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Theodore H. Spaet, M.D., Editor

ABSTRACTS OF SPECIAL INTEREST


Histologically, the duodenal and proximal jejunal lesions of celiac disease and idiopathic sprue (nontropical sprue, idiopathic steatorrhea) are identical. The ileal mucosa may be normal. This important study bears on many questions, including: (1) Does the localization of the pathology suggest that folic acid is absorbed in the proximal intestine? (2) Since the ileum may be normal, and vitamin B₁₂ is probably absorbed from the ileum, what is the mechanism of the poor B₁₂ absorption of many patients with idiopathic steatorrhea? Can it be partly due to calcium lack (R. Gräsbeck, I. Kantero, and M. Siurala. Lancet 1:234, 1959)? Is it mainly due to diarrhea (F. D. Rosenthal, H. T. Swan, and G. R. Tudhope. Gastroenterology 37:282, 1959)?—V. H.


Folic acid clearance tests were performed on 30 healthy nonpregnant women of child-bearing age, 250 pregnant women without anemia, 11 patients with normal twin pregnancy 34 to 39 weeks pregnant, and 11 patients with untreated megaloblastic anemia of pregnancy. Injected folic acid was cleared more rapidly from the plasma in pregnant than in nonpregnant women. The difference was already significant before the twelfth week of pregnancy, and the rate of clearance increased progressively from the twelfth to the thirty-sixth week. At full term, and after the expected date of delivery, clearance was significantly slower than in subjects 37 to 40 weeks pregnant, though still more rapid than in early pregnancy. There was good correlation between the incidence of abnormal clearance tests at various stages of pregnancy and the rate of growth of the fetus and uterus. Folic acid clearance was more rapid in twin pregnancy than in single pregnancy and was most rapid in the group with megaloblastic anemia. Folic acid absorption tests were carried out on 90 healthy subjects of both sexes, 45 women between 20 and 40 weeks pregnant and 10 women with treated megaloblastic anemia of pregnancy. Absorption was significantly lower in the pregnant subjects than in the normal controls, and significantly lower in those with megaloblastic anemia than in normal pregnancy. It is concluded that the increased rate of clearance in pregnancy is due to folic acid deficiency. This is probably...
mainly due to the fetal requirement exceeding the dietary intake, but it appears that impaired absorption during pregnancy may also play a part. The findings suggest that the same factors are responsible for the more severe folic acid deficiency which results in the development of megaloblastic anemia in pregnancy.—R. M. H.


The authors have previously found prolonged bleeding time, AHG deficiency and normal platelet function with respect to platelet factor 3 in cases of v. Willebrand’s disease. Twenty-six cases (13 males and 13 females) were studied by the authors. Thirteen cases had been previously described, and 13 new cases are now reported. Ten patients were treated with fraction I - 0 of normal human plasma containing high concentrations of fibrinogen and AHG. In all cases the bleeding was controlled, the bleeding time became normal, and the AHG level increased. Fraction I - 0 from patients with hemophilia A normally corrected the bleeding time, but the fraction from patients with v. Willebrand’s disease had no effect. Different fibrinogen fractions had no effect on the bleeding time of patients with v. Willebrand’s disease, but it was corrected by fraction I - 0, in which the AHG activity had been lost. It is concluded that the plasma factor correcting the bleeding time in v. Willebrand’s disease is not identical with AHG or fibrinogen.—C. W.


Based on the previously reported erythrogenic effect of testosterone in patients being treated for breast carcinoma and myeloid metaplasia, the authors have studied the effects of testosterone in the treatment of aplastic anemia in children. This mode of therapy was given in conjunction with blood transfusions and corticosteroids. Testosterone was given orally in a dose of 1 to 2 mg./Kg. body weight per day and, in 1 patient, testosterone enanthate was administered intramuscularly in a dose of 3 mg./Kg. twice weekly. The corticosteroid employed was triamcinolone in dosages ranging from 8 to 12 mg. daily. Of 5 children who were treated in this manner, 4 demonstrated striking improvement and 1 died of gastrointestinal bleeding and overwhelming infection. The beginning of improvement in favorable cases was signified by the appearance of a reticulocytosis which occurred at time intervals ranging from 1 to 5 months following the onset of therapy. This reticulocytosis was succeeded in all instances by a rise in hemoglobin concentration so that transfusion therapy could be eliminated. The time elapsed between the beginning of testosterone therapy and the last transfusion ranged from 1 to 4 months. In the favorable cases an increase in polymorphonuclear leukocytes also occurred and reached normal levels in 2 of the patients. Rises in platelet count were variable and reached normal levels in only 1 of the patients. Bone marrow aspirations revealed a change from the typical acellular fatty marrow to a regenerating and finally hypercellular marrow with marked erythroid hyperplasia. At the time of this report it is not possible to determine the subsequent fate of these patients, the total time that therapy may be required, and the results which will develop following attempted cessation of treatment.—I. S.

LEUKEMIA


In three patients with acute lymphoblastic leukemia in remission, total body radiation with Cobalt-60 at a dosage of about 850 rads was given followed by transfusions of homologous marrow. The clinical and biologic sequelae can be grouped into several phases. During the first week, digestive disturbances and a lymphoid and myeloid pancytopenia were present. Total aplasia persisted for the next 10 to 15 days. At first there were few symptoms, but later fever, necroses and a hemorrhagic tendency occurred. One of the patients died with a respiratory complication before any evidence of marrow activity had returned. In the other two patients, the cytopenia gradually diminished between the eighteenth and twentieth day after irradiation. The clinical symptoms disappeared with reappearance of circulating granulocytes. Between the twenty-eighth and fortieth day therapy could be eliminated. The time elapsed between the beginning of testosterone therapy and the last transfusion ranged from 1 to 4 months. In the favorable cases an increase in polymorphonuclear leukocytes also occurred and reached normal levels in 2 of the patients. Rises in platelet count were variable and reached normal levels in only 1 of the patients. Bone marrow aspirations revealed a change from the typical acellular fatty marrow to a regenerating and finally hypercellular marrow with marked erythroid hyperplasia. At the time of this report it is not possible to determine the subsequent fate of these patients, the total time that therapy may be required, and the results which will develop following attempted cessation of treatment.—I. S.
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A new cytostatic compound "mannomustine" (1:6-bis(β-chloroethylamino)-1:6-desoxy-D-mannitol dihydrochloride) was used in a dosage of 100 mg. daily or on alternate days for the treatment of 45 patients with leukemias, polycythemia, reticulosis and malignant disease. It was found to be of some value in the treatment of chronic lymphatic leukemias, Hodgkin's disease, Brill-Symmer's disease, reticulosarcoma, multiple myeloma and polycythemia, but was less effective than accepted agents for the treatment of chronic myeloid leukemia. In association with steroids and blood transfusion, it was of some value in acute leukemia, especially in cases of monocytic leukemia with a high total white cell count. Toxic effects include severe bone marrow depression and gastrointestinal symptoms.—R. M. H.


Isologous adult marrow cells grafted after LD₁₀₀₀₀₀₀₀ irradiation are well tolerated but of little value in the treatment of leukemia 1210 (DBA₂ leukemia) in F₁ hybrids (DBA₂ x C₅₁BL₁). The maximum irradiation dose that can be tolerated with the marrow graft is not enough to destroy all leukemic cells. Irradiation followed by graft of adult marrow C₅₁BL₁ homologous with the leukemia, is more valuable as treatment. There appears to be (as in Barnes and Loutit's experiment: Brit.J. Haematol. 3:242, 1957) a summation of the effects of irradiation and of the immune reaction of the grafted cells. The latter is only apparent if the numbers of leukemic cells are small. Unfortunately, such a graft of adult cells after irradiation is followed by homologous disease, which is usually accepted as a reaction of the graft against the host. It is claimed by some writers that the use of embryonic marrow tissue avoids homologous disease, the grafted cells becoming tolerant to host antigens. It would seem that such embryonic cells become equally tolerant to the leukemic cells, as they have no more antileukemic action than isologous adult cells. Adult C₅₁BL₁ spleen cells cannot be used, since they induce an acute homologous disease with rapidly fatal results.—G. M.

CLINICAL TRIALS WITH 6-AZUARACIL IN MALIGNANT NEOPLASTIC DISEASES. J. H. Waelsch.

Thirteen patients were treated with 6-azauracil (AzU): 3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-hydrazine. In two patients suffering from Hodgkin's granuloma, the preparation had a favorable effect. In one patient the enlarged lymph nodes practically disappeared and after 1 1/2 years no relapse had occurred. The second patient has been taking the preparation for about 8 months, and a substantial improvement of the clinical condition occurred twice in the first 6 months. Six months after starting therapy the lymph nodes again enlarged, and combined treatment with AzU and X-ray was therefore initiated. After the administration of large doses (34 mg/Kg. body weight per day) mild neurotoxic symptoms developed. In the remaining patients with malignant disease AzU did not exert a therapeutic effect, and its administration frequently had to be stopped because it produced severe neurotoxic symptoms.—L. D.

ERYTHROPOIESIS


The investigators have studied the incorporation of (A-¹⁴C) glycine into the early labeled "hemopoietic" stercobilin in a 53 year old man with mild porphyria cutanea tarda before and after a phlebotomy of approximately 1/5 of his total circulating blood volume. The purpose of the study was an estimation of the relative amounts of stercobilin formed as a consequence of stimulated erythropoiesis in an attempt to demonstrate that a significant increase in the early fraction of stercobilin results from increased red cell formation. In this patient the data presented support this contention. In addition, it appears that porphyria cutanea tarda differs from congenital porphyria in that there is no increase in the amount of "hemopoietic" stercobilin relative to late-labeled stercobilin.

Comment: Using N¹⁵ glycine G. W. James et al. (Stercobilin and hematopoiesis. Am.J.Clin. Nutrition. 3:64, 1955) have shown that in normal man after a 800 ml. phlebotomy the amount of early label "hematopoietic stercobilin" is sig-
Urines of healthy persons and of patients with hemolytic jaundice contain 4 urobilinoid fractions: (a) Fraction bound to mucoprotein: with paper chromatography and paper electrophoresis this remains at the site of application. It may be stained with acid fuchsin by the mucopolysaccharide staining method and turns brown when treated with the anilinehydrogenphtalate reagent. (b) Fraction bound to glucuronic acid: with paper chromatography it is found between the origin and front; with paper electrophoresis using borate buffer it migrates 1.4 times faster than dextrose. It does not stain with acid fuchsin and turns brown by adding the anilinehydrogenphtalate reagent. When isolated by borate electrophoresis, it gives the carbazole reaction of Dische, characteristic of glucuronic acid. (c) A fraction bound to oligo (or mono-) saccharide, which shows similar behavior, but migrates more slowly in borate electrophoresis. (d) Free urobilinoid.—S. R. H.


Vitamin B₁₂ labeled with any of the radioactive cobalt isotopes is likely to decompose at an unpredictable rate on storage. The rate cannot be correlated with specific activity, concentration, or the nature of the isotope. When decomposition occurs, radioactivity is no longer a reliable indicator of vitamin B₁₂ content, as radioactive breakdown products are present. It is recommended that preparations of radioactive vitamin B₁₂ should be tested for radiochemical purity at intervals not exceeding one month.—R. M. H.


Co³⁰⁰-labeled vitamin B₁₂ was administered orally to each of 11 subjects without vitamin B₁₂ deficiency and 10 pernicious anemia patients. Administration was both as crystalline vitamin B₁₂ and after incorporation in pig's or calf's liver. Both groups of subjects absorbed more radioactivity from liver than from the crystalline preparation. It is tentatively suggested that the vitamin B₁₂ in the liver may be in the form of a B₁₂-peptide complex which can be absorbed in the absence of intrinsic factor.—R. M. H.


The activity of hog intrinsic factor, measured by its effect on intestinal absorption of Co³⁰⁰-labeled vitamin B₁₂ in patients with pernicious anemia, was strongly inhibited by the sera of some patients with pernicious anemia, but not by normal sera. The oral administration of hydrocortisone, 80 mg. daily for 28 days, to three pernicious anemia patients significantly reduced the inhibitory effect of their sera, but did not affect their own capacity to absorb vitamin B₁₂. In the 1 case tested, the inhibitory effect of the serum was fully restored one week after withdrawal of hydrocortisone. A known inhibitory serum did not interfere with the absorption of vitamin B₁₂ by a normal subject.

It is suggested that the inhibitory factor in these sera may be a true antibody, though precipitin tests against intrinsic factor preparations have not given significant results. The possibility of its being an autoantibody is mentioned.—R. M. H.


Thirteen patients who had previously been satisfactorily maintained on parenteral vitamin B₁₂ for at least 1 year, and 9 patients with previously untreated pernicious anemia, were treated orally with a preparation containing vitamin B₁₂ and an extract of hog duodenal mucosa for periods up to 5 years. Normal serum levels of vitamin B₁₂ were not maintained in the majority of the patients, three of whom relapsed after 2 1/2 to 5 years’ treatment. Skin tests showed no relationship between skin sensitivity to the hog duodenal extract and refractoriness to treatment.—R. M. H.


An insoluble complex of 500 μg. of vitamin B₁₂, zinc and tannic acid proved to be slowly absorbed on intramuscular injection into normal
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humans. It produced elevated serum vitamin B₁₂ levels sustained at more than twice baseline levels for at least 4 weeks. One thousand µg of vitamin B₁₂ alone produced a much greater initial elevation, but high serum levels were sustained for only a week. Because of the slow absorption of the complex, the preferable initial therapy of patients with vitamin B₁₂ deficiency will probably continue to be with injections of crystalline vitamin B₁₂, especially in the presence of neurologic damage. The complex may prove to be a particularly useful preparation for maintenance therapy, provided it produces no local or systemic toxicity.—V. H.


The dynamics of the iron transfer from plasma to reticulocytes was studied in a number of beautifully designed and carefully executed experiments. Iron chelated to iron binding protein (IBP) is transferred to reticulocytes but not to mature red cells. This transfer is dependent on the extent of IBP saturation and may represent a competition for iron between IBP and iron-binding receptors on the reticulocyte membrane. Free iron, not bound to IBP, is adsorbed on the surface of both mature and immature red cells; but this loosely adsorbed iron is much less efficiently utilized for heme synthesis than iron transferred by means of IBP. The uptake of IBP-iron is a function of the reticulocyte membrane and is not primarily linked to heme synthesis. Thus, lead will block iron incorporation into heme without blocking the transfer of iron to the cell membrane. Liver slices were found to pick up iron from IBP, but only at high IBP saturation. Cobalt was found to be bound to IBP and to a limited extent to be transferred to reticulocytes, but it was concluded that this process probably would not occur under physiologic conditions and not be involved in the induction of cobalt polycythemia.—A. J. E.


The enzymic formation of heme in vitro, using protein-bound iron in human serum or rat plasma or liver as an iron source, was much increased when ascorbic acid or reduced glutathione was added to the system. The optimum concentrations of these substances were within the range of their normal concentrations in human and rat liver. It is suggested that they take part in the transfer of iron from ferritin and hemosiderin in the liver for heme biosynthesis.—R. M. H.

STUDIES ON FERRODYNAMICS. I. GASTROINTESTINAL ABSORPTION OF Fe₅⁹ IN THE RAT UNDER DIFFERING DIETARY STATES. II. THE UPTAKE AND DISTRIBUTION OF LABELED Fe₅⁹ IN EXPERIMENTAL INFUSIONAL SIDEROSIS. J. P. Wack and J. P. Wyatt. From St. Louis University School of Medicine, St. Louis, Mo. Arch.Path. 67:237, 67:243, 1959.

These two papers report a study of the high specific activity of radioactive iron in two different experimental approaches in the study of ferrodynamics. In the first, the radioactive iron was administered by stomach tube and 12 hours later the animals were killed. The precise areas of absorption of the gastrointestinal tract was undertaken by autoradiographic procedures. In the second group of experiments, intravenous injections with 10 µc. of ferrous ascorbate containing 0.0094 mg. of iron were given and the iron uptake measured by means of a low-background well-type scintillation counter. The results indicate that the stomach plays a minor role in iron absorption in reversible states of iron storage in the rat. The duodenum is the first and the main site of absorption in all animals. Absorption may take place in the ileum and colon in animals on an iron-deficient diet and those placed on low protein, high-iron diets. Alterations from the normal in terms of depressed hemoglobin reading, iron insufficiency or protein depletion may remove the mucosal block. The degree of alteration determines the extent of the anatomic zone of intestinal iron absorption. Massive amounts of injected iron rapidly supply the demands of the erythropoietic system and open up the liver as a storage depot. After the demands were met, all of the radioactive tracer dose was evenly distributed throughout the peripheral portions of the liver lobules. A "functional blockade" of the marrow is readily produced with intravenously administered saccharated iron. Carbon block did not interfere with the marrow uptake of iron. X-radiation suppressed bone marrow uptake of iron to a degree.—O. P. J.


The absorption of iron was studied during continuous intraocular infusion of a solution of ferrous sulphate labeled with Fe₅⁹. The rate of absorption was determined by the plasma Fe₅⁹ activity and
the outflow of iron from plasma using Fe$^{55}$ simultaneously administered intravenously. By giving ionized iron intravenously the rate of absorption was immediately diminished to a marked degree. By giving iron-free transferrin intravenously a marked increase in the absorption rate was observed. During intragastric infusion for several hours at different concentrations there was nothing to indicate an exhaustion of any transfer-system for iron. It is concluded that the absorption rate of iron is regulated by concentration gradients between plasma, mucosal cells and intestinal lumen.—C. W.


In rabbits, blocking of reticuloendothelial system by Evan’s blue resulted in marked hyperferremia. Animals previously injected with Evan’s blue did not show the hypoferremic effect, otherwise observed with injections of turpentine oil.—J. B. C.


A study of 341 patients who had undergone partial gastrectomy for peptic ulcer showed a progressive fall of hemoglobin over a 10 year period after operation in both male and female patients, and after both Billroth and Poyla operations. The fall was steepest in women under 50 years of age. No such fall occurred during a similar period in 127 patients with peptic ulcers who had not undergone operation. There was no significant difference in the incidence of alimentary bleeding, as judged by tests for focal occult blood, between the postoperative patients and those not given operation. The postgastrectomy anemia was predominantly of the iron deficiency type, and the mean dietary iron intake of anemic male patients was significantly lower than that of nonanemic male patients, though probably not low enough to be the sole cause of the anemia. In women, the mean iron intake was below the level required for the maintenance of a normal hemoglobin level. Nearly all cases eventually responded satisfactorily to oral iron therapy. In the second paper, studies on the alimentary absorption of Fe$^{59}$ are presented. It is shown that the absorption of iron is not altered by partial gastrectomy when given as ferrous sulphate with the patient fasting; the absorption of organically bound Fe$^{59}$ in the form of rabbit hemoglobin given with a full meal, however, is significantly decreased 4 to 6 weeks following this operation. Patients anemic after partial gastrectomy failed to show an increased absorption of organic iron administered with food, as compared with nonanemic peptic ulcer patients before surgery. Anemic patients with intact stomachs absorbed significantly more iron than either of these two groups. It is suggested that this failure to increase absorption of organic iron from the diet plays an important part in the pathogenesis of the anemia which follows partial gastrectomy.—R. M. H.


The rarity of iron deficiency anemia in South African Bantu infants has been ascribed to the high iron content of their diets which is derived from the iron utensils used in cooking. In infancy and childhood, however, there appears to be a significant incidence due to dietary deficiency. Sixty such cases were found between the ages of 3 months and 3 1/2 years, and of these 82 per cent occurred between the ages of 6 and 18 months. In none was occult bleeding present at the time of study. The majority were being fed on maize porridge and water with occasional supplements of milk and other foods. None of the mothers used iron cooking pots. The iron content of some of the maize mixtures being used was determined, and the total daily intake of iron was found to be between 2.1 and 4.5 mg. Clinically all the subjects were underweight and 54 appeared to be malnourished. All the malnourished patients showed depigmentation of hair, 20 had an acute nutritional dermatosis, 24 were edematous and 17 were markedly wasted. Hemoglobin values varied between 3.6 and 9.9 Cm. H. in 24 were edematous and 17 were markedly wasted. Hemoglobin values varied between 3.6 and 9.9 Cm. H. per cent and the MCHC from 22 to 30 per cent. The therapeutic response to oral iron was found to be unpredictable. With intramuscular iron, however, a highly satisfactory response was obtained in all the infants in which it was used. From the results of this study intramuscular iron would appear to be the treatment of choice in nutritional iron deficiency anemia occurring in infancy.—T. H. B.

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Histochemical and quantitative chemical data show that the uptake of iron from iron-enriched diets is much greater in rats fed "poor" diets than in rats fed "good" diets. Dietary siderosis in rats is characterised by an accumulation of histochemically demonstrable iron in liver cells prior to its appearance in hepatic phagocytes or in other mesenchymal cells. The dietary siderosis in rats and perhaps in man is not due to iron overload alone, but may rather be attributable to some effects of "bad" diet on intracellular metabolism.—W. J. M.


Sections of 728 unselected liver specimens obtained at necropsy from West African Negroes in Ghana were examined. About 40 per cent showed varying degrees of iron excess. In 94 instances iron was present in Kupffer cells only, the greatest incidence being in younger subjects. In a further 146 cases the iron was found in periportal parenchymal cells alone or in combination with varying amounts in Kupffer cells. Forty-four livers showed large amounts of iron in portal tracts, parenchymal cells and Kupffer cells, and in a further 24 there was in addition evidence of cirrhosis. Other tissues were examined in 22 cases with heavy iron deposits in the liver. In 5 of these it was not possible to differentiate the findings from those present in idiopathic hemochromatosis.—T. H. B.

STUDIES ON THE GENESIS OF THE MACROCYTOSIS IN THE HYPOPLASTIC ANEMIA DUE TO CHRONIC BENZENE POISONING. P. Corini, R. Colombi and D. Pecorari. From the Università, Pavia, Italy. Lavoro Umano. 11:121, 1959.

The presence of a macroerythroblastosis in all stages of maturation was observed in the bone marrow of 5 patients with chronic hypoplastic anemia due to benzene poisoning, by means of cytometric investigations. In the proerythroblasts the macrocytosis was due to the pathologic enlargement of both nucleus and cytoplasm, but in the more mature stages it was due only to the enlargement of the cytoplasm. The phenomenon is interpreted as the consequence of the antimitotic action of benzene, which causes a prolongation of the interphase, without preventing completely the synthesis of proteins.—P. d. N.

HEMOLYSIS


In transfusion of small infants, administration of blood by the "push technic" is common. In this technic, the blood is delivered under pressure by means of a syringe and, usually, a needle of
small gage. The authors of this paper present a case report of an infant in whom transfusion of blood by the push technic was followed by hemolysis of sufficient degree to induce hemoglobinuria. Experimental studies demonstrated that the degree of hemolysis could be related to the increase in the rate of flow and to the decrease in the caliber of the needle. Increasing age of the blood used for transfusion further increased the degree of hemolysis employing this technic. The authors suggest that where the push technic is employed, the blood should be introduced under general pressure and through as large a bore needle as is possible.—I. S.


A woman with reticulum cell sarcoma repeatedly suffered severe hemolytic reactions after the transfusion of apparently compatible blood. Cr51-labeled donor cells were shown to be completely eliminated within 24 hours, although they were compatible with the patient's serum by all known in vitro tests for blood group antibodies.—R. M. H.

STORAGE STABILITY OF BLOOD WITH UNSTABLE GLUTATHIONE. A. Szeinberg, Ch. Sheba and A. Adam. From Government Hospital, Tel-Hashomer, Israel. Israel M.J. 17:285, 1958.

Storage stability of blood with unstable glutathione and deficient glucose-6-phosphate dehydrogenase activity has been compared with that of normal blood. During 30 days storage under the usual blood bank conditions, no hemolysis was observed and no change in glutathione level, its stability and in the activities of glucose-6-phosphate and 6-phospho-glucocatonic dehydrogenases or glutathione reductase occurred in any of the control or test samples.—B. R.

AN IN-VITRO ABNORMALITY OF GLUTATHIONE METABOLISM IN ERYTHROCYTES FROM NORMAL NEWBORNs: MECHANISM AND CLINICAL SIGNIFICANCE. William H. Zinkham. From the Department of Pediatrics, Johns Hopkins University School of Medicine, and Harriet Lane Home of Johns Hopkins Hospital. Pediatrics 23:1, Pt. 1, 1959, pp. 18-32.

The occurrence of acute hemolytic anemia in certain individuals after administration of Primaquine and other chemically related drugs has been found to be associated with a deficiency of reduced glutathione in the red cells. In vitro, the level of reduced glutathione is markedly reduced after incubation of the erythrocytes with acetylphenylhydrazine. The abnormalities of glutathione metabolism in the sensitive individuals have been attributed to genetically determined deficiency in the enzyme glucose-6-phosphate dehydrogenase. Because of the unusual susceptibility of normal newborns and prematures to the hemolytic effects of certain drugs, studies on glutathione metabolism and glucose-6-phosphate dehydrogenase activity in the erythrocytes of newborn infants were carried out. The studies were performed on the erythrocytes of 66 normal, full-term Negro and white infants with an age range of 1 to 84 hours. Glutathione concentrations in the red cells of the newborn tend to be higher than those observed in normal adults. However, the glutathione stability test disclosed a marked abnormality. After incubation of the erythrocyte with acetylphenylhydrazine, the levels of reduced glutathione in the majority were distinctly abnormal and were similar to those found in the Primaquine-sensitive adults. An abnormal glutathione stability test was found in all infants less than 12 hours of age; by 36 hours of age the results of the glutathione stability test approximated those observed with normal adults. In contrast to the findings in individuals with genetically determined glutathione deficiencies, the normal newborn infants do not have diminished glucose-6-phosphate dehydrogenase activity in the erythrocytes. In fact, the enzyme activity in the newborn is higher than in the adult. Thus the abnormality of glutathione metabolism in the erythrocytes of the newborn could not be associated with a deficiency of the enzyme. The abnormal glutathione stability tests in the newborn infants were not affected by the in vitro addition of alphatoxopherol or adult plasma. However, the abnormality in the glutathione stability test could be eliminated by the addition of glucose in concentrations from 30 to 160 mg./100 ml. of blood. When blood of the newborn infant was incubated with menadione sodium bisulfite, a steady fall in glucose concentration occurred. A precipitous fall in reduced glutathione occurred when the level of glucose had decreased to between 10 and 12 mg./100 ml. In the normal adult, a similar relationship between reduced glutathione and glucose was evident, although a longer incubation period was necessary. However, further studies indicated that the concentrations of glutathione in the erythrocyte of the newborn are regulated by factors other than the concentration of available glucose. If blood from the normal newborn was incubated anaerobically without addition of menadione sodium bisulfite, reduced glutathione remained constant even though concentrations of glucose less than 10 mg./100 ml developed. In the study of the 66 newborn infants, 3 Negro male babies with de-


Human carbonmonoxyhemoglobin at pH 11 dissociates into half molecules of molecular weight 36,000 ± 2,000, determined by the Archibald method of approached sedimentation equilibrium. This dissociation into subunits is completely reversible by neutralization, even after periods of 36 hours. Dissociation at pH 11.6 leads to irreversible changes in the molecule. Some of the hydrodynamic properties of the hemoglobin subunits at pH 11.0 are discussed.—A. I. C.


The effect of Pb on the activity of antigens A, B, M, N, P, H, D was followed by agglutination and drying tests. It was shown that the binding of lead does not influence the activity of receptors A, B, M, N, and P. Receptor D is inactivated by lead. The reaction is very sensitive, and inactivation occurs with the use of a very small amount of lead. A concentration of 10^-9 μg. of lead per erythrocyte suffices for complete suppression of the above effect. This differential reaction of erythrocytes to lead confirms the different chemical basis of D as opposed to A, B, M, N and P. According to the literature, receptor D is a lipoprotein hapten, and on the basis of this we may conclude that lead is bound with lipoprotein components located on the inner surface of the erythrocyte membrane.—L. D.


A 25 year old man presented hemolytic anemia in the course of Mesantoin treatment given for epilepsy. The patient clinically showed splenomegaly and jaundice. The hemoglobin fell to 3 Gm./100 ml. and the reticulocyte count reached 20 per cent. The direct Coombs tests was negative, but the indirect Coombs test was positive with the use of the patient's own red cells and serum, in accordance with previous reports. The hemolytic process disappeared after 20 days of steroid treatment.—M. L.


A hitherto undescribed hemoglobin variant which resembles hemoglobin-S and hemoglobin-D on chromatography at pH 6, but hemoglobin-L or hemoglobin-P on paper electrophoresis at alkaline pH, was found in a woman from the Belgian Congo, belonging to the Lugbara tribe, and in an American Negro. ("Stanleyville I") Another hemoglobin resembling hemoglobin-D was seen in two unrelated Bantu families; on amberlite-resin chromatography at pH 6, this moved behind hemoglobin-S and hemoglobin-D, but was faster than hemoglobin-L ("Stanleyville II").—R. M. H.


Both groups of workers describe the properties of a newly discovered slow-moving component of cord blood hemoglobin from infants in Athens and Singapore, respectively. Comparison of these properties suggests that the abnormal variant is the same in each case. Fessas et al. suggest the name "Alexandra" for their new fetal haemoglobin. —R. M. H.

PERSISTENCE OF HAEMOGLOBIN "BART'S" BEYOND INFANCY IN A CHILD WITH THALASSAEMIA. C.
The persistence of hemoglobin "Bart's" beyond thalassemia major included 7 per cent of hemoglobin F and 6 per cent of hemoglobin "Bart's." The persistence of hemoglobin "Bart's" beyond early infancy in conjunction with thalassemia is put forward as further evidence in favor of its being a variant of fetal hemoglobin.—R. M. H.


Cultures of a variety of bacteria on blood agar plates incorporating red cells containing different types of hemoglobin were followed to determine the ability of the individual hemoglobin types to support surface growth. Homozygous Hgb S erythrocytes seemed less capable than normal blood of fostering the growth of several organisms.—A. I. C.


A case of sickle cell/hemoglobin-J disease is reported in a man of fair complexion. The resulting hemolytic anemia was mild and well compensated. The family history suggested that the hemoglobin-S was contributed by the patient’s mother, who was of Negro stock, and that the hemoglobin-J was from the father, who was thought to be a Caucasian from North America.—R. M. H.

HEMOGLOBIN S-HEREDITARY SPHEROCYTOSIS. B. Jones and W. G. Klingberg. From the Washington University School of Medicine, St. Louis, Mo. J.Pediat. 54:375, 1959.

Two teen-aged Negro patients with the combination of sickle cell trait and hereditary spherocytosis are presented. Each had marked splenomegaly, a severe hemolytic anemia and spherocytosis. Splenectomy was performed with good results in both.—A. I. C.


A method for preparing human red cell "ghosts" is described. Their hemoglobin content, determined as cyanmethemoglobin after dissolving the "ghosts" in 40 per cent urea, amounted to 49 to 57 per cent of the dry weight. It is suggested that this hemoglobin is a component part of the cell membrane; the sickling phenomenon is adduced as support for this concept.—R. M. H.


Human, horse and dog hemoglobins were studied with the use of cation exchange cellulose columns. The effects of temperature, dilution and treatment with 4 M urea were also investigated. Results comparable to those reported by Huisman were found for Hgb A, although different gradients of elution were used in the two laboratories. An additional component, called V by Huisman, was found to be separable into a nonheme protein and a hemoglobin component. Horse hemoglobin exhibits similar heterogeneity, but dog hemoglobin is essentially homogeneous. Increasing the temperature of chromatography results in elution of the fractions at a lower pH and an increase in heterogeneity. Human and dog hemoglobins are relatively resistant to dissociation by 4 M urea, although the human pigment undergoes some unfolding. Horse hemoglobin readily dissociates under the same conditions.—A. I. C.


The authors describe an interesting set of experiments in which Hgb. A, S and C have been labeled, subjected to mild acid dissociation and recombined at neutral pH. Studies with both ferri-heme labeled protein and C14-labeled globin demonstrate that the hemoglobin molecules dissociate into unlike portions (asymmetric dissociation). Since hemoglobin is believed to be composed of two pairs of dissimilar polypeptides, dissociation under these conditions would result in approximate half molecules, one containing two polypeptide chains of one type, the other two chains of the second type. The relationship of these findings to hemoglobin structure, position of the four hemes and to genetic theory is discussed.—A. I. C.
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An additional family with the Lepore hemoglobin is described. The combination of this anomaly with that of classical thalassemia resulted in a moderately severe thalassemia-like disease with manifestations entirely similar to those found in the original family described by the same authors. The Lepore trait is essentially asymptomatic but results in a morphologic picture similar to the thalassemia trait. Lepore hemoglobin has not been demonstrated by paper electrophoresis; hence the use of starch block studies becomes more important in any investigations related to the field of the thalassemia syndromes.—A. I. C.


A young woman with Cooley's anemia experienced three observed episodes of subacute exacerbation of the anemia. Each of these episodes was associated with bone marrow failure. On each occasion, there was a striking hematologic response to the administration of large doses of folic acid, but no response to B12 or pyridoxine. Although the response to folic acid was well established, none of the usual criteria for PFA deficiency was found. The authors postulate the existence of a relative deficiency state of folic acid brought about by a supranormal need for the metabolite. The response to folic acid was accompanied by severe bone pain, presumably due to expanding pressure of rapidly proliferating erythroblasts within a marrow cavity already fully occupied by hypertrophied erythroid tissues.—A. I. C.


The cytoplasm of foam cells in a thalassemic spleen as originally described by Whipple and Bradford (Am.J.Dis.Child. 44:336, 1936) was found to contain a large amount of acid mucopolysaccharide of the chondroitin sulfuric acid type.—J. B. C.


In all anuclear red blood cells, glycolysis is responsible for the maintenance of unequal ion distribution and of the apparent impermeability to cations. If glycolysis is blocked by inhibitors (NaF, NaF + Na2SO4, iodoacetic acid + adenosine) the rate of K outflow from the cells and that of Na+ influx is very high: 70 to 170 mg. K+ per 100 ml. erythrocytes per hour. This rapid outflow of K+ can be prevented with ethylenediamine tetra acetate (EDTA), in the presence of which the rate of K+ outflow is reduced to 6 mg./100 ml. erythrocytes per hour. This effect of EDTA is based on a binding of the ionized Ca++ of the serum. Other Ca++-binding agents, such as Calgon and oxalate, have a similar effect. CaCl2 increases the rate of K+ outflow. Citrate has no inhibitory effect, because even at high citrate concentrations there may be present sufficient amounts of Ca ion to start K+ outflow. The authors conclude that in response to glycolytic inhibitors the K+ outflow from the erythrocytes will be increased only when the system contains Ca ions. This indicates the significant role of Ca++ in the maintenance of the physiologic ion balance of the blood.—S. R. H.

HAPTOGLOBINS AND HEMOGLOBINS IN AUSTRALIAN ABORIGINES, WITH A SIMPLE METHOD FOR THE ESTIMATION OF HAPTOGLOBINS. O. E. Budtz-Olsen. From the University of Queensland, Australia. J.689, 1958.

This paper reports the distribution of hemoglobin types and haptoglobin types in two groups of Australian aborigines, one in Central Australia and the other in Northern Queensland. It also describes a simple method for the estimation of the haptoglobin groups. No abnormal hemoglobins were detected in either group. However, the haptoglobin distributions in these two groups were entirely different. They were also different from the distribution in white peoples of European origin and in two groups reported in West African Negroes. Some of the ethnologic implications of these findings are discussed.—G. C. de C.


The distribution of haptoglobin types in 406 random American Negro donors was studied. A lower incidence of "ahaptoglobinemia" and a higher incidence of the Hp2 gene was found than those reported for African natives. Possible reasons for these differences are the freedom of the American group from malaria, the origin of the members of this group from many different parts of Africa and intermarriage of American Negroes with European stock.—R. M. H.
HEMOSTASIS


Ether-extracted normal and hemophilic (A, B) plasma showed a marked drop in factor V, prothrombin, and fibrinogen. The changes were of a “denaturation” type. Factor VII and immediate antithrombin were not changed by extraction. Normal and hemophilic (A, B) extracted plasma have a lowered thromboplastic activity involving factor V and antihemophilic globulin. The thromboplastic activity of normal and hemophilic A serum was unchanged by extraction, nor was there any change in the defect in hemophilic A plasma. There was also no effect on the defect in hemophilic B blood. Comparison of plasma and serum before and after ether extraction suggests the absence of any immediate antithromboplastin (antithromboplastinogen) in either normal or hemophilic (A, B) plasma and serum. The method of ether extraction is not suitable for studying the presence of antithromboplastinogen by ordinary coagulation tests.—L. D.


The activity of various coagulation factors was studied after defibrination of oxalated plasma with thrombin. It was determined that after addition of thrombin to plasma there is a considerable loss of AHG and factor V activity. No changes in prothrombin content and in the activity of factor VII were observed. From results with the thromboplastin generation test using defibrinated plasma before and after recalcification, it was concluded that PTC activity in defibrinated plasma is lower than in normal serum. The possibility of interference by thrombin and the importance of these changes were concisely discussed.—L. D.


Antithrombin VI is a product of limited proteolysis of fibrinogen. It slightly lengthens the clotting time of whole blood and recalcified plasma, and it considerably prolongs the thrombin clotting time of plasma. Purified antithrombin preparations do not inhibit thromboplastin generation or influence the conversion of prothrombin into thrombin in diluted plasma, in the presence of calcium ions and tissue thromboplastin. It could be shown that antithrombin inhibits competitively the action of thrombin on fibrinogen. During incubation of antithrombin VI with thrombin the antithrombin activity decreases progressively. Thrombin clotting time in a system containing antithrombin VI is shortened after addition of protamine sulfate, however, the effect of protamine sulfate against heparin is far more pronounced. It is difficult to determine whether the protamine sulfate effect is specific. To date no electrophoretically pure preparations have been obtained.—E. K.


Immunochemical precipitation in gel demonstrates three antigenic groupings in the human fibrinogen molecule. By this method it is possible to distinguish fibrinogen, the products of fibrinolysis and those of fibrinogenolysis.—G. M.


The split products of fibrinogen and fibrin after action of plasmin can be identified by immunochromical technics (ring-test, double diffusion in agar and immunoelectrophoresis) with anti-fibrinogen serum.—G. M.


The authors described in their previous papers the appearance of deoxyribonucleic acids in the cytoplasm of megakaryocytes and blood platelets in numerous cases of idiopathic thrombocytopenia and in pancytopenia of undetermined etiology. This phenomenon is not observed in healthy persons or in symptomatic thrombocytopenias. The aim of the author was to determine whether the positive Feulgen reaction changes after splenectomy. In five cases of “spontaneous” thrombocytopenia and in five patients with pancytopenia showing a positive Feulgen reaction in the blood platelets, a gradual regression of nuclear substance present in the blood platelets was observed following splenectomy.—E. K.
ABSTRACTS


The platelets of 21 normal subjects contained 6.2 to 9.8 x 10⁻⁹ mgm. taurine per platelet. The taurine content of platelets increased by 20 to 40 per cent on incubation for 15 hours with cystine, and somewhat less on incubation with cysteic acid.—R. M. H.


The authors describe an absorption technic which permits demonstration of Kell antigen in blood platelets. Red blood cells in platelet suspensions may interfere with the test. This can be obviated by treating platelet suspensions with 0.5 N acetic acid, hemolyzing red blood group antigens, which then can be eliminated by washing. This series of experiments, which continues the previous ones devoted to other Rhesus antigens, such as D, C, c, Cw and E, leads the authors to conclude that all blood group antigens are present in platelets.—G. M.


Rabbits were bled 40 ml./Kg. from the central artery of the ear under local anesthesia over a 15 to 30 minute period. During the first hour after initiation of bleeding, platelet count doubled; fibrinogen, labile factor and complement decreased about 50 per cent and prothrombin time activity decreased 70 per cent. There was slight shortening of clotting time in thromboplastin generation test. Streptokinase-activateable fibrinolysin decreased and serum antifibrinolytic activity increased; no spontaneous fibrinolytic activity was observed. During subsequent hours, platelet count decreased to 50 per cent of control value, TGT became clearly prolonged, the defect being found in the plasma; fibrinogen remained low, labile factor and prothrombin time were normal, SK-activateable fibrinolysin increased, antifibrinolysin decreased below control values and spontaneous lysis was not noted. Values returned to control levels at 48 to 72 hours. Three hours after hemorrhage, lungs showed hemorrhagic areas, often triangular. Histologic examination demonstrated intra-alveolar hemorrhage; and pulmonary, hepatic and renal veins contained fibrin and amorphous (platelet?) material. In animals splenectomized 3 weeks before hemorrhage, histologic abnormalities and changes in clotting factors were much less pronounced. Many of the changes are attributed to intravascular clotting, perhaps induced by splenic contraction.—M. B. Z.


In 30 patients, clotting factors were studied before and immediately, two and 24 hours after open heart surgery with heparin and subsequent protamine administration. The following measurements showed a statistically significant postoperative decrease: platelet count, thromboplastin generation, prothrombin time activity, and fibrinogen concentration. Fibrinogen returned to normal at 24 hours. No difference was noted between eight of these patients who bled abnormally and 22 who did not. Complete lysis of endogenous fibrin in recalcified plasma occurred in less than four hours in two bleeders and two nonbleeders. Multiple defects were also found in eight patients undergoing closed heart or noncardiac sur-
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A common denominator for bleeding was not evident, but in some patients one defect was sufficiently severe to account for bleeding. At the time that excessive bleeding began, bleeders had received about the same amount of blood as the nonbleeders—between 4000 and 7750 ml. Because of pathologic hemorrhage, they subsequently required more blood.—M. B. Z.


Platelet counts, platelet stickiness, plasma fibrinogen, thromboplastin generation and prothrombin times were compared in 22 healthy men, 30 men with angina pectoris on exertion, 7 patients with acute coronary insufficiency and 10 with cardiac infarction. Values for platelet stickiness, thromboplastin generation and fibrinogen showed a significant increase in patients with angina pectoris (compared with healthy controls) and a further increase in patients with cardiac infarction. The corresponding findings in patients with acute coronary insufficiency were not significantly different from those in patients with angina pectoris on exertion, but the mean platelet count of patients with acute coronary insufficiency was significantly higher (at the 5 per cent level) than that of normal subjects and patients with angina pectoris. The differences between the means of normals and patients in the platelet stickiness, fibrinogen and thromboplastin generation tests remained unaltered on repeated testing of 29 patients with angina pectoris and 15 normal subjects, and the variation within normals and patients did not differ significantly. This carefully controlled investigation leads to the firm conclusion that "The blood of patients with ischaemic heart disease, studied in vitro, shows a phasic hypercoagulability which is entirely in keeping with a variable hyperthrombotic state."—R. M. H.


The ingestion of three whole eggs or their total lipids produced a significant shortening of the plasma stypven time after 3½ to 4½ hours in nine middle-aged men. Egg lipids from which either the total phospholipid or the phosphatidyl ethanolamine had been removed were equally effective; these results thus do not support O'Brien's suggesting that the shortening of the stypven time after eating eggs is due to their phospholipid content. The whole blood clotting time in siliconed tubes was not significantly shortened by the ingestion of any of the egg lipid preparations in the amounts used.—R. M. H.

EXPERIENCES WITH THROMBELASTOGRAPHY. L. Donner and O. Setkova. From the University Hospital, Prague, Czechoslovakia. Vnitřné lék. 5: 817, 1959.

Two-hundred and ninety-eight various patients were investigated in whom 515 trombelastograms were performed. The normal thrombelastogram values for whole blood and for citrated plasma are given. The value for whole blood is r: 9 to 15 min.; k: 5 to 8 min.; ma: 45 to 55 mm. In 10 cases of hemophilia A and in 3 cases of hemophilia B the r values ranged from 18 to 120 min., the k value from 10 to 70 min., and ma from 45 to 55 mm. Following transfusion of fresh frozen plasma, the shortening of r values and k was conspicuous during the first 24 hours, without any improvement of prothrombin consumption. In the majority of hypoprotrombinemias the values of r and k were prolonged. In 6 cases of essential thrombocytopenias and in 15 cases of secondary thrombocytopenias the k value was prolonged from 10 to 36 min., and the ma value decreased from 19 to 40 mm. Platelets from cases of thrombocytic purpuras concentrated to normal value and added to the plasma of patients with essential thrombocytopenia corrected the pathologic pattern of TEG in some of them only. In 8 thrombopathies the TEG, when performed in a normal way, was not conclusive. During treatment with Tromexan the TEG changes such that the values of r and k are prolonged and later on ma decreases. The fall of prothrombin level as measured by Quick's method and the changes of TEG were found to show poor correlation.—L. D.