an inadequate diet and excessive consumption of alcohol, but not with biochemical evidence of severity of liver disease or steatorrhea.—F. A. K.


Co$^{57}$-labeled B$_{12}$, tube-fed with normal human gastric juice to guinea pigs, was found in both the supernatant and sediment of a saline extract of intestinal mucosa. The Co$^{57}$-labeled material was not ultrafilterable and functioned as an intrinsic factor-B$_{12}$-Co$^{57}$ complex in an in vitro assay for intrinsic factor. These observations suggested that B$_{12}$ is taken into the gut wall in a bound form.—F. A. K.


Women less than 16 weeks pregnant were allocated at random to one of 3 groups receiving the following: 1) lactose 2) 100 mg. ferrous fumarate, or 3) 100 mg. ferrous fumarate with 10 $\mu g.$ folic acid. The group receiving only lactose showed the highest incidence of iron deficiency anemia, the greatest proportion of hypersegmented neutrophils in peripheral blood films, the lowest serum *L. casei* values and the highest incidence of megaloblastic change. The data were interpreted as indicating that iron deficiency produced additional stress on folate metabolism in pregnancy which lead to an increase in the incidence of megaloblastic anemia, that the frequency of megaloblastic anemia of pregnancy could be reduced by adequate iron supplementation and that a folic acid supplement larger than 20 $\mu g.$ per day would

The etiology in 120 cases of iron deficiency anemia was: nutritional 25, hookworm 52, hemorrhages 39, hook-worm plus hemorrhages 4. Hookworm anemia was predominantly, if not entirely, related to blood loss. The incidence of abnormal hemoglobin traits and G-6-PD deficiency did not appear to be significantly different from that in normal populations. Iron deficient red cells did not appear to show any resistance to induced infection with P. vivax malaria. —J. B. C.


The villi were abnormal in 3 of 7 cases. In 4, the walls of vessels in the submucosal coat appeared to be thinner than normal. Repeat biopsy after therapy in one case indicated restoration of a normal pattern. —J. B. C.


Activities of succinic dehydrogenase, aconitase and catalase were determined in whole blood. A decrease in aconitase activity was noted in 7 of 30 cases. —J. B. C.


Four marasmic infants fed a high-caloric, low-copper diet developed hypocupremia associated with marked neutropenia and hypochromic, slightly macrocytic anemia. Mild reticulocytosis accompanied the anemia which developed despite the administration of iron. After several months on the diet, scurvy-like bone changes appeared. Ascorbic acid and folic acid produced a transient, incomplete erythropoietic response in several infants. Vitamin B₁₂ effected an improvement in hematologic and bone status in two. Complete reversal of the abnormalities was most dramatic following the administration of copper. —J. B. S.


Unilateral hydronephrosis was induced in 12 rabbits. Eight developed true erythrocytosis and showed striking hypertrophy and degranulation of the juxtaglomerular apparatus. Four did not develop erythrocytosis and their juxtaglomerular apparatuses were atrophic. Various mechanisms which might induce the apparatus to secrete erythropoietin were discussed. —C. R. M.


G-6-PD deficiency was present in 14 per cent of full term male infants with jaundice not associated with fetomaternal blood group incompatibility. Among nonicteric male infants, the incidence of G-6-PD deficiency was 1.3 per cent. Evidence of perinatal hypoxia was present in 5 of 7 jaundiced male infants. The combination of G-6-PD deficiency and hypoxic liver damage would appear to be etiologically related to "physiologic" jaundice in full-term male Bantu infants. —J. B. S.


G-6-PD deficiency was detected in 6 of 20 cases of neonatal jaundice and in 2 of 36 cases with hemolytic anemia. —J. B. C.


Instability of GSH in some patients with Hb E-thalassemia disease was not related to de-
ficiency of either G-6-PD or glutathione reductase activity. It was postulated that deficiency of TPN may contribute to GSH instability.—J. B. C.


In Hb E-thalassemia disease in which reticulocytes varied widely, there was no general correlation between reticulocyte level and enzyme activity. In iron deficiency and nutritional macrocytic anemia, there was suggestive correlation between enzyme activity and reticulocyte level.—J. B. C.


A careful study of physicochemical factors affecting the osmotic fragility test: temperature, pH, amount of hemoglobin, pCO2, degree of oxygen saturation, time for hemolysis and in vitro age of blood. The importance of a constant pH is emphasized. A test which takes these factors into account and normal values are presented.—S.-A. K.


The effect of temperature variation on the fragility of normal red cells in 0.40 per cent NaCl under standardized conditions was studied. The thermal fragility test correlated well with the osmotic fragility test, was easier to perform and may replace the osmotic fragility test. Comparisons of the 2 tests in pathologic states have not yet been made.—S.-A. K.


In 45 patients (14 with hemolytic anemia), red cell life span was determined with Cr51 and erythrocyte plasticity in vitro with the "Erythrocyte Filtrability Test." In most patients, a highly significant correlation (r = -0.849; 0.01 > p) was found between these two parameters. The suggestion was made that determination of erythrocyte plasticity might be used to provide reliable indirect estimates of the actual life span of red cells.—E. R. J.


Areas were determined planimetrically in enlarged photomicrographs of blood from 7 normal males. Mean reticulocyte area was 13 per cent larger than mean erythrocyte area. The volume of normal reticulocytes was computed to be 20 per cent larger than that of the average red cell. Reticulocytes formed in response to severe erythropoietic stimuli were considerably larger than normal reticulocytes. When coupled with narrow reticulocyte transit time, the data provided evidence that the orthochromatic normoblast is the major source of normal reticulocytes, whereas large reticulocytes found during rapid regeneration following hemorrhage or acute hemolysis are derived from earlier precursors, particularly polychromatophilic normoblasts which have skipped one or more cell divisions.—H. H. F.

MISCELLANEOUS


Hemoglobin determination surveys made by the Proficiency Testing Service between 1954 and 1958 showed a reduction of the standard deviation to approximately 0.5 Gm. from the mean. Since that time, however, there has been decreased accuracy. In December, 1963, on a specimen containing 15.9 Gm. per cent hemoglobin, 398 laboratories showed a standard deviation of 0.8 Gm. with a range from 10.5 to 18.6 Gm. Most laboratories (92.4 per cent) were using the cyanmethemoglobin method and nearly all relied upon commercial standards. The author made a strong case for
uniformity of constants for calculating concentrations and for laboratories to prepare their own standard curves, probably most simply by iron determinations.—C. R. M.


The reaction of CO-hemoglobin with the reagents used for cyanmethemoglobin estimations is significantly slower than that of oxyhemoglobin or ferricemoglobin. This error is probably significant only with high concentrations of CO-hemoglobin when it may be of the order of 10 per cent. Where it is critical, the authors suggest that either the reaction time be extended to more than 3 hours or that a more concentrated Drabkin solution be used.—C. R. M.


The modified 13-gauge trocar has a stainless steel drill attached to the tip and aspirating holes on the side. The author claims that samples can be obtained more easily from dense bone and that the needle can be inserted by simple hand pressure.—C. R. M.


Premature infants who at birth received 10 to 40 mg. menadione were examined at age 7. The mean I.Q. of the group whose serum bilirubin never exceeded 20 mg. per 100 ml. was 95. For the group whose peak bilirubin level was 20-30 mg., the mean I.Q. was 85, and for the group whose bilirubin exceeded 30 mg., the mean was 85. A significantly higher incidence of neurologic motor abnormalities was present in patients with high bilirubin levels, particularly with levels above 30 mg. Eight infants died in the first month and both infants whose bilirubin had been above 20 mg. per 100 ml. had postmortem evidence of kernicterus.—J. B. S.


Significantly lower peak bilirubin levels were seen in healthy newborns fed 750 mg. activated charcoal every 4 hours, starting at age 4 hours. Institution of charcoal feedings at 12 hours did not decrease peak bilirubin levels in another test group. The authors suggested that enterohemepatic shunting of bilirubin occurs in the newborn and is of significance in the overall body economy of bilirubin. They felt that the first few hours may be critical in determining the size of the bilirubin pool and that charcoal-binding of intestinal bilirubin by 4 hours is necessary, if the bilirubin pool is to be kept at a minimum.—J. B. S.


Bilirubin removal and equilibration during exchange transfusion was studied with Cr51 and 131I tracer dilution technics. The dilution of the original circulating blood volume could be described accurately by a simple exponential equation. The equilibration rate for extravascular bilirubin was a constant proportional to the infant's plasma volume per Kg. and the initial serum bilirubin concentration. The percentage rate of bilirubin removal depended upon the volume of blood used, but not upon speed or the increment volume. Diffusion of labeled albumin into extravascular space during the exchange appeared to be much slower than diffusion of extravascular bilirubin into the vascular space. There was no correlation between rate of bilirubin shift during exchange and either the post-exchange rebound or the number of exchanges required. This discrepancy was taken as an indication that there may be two extravascular bilirubin pools: one, a labile pool which equilibrates instantly, and another more stable pool which is largely responsible for the postexchange bilirubin rebound.—J. B. S.

THE EFFECT OF SUBSTITUTED 2,4-DIAMINO-PYRIMIDINES ON THE RAT FETUS IN UTERO. J. B. Thiersch. From Washington University School
From the Statens Serum Institut, H. Olesen.

(Abstracter's note: Does true folic acid deficiency agglutinmns. No such effects were demonstrable when it was given to 2 patients, nor did it change their clinical state.—S.-A. K.)


In vitro, D-penicillamine reduced the cold agglutinin titer and the concentration of 19 S proteins and blocked the sensitization of red cells by cold agglutinins. No such effects were demonstrable when it was given to 2 patients, nor did it change their clinical state.—P. G. R.


Isoantibodies were found in children receiving injections of gamma globulin and in those who had received transfusions at birth: 9 of 14 receiving repeated injections had antigamma globulin antibodies; ½ of all exposed to gamma globulin by transfusion or injection, but only 6 per cent of unexposed children, had antigamma globulin antibodies. Females appeared to have a higher incidence of sensitization than males. These antibodies may be implicated in the rare anaphylactic reactions to gamma globulin which have been reported.—H. H. F.


Hybrid univalent 6.5S antibody molecules, formed by recombination of half-molecules of rabbit antibody to ovalbumin with those of normal rabbit γG-globulin, fail to fix complement in reactions with homologous antigen and block complement fixation by intact antibody to ovalbumin. Molecules of antibody reconstituted in the absence of other protein retain the capacity to fix complement. The data suggest that small complexes containing excess univalent antibody do not fix complement and that lattice formation is required for fixation.—H. H. F.


A macromolecular nonimmunogenic material, multi-chain poly-DL-alanine, induced tolerance towards the poly-DL-alanyl determinant. Investigation of immunologic tolerance with well-defined synthetic polypeptides and polypeptidyl proteins may lead to a better understanding of the nature of this phenomenon.—H. H. F.


The DNA-P content of rabbit bone marrow is 5.8 x 10⁻⁹ µg./cell, that of RNA-P is 3.9 x 10⁻⁷ µg./cell and the RNA:DNA ratio is 0.67. Glycerol, in concentrations of 5 per cent and more, markedly decreased RNA content, but had no influence on DNA level. Glycerol-treated cells continued to incorporate C¹⁴-formate and P³² into RNA and DNA, even though the rate was considerably diminished. Washing procedures further decreased the ability of glycerol-treated cells to incorporate radioactive isotopes. The RNA:DNA ratio in glycerol-treated cells underwent an unusually rapid decrease during incubation. Increasing the glycerol concentration up to 40 per cent permitted the isolation of both purified and cytoplasm-contaminated nuclei.—S. R. H.

The site of paraprotein production was demonstrated by electronmicroscopy in a typical case of Waldenström-syndrome. In sections of different organs and in electrophoretograms, a paraprotein, extremely rich in PAS-positive carbohydrates, was detected. By ultracentrifugal, viscosimetric and degradative examination, this protein proved to be a macroglobulin. In serum and lymph node homogenate, beta2m globulin was detected.—S. R. H.


Two types of L.E. cells were distinguished: 1) a fine granular nucleus with the included mass totally homogeneous and of uniform density with a complete, fine enveloping membrane; 2) an included mass with coarse granules and variable density also containing cytoplasmic granules from polymorphonuclear leukocytes and an enveloping membrane of double contour. The presence of cytoplasmic granules indicated that some cytoplasmic material was mixed with nuclear material, in contrast to the general belief that only nuclear material constitutes the included mass.—M. J.


A quick and simple method is described. Colchicine, 0.3 ml. and 0.6 ml. of 0.03 per cent, is given to mice and rats, respectively. After 2 hours, the animals are sacrificed, bone marrow is aspirated from the femur, suspended in 5–10 ml. saline and centrifuged (1,000 rpm, 5 min.). Five–10 ml. 0.7 per cent sodium citrate are added and allowed to stand 5 min. at room temperature. After centrifugation (500 rpm, 4 min.) 4–5 ml. of 3 parts absolute ethyl alcohol: 1 part glacial acetic acid are added dropwise with gentle shaking. After one hour in the refrigerator, centrifuge gently, add 4–5 ml. of 45 per cent acetic acid and repeat after several hours. Drops (1–2) of suspension are placed on a slide, dried at 37 C. and stained with acetoorcein.—S. R. H.

Erratum


In the August 1965 issue of Blood, a line of type was inadvertently omitted and a line repeated in the editorial, “Therapy of Acute Leukemia, 1965.” The second paragraph of the editorial (p. 220) should read:

Following the introduction of the folic acid antagonists, corticosteroids and 6-mercaptopurine, there occurred an hiatus during which, although many agents were tested, little progress was made. Comparatively recently, the chemotherapy of acute leukemia has again advanced, this time in two directions. One has been the introduction of other effective antileukemic agents; the other parallels recent developments in the use of antibiotics, and might be termed "new ways with old drugs." An excellent review by Freireich and Frei of some of these advances has recently appeared.1
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