ABSTRACTS

ERNST R. JAFFÉ, M.D., Editor

ABSTRACTERS

P. Barkhan, M.D., London, England
T. H. Bothwell, M.D., Johannesburg, South Africa
T. E. Brittingham, M.D., Nashville, Tenn.
Jacque Caen, M.D., Paris, France
J. B. Chatterjea, M.D., Calcutta, India
Pietro deNicola, M.D., Pavia, Italy
Ludvik Donner, M.D., Prague, Czechoslovakia
A. J. Erslev, M.D., Philadelphia
H. Hugh Fudenberg, M.D., San Francisco
Katsuhiro Fukutake, M.D., Tokyo, Japan
Robert Goldstein, M.D., New York City
Ira Green, M.D., New York City
F. W. Gunz, M.D., Christchurch, New Zealand
Susanna R. Hollan, M.D., Budapest, Hungary
Harry S. Jacob, M.D., Boston, Mass.

HEMOSTASIS


The authors studied 10 patients with well-documented myelofibrosis and found a significant history of bleeding in 6. Studies of the coagulation mechanism suggested that there was a defect involving the platelets in many of the patients, but the precise mechanism could not be established. In fact, it did not seem that a single defect could explain all the findings.—C. M.


This very detailed paper is based on a study of a 13 year old boy with congenital afibrinogenemia. His platelets showed both similarities to, and differences from, normal platelets and it appears that fibrinogen is not necessary for initiation of platelet agglutination, if the thrombin level is high enough. The original paper should be consulted for details, discussions and excellent electron micrographs.—C. M.


Referring to the observation of Schönlein-Henoch syndrome in three siblings, aged 10 to 14, three weeks after tonsillitis, the authors discuss the possibility of familial and hereditary factors in this condition and emphasize the role of streptococcal tonsillitis in its etiology.—E. K.


Like bentonite, attapulgite activates the contact factor, Factor XII. At higher concentrations, the other factors of coagulation are adsorbed in a well-defined order: VII + X, VIII, IX, X, XII, II. Unlike bentonite, attapulgite activates Factor V instead of adsorbing it. The coagulation accelerating properties, due to the activation of Factors XII and V, lead to a net shortening of the coagulation time. This substance eventually may be useful as a local hemostatic agent in gastroenterology.—G. M.

THE RATIO OF ERYTHROCYTE VOLUME TO PLASMA AS A FACTOR REGULATING HEMOSTASIS. H.
ABSTRACTS


Blood clotting was studied with a series of "artificial bloods" with different concentrations of erythrocytes and plasma prepared from the same blood. Low concentrations of erythrocytes enhanced coagulation. At values exceeding the upper limit of the normal hematocrit, the formation and consolidation of the clot was markedly delayed and impaired, the increase in the density of the clot was markedly delayed and impaired, and the beginning of clotting was slightly retarded. The hematocrit must be regarded as one of the regulators of hemostasis.—E. K.


The same amount of hexose, using the anthrone method, was found in insoluble and soluble fibrin, whether or not prepared in the presence of Factor XIII: FSF. The authors concluded that thrombin alone or associated with fibrinase was unable to liberate hexoses.—J. C.

IN VITRO AND IN VIVO THROMBOELASTOGRAPHIC ALTERATIONS INDUCED BY 0.4 PER CENT CdSO₄ SOLUTION. R. M. Ahuir. From the Instituto Provincial de Sanidad, Valencia, Spain. Sangre (Barc.) 10:207-226, 1965.

Thromboelastographic patterns of normal plasma treated in vitro with 0.4 per cent CdSO₄ solution showed shortening of r and k and diminution of ma. Factors II and VII were adsorbed and Factors V and VIII remained. Patterns of hemophilic plasma showed correction of r and k. The i.v. administration of 1 to 5 ml to hemophiliacs produced correction of r and k which was similar to the effect of transfusing 100 ml of blood. It was claimed that clinical improvement was obtained. The author postulated that CdSO₄ acted by neutralizing the inhibitors of intrinsic thromboplastin.—E. S.


Among 36 carriers of hemophilia A, a low level of AHG was found in the 6 carriers of mild hemophilia and in 11 of 30 carriers of severe hemophilia. The authors concluded that the two types of hemophilia are transmitted in different ways. In studies of two sisters, both mothers of hemophiliacs, one was found to have a low level of AHG and the other a level in the normal range. The authors concluded that, using only the determination of AHG, the diagnosis of the carrier state was unpredictable. Infusion of plasma from hemophiliacs into carriers did not increase AHG in the recipients. The defect in carriers was different from the one found in von Willebrand's disease.—J. C.


The authors found that with doses of venom above $10^{-2}$ mg./ml. there was coagulant activity, but an anticoagulant activity was noted with doses of $10^{-4}$ to $10^{-2}$ mg./ml. The coagulant activity present for the most part in Sephadex filtration fraction I seemed to effect a direct activation of Factor II and was similar to the activity found by Nahas in Echis coloratus.—J. C.


The formula obtained for the kinetics of the fibrinolytic activity of Factor XII was similar to the Michaelis-Menten formula for enzymatic reactions. Studies were performed with the fibrin plate technic.—E. S.


Coumarin derivatives are widely used as anticoagulants. Observations on 25 rabbits of both sexes, however, showed that coumarin, injected intravenously or intraperitoneally in doses of 0.05-0.10 Gm./Kg., significantly shorten the clotting time, plasma recalcification time, prothrombin time and thrombin time.—E. K.

LEUKOCYTES

MYELOFIBROSIS ASSOCIATED WITH TUBERCULOUS LYMPHADENITIS. S.-M. Samuelsson, A. Killander,

In a series of 10 cases of myelofibrosis, four had tuberculous cervical lymphadenitis; 24 cases of coinciding myelofibrosis and tuberculosis were reviewed.—S.-A. K.


In 13 cases of Hodgkin’s disease, some transformation of lymphoid cells in the presence of phytohemagglutinin occurred. This property evolved in parallel with the change in the Cuti reaction (8–12 per cent transformation of lymphoid cells in patients with Cuti −, 30 to 40 per cent in patients with Cuti +).—J. C.


Twelve cases of malignant lymphoreticular disease (reticulalymphosarcoma, Hodgkin’s disease, Hodgkin’s sarcoma) were reported. In each case, the disease was in an advanced stage and could not be influenced by the usual therapy. When 500 mg. of Endoxan together with prednisolone were administered intravenously per day (total dose 5,000 to 8,000 mg.), a complete remission was observed in almost all patients. The subjective side effects were negligible; alopecia developed in two patients. Transient leukopenia and thrombopenia were observed.—S. R. H.


The basic disorder revealed by autopsy of a male aged 55 showed the classic histologic features of macrofollicular reticulosarcoma and the simultaneous presence of previously benign and later definitely malignant alterations and transition forms. Specific morphologic changes were found in lymph nodes and spleen. In the gastrointestinal tract thickening of the mucous membrane, diffuse polyposis and diffuse infiltration of the stomach and multiple plaques in the intestines were observed. In the cirrhotic liver, characteristic histologic alterations of Brill–Symmers disease were detected.—S. R. H.


One hundred animals were divided into 4 groups: 50 control animals (Group 1), 10 injected with complete Freund’s adjuvant (Group 2), 10 injected with antigen prepared from lymph nodes of normal guinea pigs (Group 3) and 39 injected with the same antigen emulsified in adjuvant (Group 4). Most of the injected animals showed a 10 per cent decrease in serum proteins. By microimmunoelectrophoretic technics, a reinforcement of IgM in group 2 and 4 was shown. By micrdiffusion in agar, 6 per cent of the guinea pig sera of group 4 gave precipitation lines with the antigen. Using passive hemagglutination, 30 to 40 per cent of all animals had positive results with the antigen at a titer of 1:40. Using complement fixation, positive results were obtained in 10 and 20 per cent of animals of group 3 and 4, respectively. Histologic studies of the lymph nodes from group 2 and 4 showed hyperplasia of the reticular tissue with decreased size of follicles. In a few animals of group 4, giant cells were observed. In group 3, hypertrophy of lymphoid follicles was observed. Nonspecific modifications of serum proteins were detected in some animals belonging to group 2 and 3 and in a greater number of animals of group 4. Circulating isoaantibodies could be compared to those detected in 2 cases of lymphosarcoma. The histologic alterations of the lymphatic organs could not be compared with the alterations detected in humans.—E. S.


Thymectomy prevented the development of methylycolanthrene-induced lymphoid leukemia in DBA mice and isogenic thymuses grafted subcutaneously before the application of the drug restored the incidence of leukemia in thymectomized
ABSTRACTS

The grafted thymuses were seldom infiltrated by the leukemic process, suggesting an indirect influence of the graft in the pathogenesis of leukemia. The incidence of skin carcinomas induced by methylcolanthrene was not influenced by the presence or absence of the thymus.—E. S.


Neonatal thymectomy leads to a wasting syndrome with disordered development of immune reactivity. Miller considers the fundamental lesion to be lymphoid aplasia. De Vries suggests that the fundamental lesion is failure of the immunologically competent cells to recognize the self-antigens peculiar to the subject and that the lymphoid aplasia is secondary. The authors have studied L. E. cells and antinuclear antibodies in mice 6 weeks and 3 months old who had been thymectomized at birth. The L. E. tests were negative, but serum antinuclear antibodies were found more frequently in thymectomized mice than in sham operated or normal mice of the same age. These results would appear to be in good agreement with the pathologic studies of de Vries and they raise the question of control by the thymus of the establishment of specific tolerance of the normal subject to his own antigens.—C. M.


This complex and severe developmental anomaly is associated frequently with hemoglobin abnormalities and with anomalous nuclear projections in the neutrophils and eosinophils. These projections resemble drumsticks and sessile nodules, are frequently multiple and do not appear to be artifacts. The suggestion is made that one or more of the D1 chromosome genes may control neutrophil and eosinophil nucleus organization. Other chromosome abnormalities, however, (C, 21 and X) also appear to exert an effect on this process. —C. M.


The authors present a detailed morphologic study of "Russell" and other bodies in myeloma cells (with excellent illustrations) and suggest that they are all very similar. Both intranuclear and intracytoplasmic bodies originating in the cytoplasm, probably in the Golgi apparatus. The inclusions are seen in all types of myeloma cell and the separation of morular from grape-cells does not seem to be justified.—C. M.

ERYTHROCYTES


A 54 year old white woman with a 15 year history of intermittent meta- and sulfhemoglobinemia with moderate to severe Heinz-body hemolytic anemia was reported. The condition remained moderate to severe, despite exclusion of exogenous oxidant chemicals. Hemolysis, which was shown by cross transfusion studies to be due to an "extracorporeal" defect, was improved by splenectomy. A dramatic normalization in the level of methemoglobinemia which at times reached as high as 6 Gm. per cent, followed a course of neomycin therapy. This salutary effect persisted for several months, despite discontinuance of the drug. A peculiar flora of intestinal bacteria may have been responsible. (Abstracter's note: This case is reminiscent of one reported by Selwyn (Brit. J. Haemat. 1:173, 1955) in which Heinz-body hemolytic anemia was markedly aggravated in a patient taking phenacetin who developed spontaneous intestinal obstruction).—H. S. J.


The fall in red cell AChE associated with acute hemolytic anemia in a patient exposed to hyperbaric oxygen led to studies of the role of H2O2 and lipid peroxides on the enzyme in vitro. AChE activity of red cell extracts and of intact red cells was inhibited by gaseous diffusion of H2O2 and by
addition of preformed lipid peroxides prepared by ultraviolet irradiation of red cell lipid extracts. Oxygen alone produced no inhibition of enzyme activity. It was concluded that AChE of red cells was inhibited in vivo by formation of lipid and, possibly, other peroxides. The observation by these authors (Nature 210:91, 1966) that PNH red cells form lipid peroxides excessively in vitro may explain the diminished AChE in red cells of this disease.—H. S. J.


The lipid composition of red blood cells from healthy adults and from patients with acanthocytosis, paroxysmal nocturnal hemoglobinuria, congenital hemolytic anemia and hereditary sideroblastic anemia were studied. The normal and abnormal erythrocytes differed in their cholesterol and esterified fatty acid content and in the fatty acid distribution of the phospholipids. Correlations between membrane permeability and the changes in lipid composition of the erythrocytes were discussed.—S. R. H.


Clinical and hematologic data from four members of a family suffering from hereditary sideroblastic anemia were presented and the results of some special biochemical and electron microscopic studies were discussed. Definite changes were found in the lipid composition of the red cell membranes of three patients with hereditary sideroblastic anemia and a patient with acanthocytosis and secondary sideroblastic anemia. It was surmised that the changes in the permeability of cellular and subcellular membranes played an important role in the development of the intracellular iron overload.—S. R. H.


The case of a 22 year old male was presented in which three separate courses of pyridoxine produced remission of the sideroblastic anemia and in each case caused normalization of extremely low levels of serum cholesterol (90 mg. per cent) and of total serum lipids (300 mg. per cent). Other reported cases of pyridoxine-responsive anemia in which hypcholesterolemia had been noted were reviewed. The patient developed thrombophlebitis during many attempts to administer pyridoxine and he finally succumbed to pulmonary emboli. The nature of the thrombotic diathesis is unknown, but its possible relation to alterations in serum lipids is intriguing.—H. S. J.


Rats fed ethionine lose weight, but show a rise in hematocrit and hemoglobin levels. The animals also have an elevated serum iron and a decreased iron binding capacity, resulting in a high degree of saturation. This trend ceases as ethionine feeding continues and the avidity of the liver for and its content of iron decreases. In the early stages, however, the process does resemble idiopathic hemochromatosis.—C. M.


The absorption of ferrous sulfate and hemoglobin (hemolysed rabbit erythrocytes) was studied by a double radio-iron technic. In normal subjects, the absorption ratio FeHb/Fe₂SO₄ was about 1. As expected, iron absorption was increased in iron deficient non-gastrectomized patients; this increase was most pronounced for ferrous sulfate, yielding an absorption ratio FeHb/Fe₂SO₄ of 0.34. Next, 13 male patients were studied in whom partial gastrectomy had been done about 3 years previously because of duodenal or gastric ulcer. In patients with a Billroth I operation, the iron absorption pattern was as in non-gastrectomized individuals, possibly with a relative reduction of iron absorption in anemic patients. All six patients with a Billroth II operation appeared to be iron deficient. In three, iron absorption was increased, but the FeHb/Fe₂SO₄ ratio was not decreased as in non-gastrectomized sideropenic individuals. In the
remaining three Billroth II patients, iron absorption was decreased. This reduction was most marked for ferrous sulfate, resulting in an absorption ratio FeHb/Fe₂SO₄ of 1.5-2.4. It appeared that after Billroth I operations there was a slight quantitative change in iron absorption, whereas after Billroth II operations there was a quantitative, as well as a qualitative, iron absorption defect which seemed to be related to the severity of the anemia.—S. A. K.


A detailed account of atomic absorption spectroscopic determination of both serum iron and iron-binding capacity is presented. The accuracy is of the order of ± 1 μg., but the method obviously calls for considerable skill and careful control. Direct estimation is possible only above 200 μg. per 100 ml. and a chelation technic is necessary below that level. While the effects of hemolysis are less than with other methods, it still introduces a significant error, particularly in aged serum.—C. M.


The production of CO in vivo has been shown to result mainly from catabolism of the α-methylene bridge carbons of heme molecules. A rebreathing apparatus for measuring CO production was described and its use in assessing blood heme destruction in 7 patients with hemolytic anemia was noted. This method estimated red cell survival which was well correlated with the results obtained with radiochromate studies (r = 0.94). Since average molar ratios of CO production to circulating heme catabolism were greater than unity in 6 of 8 studies, it was suggested that ineffective erythropoiesis also was measured by this method.—H. S. J.


The improved benzidine reaction for plasma hemoglobin determination was studied. A stable benzidine reagent, a hemoglobin standard solution and a procedure for expediting color-development at 37 C. were elaborated. A solution of sodium perborate was used for oxidizing. Color reaction was stopped and stabilized with dextrose. By varying the final volume of the reaction mixture, the accuracy was increased with low or high plasma hemoglobin values. Calibration with the hemoglobin standard should be performed in parallel with each determination. Normal values were determined in healthy adults and the clinical advantages of this method in hemolytic diseases were discussed.—S. R. H.


The need for increased recognition that ocular complications are common in SS and SC diseases is emphasized by the authors who present three patients with SC disease presenting as Eales’ Disease. This condition of recurrent hemorrhages into the retina and vitreous has rendered these and other patients with these hemoglobinopathies nearly and, sometimes, totally blind. No treatment is suggested.—H. S. J.

**STUDIES ON RELATIONSHIPS BETWEEN STRUCTURE AND FUNCTION OF HEMOGLOBIN Miwate.** N. Hayashi, Y. Motokawa and G. Kibuchi. From Tohoku University School of Medicine, Sendai, Japan. J. Biol. Chem. 241:79-84, 1966.

Functional properties of hemoglobin Miwate, in which the alpha chain histidine in position 87 which links the globin chain to heme is replaced by tyrosine, were studied to gain insight into structural-functional relationships in the hemoglobin molecule. Abnormalities included: (1) greatly diminished O₂ and CO affinity; (2) an interaction constant “n” of 1.0, signifying no potentiation of oxygen binding with increased oxygen supply; (3) an absent Bohr effect, despite the usual two reactive SH groups per molecule; and (4) a 6-fold increase in the rate of autoxidative denaturation relative to hemoglobin A. With reduction of a-linked ferriheme groups with dithionite, normal O₂ and CO affinity and the Bohr effect were regained, but no change in the value of “n” occurred. The results indicated that the substitution of a single amino acid, if strategically placed, can produce conformational changes in the whole hemoglobin molecule.
which affect differentially the Bohr effect, affinity for ligands and the shape of the O₂ and CO dissociation curves.—H. S. J.


Chromatographic and electrophoretic purification of stored human hemoglobin suspensions and analyses of the separated minor hemoglobin components which appeared with time were performed. The data support the conclusion that SH groups of the beta chains of normal A₀ hemoglobin become bound to oxidized glutathione in mixed disulfide linkage to yield A₁ (also known as A₂) hemoglobin. Reduced glutathione, by eliminating the formation of these mixed disulfides, may play an important role in maintaining the physical and physiologic integrity of normal hemoglobin. This conclusion also has been reached by Allen and Jandl in studies on red cell senescence (J. Clin. Invest. 39:1818, 1960. and 40:454, 1961).—H. S. J.


In this timely review article, 444 papers are cited in the description of epidemiologic and clinical aspects of polycythemia. Accepted classifications have been strengthened by separating clearly benign erythrocytosis from polycythemia vera and secondary polycythemia.—A. J. E.


After comparative examination of over 80 patients with polycythemia vera (Vaquez-Osler’s disease) and idiopathic metaplasia, a number of characteristic hematologic differences could be established. Special attention was paid to polycythemia vera and a polycythemic stage in nearly 30 per cent of patients with idiopathic myeloid metaplasia which terminated sooner or later with anemia, leukemia or even erythremia. Clinical and laboratory findings suggested that Vaquez-Osler’s disease and a polycythemic stage of myeloid metaplasia differ significantly and, therefore, should be treated in different ways.—S. R. H.


The data from these two papers support the hypothesis that a high hematocrit with increased viscosity of whole blood provides unfavorable coronary perfusion and may contribute to coronary heart disease. Statistically, there was a difference between the hematocrits of patients with chronic coronary disease and the healthy controls of the same age and sex. Clinically, there appeared to be a decrease in the frequency of the anginal attacks and increased effort tolerance following reduction of the hematocrit by phlebotomy.—A. J. E.


Careful studies of erythropoietin were made before and after renal homotransplantation in ten patients. Five patients exhibited a significant increase in erythropoietin titer after transplantation, an increase which was not related significantly to the usual parameters of renal function. These studies confirmed what has generally been observed, that the denervated human renal homograft can release erythropoietin shortly after its transplantation.—A. J. E.


ON THE MECHANISM OF ERYTHROPOIETIN-INDUCED DIFFERENTIATION. IV. SOME CHARACTERISTICS OF ERYTHROPOIETIN ACTION ON
ABSTRACTS


These two papers described the employment of in vitro technics in the study of the action of erythropoietin. Bone marrow suspensions from Sprague-Dawley rats were used and the effect on membrane synthesis and hemoglobin synthesis of various metabolic inhibitors was studied. Although there have been no definite conclusions, the technic was very promising—A. J. E.


The fall in O2 tension which occurs at an altitude of 2,000-2,350 meters above sea level initiates the erythropoietic response in rats, as was shown by Fe59 studies.—E. S.


The effect of gamma irradiation on the response to erythropoietin was studied in order to elucidate the kinetics of stem cells. The effect of irradiation was found to depend on the moment irradiation was given in relation to the erythropoietin injection. Attempts were made to explain the clear-cut time effect relationship.—A. J. E.


Erythremia is induced by placing mice in a box with increasing concentrations of CO. A hematocrit of about 80 per cent is obtained after 17 to 20 days. Seven to 8 days after cessation of hypoxia, reticulocytes are absent and spontaneous Fe59-incorporation is less than 0.3 per cent of the injected dose. No endogenous erythropoietin stimulation is present as long as the hematocrit remains above 55 per cent. The optimum time for assaying erythropoietin has been studied. Dose fractionation increases the effect of a given quantity of erythropoietin. It is suggested that this is due to an erythropoietin-induced increase in the number of erythropoietin-sensitive cells. The sensitivity of the method is about 0.03 units erythropoietin, standard B.—S. A. K.


Ribosomes prepared from reticulocytes of thalassemia incorporate significantly less amino acids into protein than do ribosomes from other chronic hemolytic anemias. This defect is not corrected by addition of supernatant fractions from cells of non-thalassemic subjects or from rabbit reticulocytes. The ability of thalassemia ribosomes to respond to polyuridylic acid-directed phenylalanine incorporation is normal. These results suggest that the primary defect in thalassemia involves a decrease or defect in messenger RNA for hemoglobin A.—H. S. J.


Studies of rabbit marrow using a colchicine technic revealed a duration for mitosis of 44-83 minutes (mean 66.5) in “erythroblasts.” These cells were not subdivided into classes or by degrees of maturity because the authors felt that this was not justified. Discussions of the relative merits of this and other methods and of factors involved in the calculation were presented.—C. M.


This very interesting paper contains descriptions of autophagic vacuoles in human erythrocytes and has numerous electron micrographs illustrating their properties. These vacuoles are lysosomes in that they have limiting membranes and show acid phosphatase activity. They are common in reticulocytes, but much less so in mature erythrocytes when the spleen is present. They are increased in the erythrocytes of splenectomized individuals and of those with abnormalities of erythropoiesis and, especially, where both conditions are present. Siderocytes are conspicuous in splenectomized individuals when compared with those who have functioning spleens.
and so are the vacuoles. The vacuoles represent a mechanism by which the spleen can remove iron from intact erythrocytes, as has been suggested previously on the basis of other studies.—C. M.

MISCELLANEOUS


In 15 patients with cirrhosis, the authors, in addition to the leukemoid symptomatic reactions of superinfection and neoplasms, noted the possibility of extensive medullary plasmocytosis and the frequency of pancytopenia, anemia, thrombocytopenic purpura and splenomegaly. These findings may indicate the presence of inapparent disease of the liver.—J. C.


Cryoglobulins with rheumatoid factor activity had no other antibody effect characteristic of the total serum or its normal gamma globulin fraction. The normal antibody content of sera with cryoglobulinemia and rheumatoid factor activity was not smaller than that of controls. The presence of large quantities of pathologic proteins in the serum did not decrease the level of normal antibodies in all cases.—S. R. H.


A total of 554 patients with serum M-components was studied retrospectively: 318 cases were of type γG, 109 γA, 95 γM, 3 γD, 18γA. A definite diagnosis of myeloma was made in 194 patients and this diagnosis was probable in another 50. Reticular neoplasia was diagnosed in 64 patients, “other conditions” in 197 cases, and 49 patients had cancer. The frequency of myeloma increased with the concentration of the M-component: among patients with less than 1 Gm. per 100 ml. of γG- or γA-M-component, 12 and 27 per cent, respectively, had myeloma. When the pathological γ-G was above 2 Gm. per 100 ml. or the γ-A above 1 Gm. per 100 ml., about 80 per cent of the patients had myeloma. Similarly, the frequency of reticular neoplasia increased with increasing concentrations of M-component. Two patients with γD-M-component had myeloma.—S. A. K.