HEMOSTASIS


In a family of 5 generations with 47 members, 10 had undue bleeding which in 8 was associated with thrombocytopenia. Seven thrombocytopenic members were males; splenectomy corrected the disorder in 3. The only affected woman recovered spontaneously. The condition responded poorly to steroids. The authors suggested that the disease was inherited as a mendelian dominant with incomplete penetrance in the female. There was no detectable linkage between Xga blood groups and the gene for thrombocytopenia.—P. B.


Naphthionin (sodium-α-naphthylamine-4-sulphonate), intravenously or intramuscularly, shortened the bleeding time in normal subjects and in thrombocytopenic patients. It also appeared to be clinically effective in controlling hemorrhage in 3 patients with thrombocytopenia. Aplastic anemia and thrombocytopenia following Thiotepe failed to respond. The hemostatic action of Naphthionin was attributed to an effect on fibrinogen. Naphthionin was ineffective where hemorrhage was not due to thrombocytopenia.—P. B.


Adenosine diphosphate (ADP), ATP, 5-hydroxytryptamine and noradrenaline were used to compare platelet-clumping activity before and 10 and 30 days after operation in 18 patients. Ten days after operation, there was an increase in initial rate and in maximum intensity of clumping; at a month, the initial rates had returned to pre-operative values. The maximum response to ADP was similar. With ATP and noradrenaline, the maximum intensity remained higher a month after operation than before operation.—P. B.


By using Coons’ fluorescent antibody technic, the plasma thin layer was demonstrated to cover the inner surface of vascular endothelium of...
heparinized rats treated with either bovine thrombin, EACA or trypan blue. The layer was seen more frequently in small arteries than in veins of the kidney, but was present in both vessels of the lungs. A small number of platelets and tiny fibrin threads occasionally adhered to the inner surface of the endothelium, even in heparinized rats, and were increased after treatment. The endothelial plasma thin layer may be composed of fibrin, platelets and globulin, possibly as a result of intravascular coagulation. Microcoagulation may occur spontaneously as a physiologic phenomenon. —K. F.


The authors showed that Hageman factor acts as a plasminogen activator and that a mixture of Hageman factor and euglobulin precipitated from human plasma has a marked fibrinolytic activity. Factor XII also appears to act as a proactivator activator.—E. S.


Both in vitro and in vivo, the active isomer of A.M.C.A. appears to be at least 10 times more potent than epsilon-amino-caproic acid. In systemic therapy, much lower effective dosage levels can be used (less than 3 Gm. per day). A clinical trial is in progress to establish its therapeutic value.—P. B.

ERYTHROCYTES


Six patients are reported who manifested pancytopenia following drug administration (usually chloramphenicol) and in whom red cells developed the characteristic abnormalities of PNH several months after the aplastic syndrome. Four of the patients, later in the course of illness, manifested all the features of PNH. Five patients, similarly affected, have been previously described by others. The relationship of these two diseases remains obscure. (Abstracter’s note: Reticulocytosis of 1–3½ per cent during the “aplastic” period was noted and is distinctly atypical in the usual drug-induced aplastic anemia. This atypical feature will be looked for with interest in future case reports.) —H. S. J.


Three male and 1 female Sephardic Jewish patients are described with chronic, easily detectable low-grade hemolysis, but without anemia, whose red cells contain G-6-PD levels which were moderately to severely reduced. It is suggested that these cases form a distinct entity within the population of G-6-PD-deficient subjects, differing both from the syndrome of congenital nonspherocytic hemolytic anemia in children whose red cell survival is severely curtailed and from the larger group of asymptomatic (especially Negro) individuals whose decrease in red cell survival is only barely perceptible.—H. S. J.


The incidence of G-6-PD deficiency and neonatal jaundice in all male infants born in one year in part of the island of Lesbos was studied; of 634 tested, 4.6 per cent were found to be deficient. Hyperbilirubinemia of 16 mg. per 100 ml. or over was observed in 34 per cent of deficient newborn males and in 9.1 per cent of those with normal enzyme activity (more than half of them without incompatibility or prematurity). It was postulated that in this population a second factor increases the incidence of neonatal jaundice, especially when it is combined with G-6-PD deficiency.—P. B.

EXERTIONAL HAEMOCLOBINURIA: A REPORT ON THREE CASES WITH STUDIES ON THE HEMO-
that, despite adequate treatment, severely affected babies and those delivered prematurely in the first 3 months of life. It was concluded that this effect was due to stimulation of hydrochloric acid production. These findings may be relevant to the well-established association between excessive consumption of alcohol and the development of iron overload.—P. B.

ABSTRACTS

A vitamin-B_{12}-tannin complex in an aluminum-monostearate suspension (B_{12}-T.A.M.) for slow release was given intravenously to 9 patients with pernicious anemia, 1 with Crohn’s disease and 1 with nutritional deficiency. A single dose (1000 μg vitamin B_{12}) produced an effective remission in 7; the others required 2 doses. A maintenance dose of 1000 μg every 2 months appeared adequate for most patients, as judged by serum vitamin B_{12} levels. Some patients may need more. The reticulocyte peaks reached higher levels with B_{12}-T.A.M. than are commonly seen with the usual aqueous solutions of cyano- or hydroxocobalamin.

—P. B.

Folate Deficiency in Acute Tropical Sprue.


Observations on 40 patients with tropical sprue and anemia were described: bone marrow was megaloblastic and serum folate activity was reduced in every patient. Most patients had a full hematologic response to small doses of folic acid (200 μg daily, intramuscularly for 2–4 weeks). Suboptimal response in 2 patients was due to associated iron deficiency. Every patient had malabsorption of vitamin B_{12} and reduced serum B_{12} levels. There was no hematologic response in 5 patients treated with 1 μg B_{12} daily, intramuscularly for 2–4 weeks.—F. B.


Plasma clearance of intravenously administered folic acid was abnormally rapid in 5 patients with untreated hyperthyroidism. In 3, basal serum L. casei folate activity was subnormal. Studies, repeated at intervals after partial or complete treatment, suggested that hyperthyroidism in man is associated with depletion of folate stores and that subclinical deficiency may be attributed to increased demand for folic acid in the hypermetabolic state.—P. B.


The patient (11 weeks postpartum and breast feeding) had normal serum vitamin B_{12} levels, but L. casei folate activity was low. After a control period with no uncooked green vegetables, a liquid extract of fresh lettuce was given for a week without response. Thereafter, 3 whole lettuces were fed daily for 6 days; reticulocytosis of 14 per cent occurred and the hemoglobin level and hematocrit began to rise. The marrow was much less megaloblastic. No secondary reticulocyte rise was noted with pteroylglutamic acid.

—T. H. B.


A method is described with L. casei as test organism and a modified medium in which a greater growth response occurs than in media previously described.—F. B.


The ratio of circulating hemoglobin concentrations in patients with 2 or more major hemoglobins accurately reflected the synthetic rates of these components, as studied in reticulocytes and erythroblasts incubated in vitro. Neither erythroid stimulation by phlebotomy nor deficiencies of O_{2} or other metabolites in vitro failed to alter relative synthetic rates. This latter finding contrasted with the situation in umbilical cord blood where hemoglobin F is preferentially synthesized relative to A, under in vitro conditions of hypoxia or glucose lack (Allen, D. W. and Janell, J. H. J. Clin. Invest. 39:1107, 1960). (Abstractor’s note: Evidence that relative synthetic rates of heterogeneous hemoglobins may be altered with nutritional deficiencies in vivo have been presented by Heller et al. Blood 21:479, 1963, and by Levere et al. Nature 202:499, 1964, in patients with sickle cell trait and coexistent folic acid deficiency or iron deficiency, respectively.)—H. S. J.

Studies on Hemoglobin Biosynthesis: Asynchronous Synthesis of Hemoglobin A and Hemoglobin A_{2} by Erythocyte Precursors. R. F. Rieder and D. J. Weatherall. From Johns...
In reticulocyte suspensions, incorporation of both radioactive iron and amino acids into hemoglobin A exceeds that into hemoglobin A₂. This difference becomes very much less marked when suspensions of bone marrow are utilized, indicating that in the reticulocyte hemoglobin A₂ synthesis is retarded before that of hemoglobin A. In 3 of 4 bloods from individuals with thalassemia major, synthesis of hemoglobin A₂ exceeded that of hemoglobin A, supporting the concept that a quantitative defect in hemoglobin A synthesis underlies this disorder.—H. S. I.

**Artificial Synthesis and Hybridization of Hb M₁wate**


Methemoglobins of Hb M₁wate and Hb A were successfully reconstituted by coupling hemin of Hb A with globin of purified Hb M₁wate and vice versa. Hybridization of Hb M₁wate with canine hemoglobin yielded a black cathodal hybrid (α₉β₂) and a red anodal hybrid (α₉β₂), in addition to the original hemoglobins. The chocolate-brown color of Hb M₁wate appeared to be due to its abnormal globin with aberrant α chains.—K. F.

**Combination of Globin and Its Derivatives with Hemins and Porphyrins**


Apochemoglobin with a helical content of 50 per cent was prepared and used. Protoporphyrin, protoporphyrin, hematoporphyrin, etiohemin and hemindimethylsere combined with globin, while protoporphyrin-dimethylster and etiohemin did not. Acetylated globin bound hematin, but not protoporphyrin, while azoglobins had no capacity to combine with hemins and porphyrins. PCMB-globin, though unstable, bound hemins and porphyrins. These results emphasized the importance of the linkage between the carboxyl groups of porphyrin and the amino groups of globin.—K. F.

**Studies on the Role of the Juxtaglomerular Apparatus of the Kidney in Erythropoiesis**


Fe⁵⁰ utilization for hemoglobin production or specific activity of heme was equated with erythropoietin activity. The granularity of JGI was expressed as juxtaglomerular index (JGI). In hypertransfused rats, erythropoiesis was depressed with some reduction in JGI. Simultaneous increases in erythropoiesis and JGI were observed in bled or phenylhydrazine-injected rats and rats injected with purified erythropoietin or anemic sheep plasma. High erythropoietic activity developed 4 weeks after unilateral constriction of a renal artery. There was a marked increase of JGI in the clipped kidney, whereas that of the untouched kidney was normal. Angiotensin II i.v. resulted in increased JGI of normal or ureter-ligated rats, whereas increased erythropoiesis was variable. In bilaterally nephrectomized rats, no response was observed after angiotensin II. Increased JGI in bilaterally adrenalectomized rats was not accompanied by increased erythropoiesis. In these rats, erythropoiesis increased after injection with phenylhydrazine with a reduction of the increased JGI. There appeared to be an intimate relationship between erythropoietic activity and JGA granules.—K. F.

**Red-Cell Aplasia with Carcinoma of the Bronchus**


Carcinoma of the bronchus presenting with selective red cell aplasia of the bone marrow is described. A humoral factor in the patient’s serum inhibited iron utilization in rabbits. The factor disappeared after irradiation of the tumor, but anemia persisted.—P. B.

**Quantitative Thin-Layer Chromatography of Phospholipids in Normal Human Erythrocytes**


A method for separation and quantitative estimation of the various phospholipid fractions, using thin layer chromatography on Silica Gel G, was described. Phosphatidyl ethanolamine, phosphatidyl serine, lecithin, sphingomyelin and lysophosphatidyl ethanolamine could also be measured, but a preliminary fractionation on a silicic acid column...
was necessary. Normal values were determined from a study of 21 healthy donors, and lyso-
phosphatidyl ethanolamine was estimated in 7 subjects. The method had a number of advan-
tages over column and paper chromatographic procedures and could be used for the analysis of relatively large numbers of samples. It did not distin-
guish between plasmalogens and the corre-
sponding di-ester phospholipids. Inositol phospha-
tide could also not be separated.—T. H. B.

**IONIC PROPERTIES OF AQUEOUS DISPERSION OF PHOSPHATIDIC ACID. M. B. Abramson, R. Katz-

At physiologic pH, stable aqueous dispersions of phosphatidic acid can bind only 1.2 μequivalents of Na⁺ or K⁺ per μmole of P. Phosphatidic acid, therefore, would be a poor molecule to act as a cation carrier across biologic membranes, as originally suggested by Hokin and Hokin (Fed. Proc. 22:8, 1963). At least 2, possibly 3, Na atoms are carried per phosphate group involved in cation transport in red cell ghosts.—H. S. J.

**HUMAN ERYTHROCYTE ADENOSINE TRIPHOSPHATE D-3-PHOSPHOGLYCERATE 1-PHOSPHOTRANS-
FERASE. T. Hashimoto and H. Yoshikawa. From University of Tokyo, Japan. J. Biochem. 56: 279, 1964.**

Detailed procedures were presented for prepa-
ration of crystalline enzyme. The technic involved denaturation of hemoglobin with ethanol-chloro-
form, adsorption and elution from calcium phos-
phte gel, negative adsorption by diethylamino-
ethylcellulose and ammonium sulfate fractionation. The purified enzyme was obtained as long-rectan-
gular crystals and was specific for phosphoglycer-
ate. The turnover number was of the same order as that of crystalline yeast enzyme. Michaelis constants for the substrates were similar to values for yeast and muscle enzyme reported by others. —K. F.

**THE REDUCED PYRIDINE NUCLEOTIDE DEHYDRO-

Four enzymes with reduced pyridine nucleo-
tide dehydrogenase activity were demonstrated in human red cells, two with TPNH and two with DPNH activity. The DPNH dehydrogenase activity, missing in one type of congenital meth-
emoglobinemia, was calculated to account for two-thirds of the capacity of human red cells to reduce methemoglobin. Since heterozygotes for this disease are not methemoglobinemic, a one-
third loss in overall pyridine nucleotide dehydro-
genation does not lead to pathologic consequences. It was concluded that the only defects in reduc-
ing mechanisms likely to produce methemoglobin-
emias are absence of DPNH dehydrogenase, of DPN, or of enzymes producing DPNH.—H. S. I.
relationship also varied between antisera with the same blood group specificity.—H. H. F.


Using anaphylaxis with desensitization, specific antigens, absent in normal tissues, were revealed in tissues of patients who died of multiple myeloma. Specific antigens from various myelomic tissues (liver, spleen, kidneys) possessed immunologic affinity.—J. K.


Early termination of pregnancy resulted in a significantly higher survival rate of infants with erythroblastosis, particularly where previous infants had also been affected. The criteria used were relatively nonspecific and did not include spectrophotometric examination of amniotic fluid.—I. B. S.


Bone marrow examinations in 79 children revealed tumor cells in 21 of 30 cases of neuroblastoma, 3 of 18 with embryonal rhabdomyosarcoma, 2 of 6 with osteogenic sarcoma, 1 of 8 with Ewing’s sarcoma, 1 of 2 with retinoblastoma and none of 12 with Wilm’s tumor.—J. B. S.


In CCl₄-treated mice, spleen weight increases, and the lymphoid system exhibits proliferation of cells containing γ-globulin. In immunized mice treated with CCl₄ or allyl alcohol, these cells produce specific antibodies, indicating a quantitative alteration of immunologic reactivity. Liver injury seems to have an “adjuvant” effect.—H. H. F.


A syndrome of severe, prolonged unconjugated hyperbilirubinemia which relapses and remits in association with breast feeding was described in 7 unrelated full-term infants. Milk from the 7 mothers consistently inhibited glucuronyl transferase activity in vitro. Control milk specimens (99) did not. The steroid named in the title was crystallized and identified from inhibitory, but not from noninhibitory, human milk.—H. S. J.


Radiochemically pure biliverdin-C¹⁴ was administered intravenously to rats prepared with external biliary fistulae. The major fraction of pigment was rapidly reduced to bilirubin and was recovered from bile as conjugated bilirubin-C¹⁴. The results support the view that biliverdin is an intermediate in the degradation of hemo- globin to bilirubin.—H. S. J.


Following the injection of C¹⁴-labeled glycine or delta-amino-levulinic acid (DALA) into human subjects, bilirubin isolated from plasma or bile is rapidly labeled. With glycine-2-C¹⁴ as precursor, two early peaks of bilirubin activity are manifest—at 12–24 hours and between days 3–5; the latter appears at a time when circulating red cell heme activity is increasing maximally. With DALA, only one peak is found, at 1.5 to 6 hours, and labeling of red cell heme is minimal. The authors suggest that the early-labeled bile pigment is made up of 2 components: the first
ABSTRACTS

independent, and the second dependent on red cell heme synthesis. The suggestion is strengthened by the finding of persistent labeling of the first component in dogs following radiation-induced marrow aplasia.—H. S. J.


Removal of appendix and thymus in the newborn rabbit depresses production of antibody to bovine γ-globulin more than thymectomy alone. Neonatal appendectomy has as much effect on antibody-producing capacity as neonatal thymectomy. Neonatally thymectomized-appendectomized animals show depletion of lymphocytes in peripheral blood and failure of lymphoid development in nodes and spleen.—H. H. F.


Intranuclear inclusion bodies, plasma cells, flame cells and thesauocytes were observed in 31 patients; 26 had paraproteinaemia of γγ1A-type, 4 of γγ4-type and 1 of γγ5-type. In all patients with γγ1A-type plasma cells with intranuclear inclusion bodies were found. Many of these marrows also contained flame cells and thesauocytes. No plasma cell changes were absolutely specific for the γγ1A-group.—H. H. F.


Using mixed agglutination and mixed antihemoglo- lin reactions, A and B isoantigens have been demonstrated on spermatozoa of secretors, but not on those of nonsecretors. The sex-linked antigen, Xgα, could not be shown on spermatozoa.—H. H. F.


A simple and reproducible method is described in which the hemoglobin-haptoglobin complex is separated chromatographically from excess-added free hemoglobin.—P. B.


A large proportion of metaphase plates showed little difference between unshadowed and shadowed preparations. Major coiling could be better seen in shadowed preparations and acrocentric chromosome satellites showed up well. The centromere region of some shadowed chromosomes showed a fairly prominent dimple or crater not seen in unshadowed preparations. This technic may be of value in the more exact identification of individual chromosomes.—T. H. B.