ABSTRACTS

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ABSTRACTS OF SPECIAL INTEREST


Addition of phytohemagglutinin and of streptolysin S (both nonspecific stimulators of mitosis) to in vitro cultures of leukocytes of normal and of agammaglobulinemic subjects results in mitosis of lymphocytes and their differentiation to plasma cells. In contrast, when specific antigens to which the cell donor had been recently exposed were added to the leukocyte cultures, mitosis and differentiation of lymphocytes of normal, but not of agammaglobulinemic, donors occurred.

White blood cells from 5 patients with idiopathic agammaglobulinemia were studied, including two with "typical" sex-linked agammaglobulinemia and 3 with the "acquired" form of the disease. The data indicate that the absence of plasma cells in agammaglobulinemia is not in itself responsible for failure of antibody production, but is rather the morphologic concomitant of the primary defect (failure of antibody production on exposure to antigenic stimulation).—T. E. B.


Fresh biopsy material from a child with Burkitt's lymphoma was inoculated intraperitoneally into 4 suckling African Green monkeys in May, 1962. One monkey died shortly afterwards. Of the 3 which survived into 1964, 2 were found to have lesions of limb long bones indistinguishable from those of Burkitt's lymphoma. The existence of an infectious agent in Burkitt's lymphoma is supported by these observations.—P. B.

LEUKOCYTES


Two patients with rheumatoid arthritis developed agranulocytosis within the first 5 weeks of administration of D-penicillamine. One of the patients died. The agranulocytosis was attributed to a cytotoxic effect rather than to an immunologic mechanism.—T. E. B.

IRRADIATION OF THE BLOOD: METHOD FOR REDUCING LYMPHOCYTES IN BLOOD AND SPLEEN. B. A.
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Insertion of a beta-emitting source into the right atrium of the heart of dogs permits intensive irradiation of the circulating blood with subsequent depletion of lymphocytes in the peripheral blood, lymph nodes, and spleen.—T. E. B.


Evidence is provided for the existence of a cell-free factor ‘RLP’ (‘radiation-leukemia-protection’ factor) in sheep spleen capable of inhibiting radiation leukemia induction in C 57 BL/6 mice when injected repeatedly after the irradiation.—P. E. B.


Adult rats treated with reserpine showed involution of the thymus and impairment of both delayed hypersensitive responses and antibody formation.—P. B.


The probable transmission of lymphosarcoma in cats is reported. Electron microscopy of the tumor showed virus-like particles. The etiologic relationship between the particles and the tumor remains to be established.—P. B.


When added with phytohemagglutinin to cultures of kidney and HeLa cells, human and rat lymphocytes produced toxic changes in the cultured cells. No evidence was obtained for release of a cytotoxic agent from the lymphocytes. It was concluded that the cytotoxic activity of the lymphocytes was induced by phytohemagglutinin.—P. B.


Antisera to rabbit polymorphonuclear leucocyte granules and to rabbit erythrocytes were prepared in guinea pigs. Both antisera were hemolytic, and both showed striking cytotoxicity on leukocytes, producing not only severe morphologic changes in the leukocytes but also release of granule-bound hydrolases (lysozyme, aryl sulfatase, cathepsin) into the medium. Granulolytic activity of the antisera could be greatly reduced by absorption with either rabbit leucocyte granules or rabbit erythrocytes. It was suggested that leucocyte granules and erythrocytes have an identical or similar membrane constituent.—T. E. B.

**ERYTHROCYTES**


Intravenous injection of ethyl palmitate into mice resulted in necrosis of the spleen and depression of liver phagocytic activity. This procedure prolonged the survival of human red cells transfused into mice.—P. B.


Red cell and plasma volume were determined before and after splenectomy in 5 patients with anaemia and simple splenic hyperplasia. The anaemia appeared to be due to expansion of plasma volume.—P. B.

**Morphological Demonstration of Two Red Cell Populations in Human Females Het-

A combination of sodium bicarbonate (to combat acidosis) and magnesium sulphate (to delay blood-clotting) has been found useful in the symptomatic treatment of patients with sickle-cell anemia.—P. B.

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Direct evidence for two red cell populations was obtained by an appropriate histochemical technique.—P. B.


Erythrocyte G-6-PD deficiency has been found to have an incidence of 5.5 per cent in adult Chinese males from the southern Chinese province of Kwantung.—P. B.


Hemoglobin ΓGCRGA is abnormal in the β chain where an asparaginyl residue substitutes for an aspartyl residue in position 79. It may be written as α2β2β79Asn. The homozygous individual in whom it was found is clinically well with a normal blood picture. In the heterozygotes, HbA and HbG occur in equal proportions.—P. B.


Propane, ethane and methane were found to ‘unsickle’ sickled erythrocytes containing HbS. These compounds also prevented and/or reversed the gel formation of deoxygenated HbS solutions and reduced the degree of optical rotation. These observations are thought to be consistent with the author’s suggested sub-molecular mechanism for sickled cell formation. Each of the β-chains of the HbS molecule appears to form a binding site by an intramolecular hydrophobic bond between the two valyl residues; the first valyl of the amino terminus and the genetically introduced sixth valyl, allowing cyclization from the carbonyl of the first to the amino of the fourth by hydrogen bonding. —P. B.


A combination of sodium bicarbonate (to combat acidosis) and magnesium sulphate (to delay blood-clotting) has been found useful in the symptomatic treatment of patients with sickle-cell anemia.—P. B.


The hemoglobin of a patient with the clinical and hematological features of sickle cell anemia was found on electrophoresis to consist of three components: Hb F, Hb S and a slower band in the position of Hb E. The slow band represents a new hemoglobin and was shown to consist of the α chains of Hb Stanleyville-II (Sta-II) and the β chain of Hb S and was designated Sta-II/S. Family studies showed the independent segregation of Hbs A, S and Sta-II. Hb Sta-II was identified as an α chain mutant with an abnormality in one of the “core” peptides (amino-acid residues 93–139).—P. B.


Iron-59 incorporation into hemoglobins A and S was studied in a patient with sickle cell trait and iron deficiency. The evidence supports the hypothesis that hemoglobin S is synthesized at a rate at least equal to that of hemoglobin A.—P. B.


The Figlu test was performed in 34 asymptomatic nonwhite infants. Urinary Figlu was estimated after the giving of either a 10 Gm. or 2.5 Gm. load of histidine. When negative results were obtained, the test was repeated with larger doses. All infants who at any stage showed a positive Figlu test received 0.4 mg. folic acid daily for 14–
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28 days and the test was then repeated. The Figlu test was positive in 64.7 per cent of cases, the incidence being highest in the 3–6 month age-group. All except one of the 19 infants given folic acid therapy showed a negative result on retesting.—T. H. B.


Investigations were carried out on 51 nonwhite infants suffering from gastro-enteritis. In the whole series there was a 55.5 per cent incidence of megaloblastoid change and a 50 per cent incidence of positive Figlu tests. The marrow and Figlu findings showed agreement in 70 per cent of cases. In 7 cases the Figlu test was positive while the bone marrow was negative and in 5 instances the findings were reversed. Serum vitamin B₁₂ levels were normal in all cases. Although several reasons for the negative Figlu tests in subjects with megaloblastoid change were considered, the exact basis for these discrepancies was not established.—T. H. B.


Given intramuscularly to patients with pernicious anemia, 1000 µg hydroxocobalamin gave initially higher serum vitamin B₁₂ levels which remained higher for longer periods than did 3000 µg cyanocobalamin. Hydroxocobalamin (1000 µg monthly) appears satisfactory for maintenance therapy.—P. B.


An investigation of 55 cases of the Paterson-Kelly syndrome showed a high incidence of iron deficiency, achlorhydria, and subnormal vit. B₁₂ absorption. It is suggested that this syndrome is associated with the genetically determined gastric atrophy found in patients with pernicious anemia and that iron deficiency is a secondary feature.—P. B.


Iron stores in 1,332 consecutive unselected patients were examined, using the authors’ own modified Vim-Silverman needle for marrow biopsy. Hemosiderin was absent in approximately 30 per cent of patients. In many of these, iron deficiency had been unsuspected. The article lacks details for the marrow preparation and gives no corroborative data for the diagnosis of iron deficiency. This reviewer has found that histologic sections, which have an average thickness of only 5–6 micra, may fail to show hemosiderin, even if a neutral fixative is used. The same aspirates, prepared as described by Ruth and Finch (J. Lab. Clin. Med. 33:81, 1948) with an average thickness of 50–100 micra, show moderate amounts of iron.—R. O. W.


Iron-deficiency anemia in a group of women with gynecologic disorders was satisfactorily treated by the intravenous administration, as a single dose, of 1000–1750 mg. iron-dextran complex in 540 ml. 5 per cent dextrose (infusion in saline rather than dextrose appears to minimize the local thrombophlebitic reaction).—P. B.


Two of 6 rabbits injected once weekly for 28 weeks with 2 ml. iron-dextran into the right thigh muscle developed local sarcomata 39 months and 48 months after the first injection. One animal had lung metastases. Liver changes (accumulation of iron, necrosis, nodular regeneration, and cirrhosis) were seen in all rabbits. In view of these findings, the authors urge caution in the clinical use of iron-dextran.—P. B.

A simple clinically applicable spectrophotometric method, using Na₂EDTA as a decolorizing agent, is described for the estimation of ferrioxamine and/or desferrioxamine in urine.—P. B.


Seventeen patients with chronic bronchitis and severe dyspnea had normal hemoglobin concentrations, red-cell counts and hematocrit values. Five had low serum-iron levels but normal unsaturated-iron-binding capacity. After treatment with intramuscular iron-sorbitol, 6 of 11 patients showed an increase in hemoglobin mass. It was suggested that availability of iron for hemoglobin synthesis may be a factor which limits the physiological polycythemic response to anoxia in this disorder.—P. B.

**Demonstration of Fœtal Erythrocytes by Immunofluorescent Staining.** J. Tomoda. From the University of Nagoya School of Medicine, Nagoya, Japan. Nature 202:910–911, 1964.

An antiserum against fetal hemoglobin was labeled with fluorescein isothiocyanate and used to detect cells containing fetal hemoglobin by ultra-violet microscopy.—P. B.


As a method of forecasting severity of hemolytic disease of the newborn, liquor amnii specimens from immunized Rh negative mothers at 34–36 weeks gestation were examined for the presence of bilirubin, protein and hemoglobin. Elevated bilirubin values provided the best prognostic information. However, stillbirth occurred in 3 per cent and very severe disease in 9 per cent of patients in whom the liquor was clear. Perfect selection of cases for premature induction of labor cannot be made by examination of liquor amnii, but the procedure is felt to be of great value in management when interpreted in conjunction with the previous history.—P. B.


A diazo test was used to determine the presence of indirect bilirubin in amniotic fluid. It was concluded that liquor examination is of definite value in the prediction of the severity of hemolytic disease of the newborn and in determining the subsequent management of patients.—P. B.


Menadione sodium bisulphite produced some reduction in methaemoglobinemia. Methylene blue was most effective. Ascorbic acid had a minimal effect. The compounds were given intravenously.—P. B.


Mercuric chloride is capable of reacting with both the sulphhydryl groups and imidazole residues of hemoglobin.—P. B.


'holes' can be found in red cell membranes by electron microscopy after complement-dependent cell lysis. According to the 'one-hit' theory, production of a single lesion in the erythrocyte membrane will lead to lysis. The authors' findings provide strong support for the 'one-hit' theory of cell lysis and indicate that the sites of damage correspond with the holes seen with the electron microscope.—P. B.


Isoantibodies are capable of inducing resistance of red cells to immune hemolysis both in vitro and in vivo when they act together with some factors.
present in normal mouse serum. It seems probable that this phenomenon is wholly or partially responsible for the survival of incompatible erythrocytes in antibody-treated recipients.—P. B.


Lead increased the osmotic resistance of the red cells but did not increase their mechanical fragility. The latter factor is thought, therefore, not to be a major factor in the production of lead anemia.—P. B.

**ANOMALOUS SWELLING OF ERYTHROCYTES EXPOSED TO RESORCINOL.** H. Latta. From Western Reserve University, Cleveland, Ohio. Laboratory Invest. 13:902–915, 1964.

The author observed two distinct phases. The slower began after about 30 minutes of exposure, was due to membrane permeability to inorganic ions, resulted in hemolysis and was prevented by sucrose. The more rapid phase occurred in 20–30 minutes and was not prevented by sucrose. It was thought to be due to an alteration in the molar osmotic coefficient of hemoglobin by the resorcinol.

There are several differences between this process and immune hemolysis, the latter showing increased rigidity of the cell membrane and shrinkage of the cell, rather than swelling. For all of these experiments, rabbit erythrocytes suspended in cold buffered isotonic saline were used. In view of the changes this medium produces, it would be of interest to see whether suspension throughout in protein medium would modify the resorcinol effect.—C. R. M.

**HEMOSTASIS**


The clotting mechanism is inferred to be a cascade of proenzyme-enzyme transformations leading to an explosive generation of thrombin and fibrin formation. It is suggested that the physiological need to link the minute physical stimulus of surface contact with the final enzymatic explosion has resulted in the evolution of a biochemical amplifier in which enzymes are analogous to photomultiplier or transistor stages. There may also be positive and negative feedback mechanisms.—P. B.


After bypass operations for correction of cardiac defects, 21 of 327 patients required reoperation for severe bleeding. Of these 21 patients, 12 had normal preoperative coagulation findings and 3 had slightly reduced platelet counts. Six patients had an abnormal coagulation test preoperatively and in 5 of these postoperative abnormalities of clotting were present. There were 4 deaths attributable to bleeding. A definite surgical source of bleeding was found in 13 patients, 1 of whom had an abnormal preoperative coagulation test. When bleeding is excessive, the chest should be reopened, even when a coagulation defect is known to be present. In patients receiving 20 ml. per Kg. low molecular weight dextran the frequency of severe postoperative bleeding was lower and fewer coagulation abnormalities were detected 30 minutes after bypass.—P. B.

**THE EFFECT OF A SULFATED POLYGLUCOSE ON COAGULATION IN THE RABBIT.** R. D. Williams, D. V. Clatanoff and R. D. Coye. From the University of Wisconsin Medical School, Madison, Wis. Laboratory Invest. 13:865–870, 1964.

Sulfopolyglucin is a synthetic polysaccharide with 12–18 glucose units and a molecular weight of about 5,000. It has both anticoagulant (heparin-like) and coagulant activity. Both in vitro and in vivo, the coagulant effect seems to be due to the formation of an insoluble precipitate with fibrinogen. In vitro, heparin delayed precipitate formation, but no such effect was evident in vivo. This accounts for the combination of "bland thrombi" and a markedly reduced coagulability of the blood seen with administration of this compound. It is suggested that naturally-occurring similar compounds may have a similar effect in vivo, and this seems very likely.—C. R. M.

**THE INDUCTION OF PERMEABILITY-INCREASING ACTIVITY IN HUMAN PLASMA BY ACTIVATED HAGEMAN FACTOR.** O. D. Ratnoff and A. A. Miles. From the Lister Institute for Preventive
Infusions into rabbits of adenosine and 2-chloroadenosine, substances which inhibit platelet aggregation in vitro, suppressed the formation of platelet thrombi and emboli in injured cerebral vessels without interfering with the formation of hemostatic plugs. 2-chloroadenosine produced respiratory arrest in two animals.—P. B.

ROLE OF ADENOSINE DIPHOSPHATE IN THE AGGREGATION OF HUMAN BLOOD PLATELETS BY THROMBIN AND BY FATTY ACIDS. R. J. Haslan.


By utilizing pyruvate kinase as an enzyme capable of removing low concentrations of ADP, evidence has been obtained that ADP is of general importance in the aggregation of human platelets by human thrombin and by the sodium salts of fatty acids (palmitate, stearate, arachidate, behenate and lignocerate) in the presence of calcium ions.—P. B.

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Effect of Ethyl Chlorophenoxyisobutyrate with or without Androsterone on Platelet Stickiness. C. Symons, A. de Toszeghi and I. J. Y. Cook.


A trial of ethyl chlorophenoxyisobutyrate with and without androsterone ('Atromid' and 'Atromid S') and placebo (corn oil) was carried out on patients with ischemic heart-disease who were also receiving anticoagulant drugs. Both Atromid and Atromid S reduced both platelet stickiness and serum cholesterol concentration. Platelet stickiness and prothrombin times did not correlate.—P. B.


The authors state that comparative analysis of the fibrinopeptides can be used to examine molecular evolution and the true relationships of existing vertebrates. Proposed amino-acid sequences of fibrinopeptides A and B from seven mammals (ox, sheep, goat, reindeer, pig, human, rabbit) are given and their implications discussed.—P. B.
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In the absence of inhibitors, calcium enhances fibrinolysis. The inhibitory effect of calcium on fibrinolysis in whole blood can be explained by its effect on the inhibitors. Anti-activator is dependent on calcium for its formation. Calcium enhances antiplasmin activity.—P. B.


Methods are described by which thrombolytic therapy with local and systemic infusions of streptokinase can be initiated and controlled.—P. B.


Homologous plasma clot implanted into the subcutaneous tissue of rats stimulates development of an initial inflammatory reaction followed by granulation tissue formation, fibrinoplasia and formation of dense connective tissue. Vascular connective tissue is rich in fibrinolytic activity.—P. B.

MISCELLANEOUS


The authors describe a method of handling aspirated marrow to provide tissue sections. The method is described in detail. While it appears reasonable, it is impossible to say how it would compare with other techinics without using them in parallel.—C. R. M.


Simultaneous injection of Cr51-labeled red cells and I131-labeled plasma is used to determine total red cell mass and total plasma volume independently in animals. Simple addition of total red cell mass and plasma volume gives the total blood volume and eliminates the use of the venous hematocrit. The method allows for the determination of the total body hematocrit.—P. B.


A system is described which enables the hemoglobin estimation and leukocyte count to be made from one diluted sample.—P. B.


The author thinks that malaria may be a major factor affecting the world distribution of the Rh factor, analogous to its effect on the sickle cell, hemoglobin C, thalassemia and G-6-PD genes. The lower frequency of Rh-negative genes in malarious and tropical areas appears to support the theory.—P. B.


The authors provide a useful and valuable summary of their most recent findings on the blood groups of chimpanzees, orangutans, gibbons, gorillas, baboons, pigtail monkeys, rhesus monkeys, Celebes black apes, Java macaques and squirrel monkeys. The data include ABO groups, Lewis types, Rh-Hr types, M-like and Nf factors and P factors.—P. B.

Wasting due to neonatal thymectomy can be reversed by administration of syngeneic adult spleen cells and less effectively by syngeneic adult thymocytes. Attempts to reverse wasting by thymus grafting were unsuccessful.—P. B.


Twenty patients with myelomatosis were treated with oral melphalan (L-phenylalanine nitrogen mustard). Pain was relieved in 11 of 15 cases. A fall in the neutrophil count occurred in all cases and in the platelet count in 7 cases. Melphalan was more toxic in the presence of uremia. Melphalan appears to be of some value in the treatment of myelomatosis.—P. B.
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