ABSTRACTS OF SPECIAL INTEREST


A new method for the determination of chain anomaly in abnormal hemoglobins has been devised by combining hybridization and agar gel electrophoresis. A solution of canine hemoglobin (Hb Can) mixed with human abnormal hemoglobin (Hb X) and Hb Can mixed with human normal hemoglobin (Hb A) are subjected to dissociation and recombination. The mixtures are then subjected to agar electrophoresis. Their electrophoretograms are compared. Disagreement in mobility of the hybrid spots on the cathode side indicates chain anomaly in the abnormal hemoglobin (α₂β₂X); that seen on the anode side is indicative of chain anomaly (α₂Xβ₂A).—K. F.


Four moles of carbon monoxide are formed from each mole of catabolized hemoglobin. Normal newborn infants had a mean carboxyhemoglobin concentration of 0.42 per cent, with a range of 0.1 to 1.8 per cent. Infants with Rh-erythroblastosis had elevations ranging from 2.6 to 11.9 per cent, with the highest COHb levels being present in the infants with the lowest total hemoglobin concentrations. Infants with ABO erythroblastosis had moderate elevations ranging from 1.9 to 6.6 per cent, and infants with hyperbilirubinemia which could not be ascribed to hemolytic disease had values within the normal range. The authors suggest that measurements of carboxyhemoglobin may serve as a useful index to the presence of hemolysis.—J. B. S.


Numerous chromosomal abnormalities of both structure and number were observed in cultures of peripheral blood leukocytes from a patient given 167.5 mc. of I³¹. A striking increase in chromosomal abnormalities appeared within 20 minutes of I³¹ administration and was maximal during the period 3–12 hours after I³¹ adminis-
Description of two cases in the same family, with a total defect of G-6-PD in the brother and a partial defect in the sister. Erythrocyte survival was considerably reduced in the brother, moderately so in the sister and slightly in the mother. The anemia was classified as type 1 according to Selwyn and Dacie, on the basis of autohemolysis studies.—P. d. N.


Incubation in glucose of erythrocytes from patients with deficient G-6-PD influences the osmotic fragility as in normal and microcytic erythrocytes, in contrast to the behavior without the addition of glucose. A significant decrease in osmotic fragility was found in the G-6-PD-defective subjects, but to a lesser degree than in thalassemic subjects. During favic crisis and at least for a week thereafter, the osmotic fragility increases to normal values, and is decreased again after 3 months.—P. d. N.

DYNAMICS OF HEMOLYSIS AND HEMATOLOGICAL FINDINGS IN ELLIPTOCYTOSIS. C. Conti, G. Torloniano and M. Muzzolini. From the University, Roma, Italy. Policlinico sez. prat. 69: 1477-1497, 1962.

In 33 subjects with elliptocytic anemia or constitutional elliptocytosis from 13 Italian families, normal values of the MCV, MCH, MCHC and Hb A2 were found. With the exception of two cases, in which heterozygous sickling trait was present, no pathologic hemoglobins were detected. A slight increase of Hb F in a few anemic subjects disappeared after splenectomy. Hyperhemolysis was demonstrable in most cases. Erythrocytes exhibited a normal life in splenectomized subjects, but were sequestered in the spleens of elliptocytic or normal subjects. Extracorporeal factors damaging erythrocytes were not demonstrable. Splenectomy did not significantly modify the elliptic deformation of erythrocytes.—P. d. N.


Four patients with hereditary non-spherocytic hemolytic anemia were investigated. Two cases were classified as type I on the basis of autohemolysis tests. The other two cases were classified as type II. The patients with type I disease were found to be glucose-6-phosphate dehydrogenase deficient. The authors postulate that in G-6-PD deficiency an accumulation of GSSG inhibits the hexokinase reaction, that this results in inhibition of glycolysis, drop in red cell AMP, ADP and ATP were found to be in the normal range.—E. B.

CRYSTALLINE PHOSPHOGLYCERATE KINASE FROM HUMAN ERYTHROCYTES. T. Hashimoto and H. Yoshikawa. From the University of Tokyo, Japan. Biochim. et biophys. acta 65:355-357, 1962.

There are not many erythrocyte enzymes which have been prepared in the crystalline state. This short communication, describing the crystallization of erythrocyte phosphoglycerate kinase from human red cells, is therefore of considerable interest. The procedure includes removal of hemoglobin by ethanol-chloroform denaturation, ethanol precipitation of the enzyme and purification on calcium phosphate gel and DEAE cellulose.—E. B.

ABSTRACTS

F. Palamara. From the University, Pavia, Italy. Folia med. 44:137-154, 1961.

The effect of aniline on serum and erythrocyte enzymes and on mechanical erythrocyte resistance was studied in rats after single doses of 1 cc., in rabbits after single doses of 0.5 cc., and in the erythrocytes of 12 normal human subjects following the addition in vitro of the reagent at various concentrations. In rats, significant increases of serum lactic and malic dehydrogenase levels occurred 24 hours after injection; GPT and aldolase were less affected. After 48 hours these findings were less evident, and complete normalization occurred after 144 hours. Reduced values were observed in rabbits 48 hours after injection. In erythrocytes, slight diminution of transaminases, dehydrogenases and aldolase, and, most strikingly, of G-6-PD were observed in rats after 48 hours. After 9 days there was an increase of activity above the initial values; after 11 days normalization occurred. A reduction of mechanical resistance was observed after 24 hours in the rabbits, but the values were normal again after 4 to 5 days. In vitro, no significant modifications were observed.—P. d. N.


Severe cyanosis suddenly appeared in a 3 year old boy 30 minutes after induction of general anesthesia using cyclopropane-ether and endotracheal intubation. Vital signs remained stable and ventilatory efforts were normal. Anesthesia was discontinued, 100 per cent oxygen was administered and the patient became fully awake. Nonetheless, cyanosis persisted for 4 hours. Three weeks later the procedure was again attempted, and again cyanosis supervened within 30 minutes. Blood drawn at this time was chocolate colored and remained so despite exposure to oxygen. At this juncture, methylene blue was administered intravenously, and the cyanosis promptly cleared. It was then discovered that a preparation containing ethyl aminobenzoate (Americaine) had been used to lubricate the endotracheal tube. An aerosol of this product was sprayed into the youngster’s throat and cyanosis again appeared. Spectroscopic examination of the blood indicated methemoglobinemia. Because of the small dose necessary to induce severe cyanosis, idiosyncrasy to the agent was postulated. No studies of the patient’s methemoglobin reductase or G-6-PD activity were described.—J. B. S.


A new “private” blood group, Webb, was found in a blood donor in the course of routine testing. The antigen was discovered in 9 of 20 members of two families, each with three generations. No further examples of the antigen were found in about 3550 white Australians. The anti-Wb appeared to be a “natural” antibody and was present in only one of 2000 sera tested. Wb was not related to any of nine major blood group systems and an international comparison with many other “private” blood groups showed that the new group had not been previously described.—F. W. G.


The author showed a significant shortage of A children in O X A as compared with O X O matings. Using p = 0.2774, q = 0.1707 and r = 0.5519 as gene frequencies of IA, Ib and O, respectively, which were obtained by Kobayashi (1940), the rate of elimination of A children in O X A matings was computed at 23 per cent. However, in view of recent findings concerning prezygotic selection that 10-bearing sperm in IA Ib fathers are transmitted to children not at the rate of 50 per cent, but approximately 55 per cent, it has been calculated that the corresponding rate amounts to about 9.6 per cent instead of 23 per cent. In a population under strong selection pressure, the overall mortality rate was estimated to be about 21 per cent of all incompatible zygotes, resulting in different family sizes between compatible and incompatible matings, whereas in another population under more favorable conditions, this rate was markedly reduced to about 5 per cent. Studies on fertility data as well as segregation analyses of family data indicated that the overall effect of ABO incompatibility on mortality of fetuses was the same irrespective of the mother’s type, O, A or B, despite the fact that clinical observation of hemolytic disease of the newborn is almost always
restricted to children of O mothers. Frequency of childless couples was essentially higher among incompatible matings than among compatible matings, suggesting that miscarriages and stillbirths due to ABO incompatibility are frequently occurring in primiparae. Segregation analyses revealed that there was no significant parity effect on incompatible matings with A, B and O mothers. It was pointed out that apparently normal or heteroimmune maternal antibodies were responsible for the effect of the incompatibility. Two possible mechanisms to maintain the stable polymorphic state have been discussed.—K. F.


Forty-eight previously reported cases of aplastic anemia complicating anticonvulsant therapy are reviewed. In most instances, multiple drugs had been used, but the majority of the cases appeared to be secondary to therapy with mephénytoylhydantoin (Mesantoin) or trimethadione (Tridione). No correlation with age, sex, or drug dosage was found. Duration of drug therapy seemed to be important, and the mean duration of therapy before the appearance of bone marrow hypoplasia was 9 months. The mortality rate was approximately 75 per cent and the author concludes that the prognosis in aplastic anemia secondary to anticonvulsants is identical to that seen in idiopathic aplastic anemia. Death usually occurred within 2 months after the appearance of pancytopenia. Recovery of the bone marrow, when it occurred, was a slow, gradual process, but evidence of beginning improvement was frequently seen in the first or second month after onset. The author briefly describes his previously reported case in which bone marrow transfusion from the patient's identical twin resulted in a dramatic and rapid cure.—J. B. S.

**LEUKOCYTES**


A technic is described for obtaining cultures of macrophages from peripheral blood in the absence of significant mitosis. The macrophages seemed to develop by transformation of lymphocytes and probably of monocytes of the original inocula.—T. E. B.


By putting a test tube of blood into an ordinary vacuum bottle wrapped with aluminum foil, the blood can be maintained at or near body temperature during transit so that the viability of leukocytes for growth in culture and for counting of chromosomes seems to be preserved.—T. E. B.


The frequency distribution of the drumsticks found in 500 neutrophil leukocytes was determined in 250 Japanese females. The number of drumsticks varied from 1 to 34 with an average of 12 ± 0.4, and 6 drumsticks were found on the average in 255 ± 8 neutrophils in 227 out of 250 females. These figures are rather close to those reported for European females. However, it was shown that the number of drumsticks found in 500 neutrophils decreased slightly but significantly with age, although the presence of drumsticks was demonstrated for all ages here examined. On the other hand, no drumsticks were observed in 100 males.—K. F.

**A Practical Diluent for Electronic White Cell Counts.** G. D'Angelo and M. Lacombe.

The authors describe a simple cetrimide-citrate-saline solution for counting leukocytes with the Coulter counter. The solution seemed to provide unusually rapid and effective stromatolysis of red cells without being significantly toxic for leukocytes.—T. E. B.


Control patients given pyrexal intravenously showed 3–5 hours later an increase in granulocytes of at least 2000 per cu. mm. Patients treated with marrow suppressive agents (e.g., irradiation, 5-fluorouracil, cyclophosphamide) often showed little or no granulocyte increase after pyrexal, though their peripheral blood findings were not necessarily abnormal. In such patients marrow suppressive therapy seemed to be particularly hazardous. It is suggested that the pyrexal test is useful in the evaluation of marrow reserve and may be superior to the use of peripheral blood counts in regulating the use of the many marrow suppressive agents now employed in the treatment of cancer.—T. E. B.


Both polymorphonuclear leukocytes and macrophages ingested various $^{32}P$- and $^{14}C$-labeled bacteria in vitro. Extensive degradation of bacterial lipid, nucleic acids, and proteins occurred within both cell types. The majority of the bacterial breakdown products were then excreted into the medium by the leukocytes, but there was some evidence to suggest reincorporation of bacterial constituents into leukocyte lipid.—T. E. B.


Leukocytes infected with vaccinia virus produce a substance with the properties of interferon. Leukocytes may in this manner contribute to host defense against viral infections.—T. E. B.


Passive transfer of transplantation immunity by means of sensitized lymphoid cells enclosed within diffusion chambers was apparently demonstrated. Numerous possible explanations for the results were carefully considered. It seems likely that sensitized lymphoid cells will continue to release antibody when transplanted within the diffusion chamber, and that this antibody will be effective in rejecting the corresponding skin homograft. This paper therefore provides further evidence against the exclusive interpretation of transplantation immunity as a form of delayed hypersensitivity.—T. E. B.


Slide chambers and time-lapse cinemicrography were used to study the motility of individual lymphocytes. Cells from patients with chronic lymphocytic leukemia or with lymphosarcoma in the leukemic phase showed an increased tendency to be nonmotile and a decreased capacity to show linear, polarized, effective motion in comparison with lymphocytes from normal subjects. It is suggested that leukemic lymphocytes have a decreased functional capacity.—T. E. B.


In a group of 87 children with acute leukemia, 54 received hydrocortisone and 33 received ACTH as the initial therapy. The incidence of complete remission, partial remission, and bone marrow remission, respectively, was: hydrocortisone 39, 22, and 61 per cent; ACTH 15, 24, and 33 per cent.
The median duration of response was 63 days with hydrocortisone and 46 days with ACTH. Sixty-four of the children who subsequently completed a course of treatment with 6-MP, had remission rates similar to those seen in the group who received hydrocortisone. The mean duration of 6-MP remission did not differ significantly from the duration of hormone-induced remission, even though the longest remissions occurred during 6-MP therapy. There was no significant difference in the incidence of subsequent 6-MP response between the hydrocortisone- and ACTH-treated groups, and the responses to each hormone were independent of the responses to 6-mercaptopurine.—J. B. S.


Fifty-nine children with various types of neoplastic disease were treated with cyclophosphamide. Some degree of remission was induced in each of 12 patients with lymphoma. The responses were of short duration except in the two patients with Hodgkin's disease who obtained remissions of more than 1 year. Six of 16 children with acute stem cell leukemia developed partial or complete remissions of 2 to 9 months duration. Excellent but temporary responses were seen in three of six patients with neuroblastoma. Moderate anemia and moderate to severe alopecia or hemorhagic cystitis were seen in many of the patients who received long-term, continuous oral therapy. Gastrointestinal distress was seen in most of the children in whom the initial phase of therapy consisted of high doses of cyclophosphamide administered intravenously.—J. B. S.

HEMOSTASIS


Trypsin promptly (0–2 seconds) retarded the coagulation of fibrinogen by subsequently added thrombin. Prolonging the exposure to trypsin resulted in greater clotting interference. If added soon (0–5 seconds), soybean trypsin inhibitor blocked this action. Increasing fibrinogen relative to trypsin also obviated the action of trypsin. When thrombin was added before trypsin, clotting was hardly impaired. The same effects were obtained with plasmin, and its action could be blocked by epsilon aminocaproic acid and soybean inhibitor. Alpha-chymotrypsin action was blocked by soybean inhibitor and by p-phenyl propionic acid. The authors suggest that the data may indicate that interference with thrombin clotting of fibrinogen by trypsin, plasmin, and chymotrypsin is related to proteolysis at specific peptide sites, and not to random fragmentation of fibrinogen or to evolution of an anti-thrombin. Thus, if trypsin, plasmin, or chymotrypsin reaches the susceptible loci first, they are rendered nonsusceptible to thrombin and vice versa. From the known action of trypsin and plasmin it is inferred that the site of their blocking action is arginyl-glycyl peptide bonds. The observation that chymotrypsin splits TAMEs suggests that this enzyme acts similarly.—R. G.


The importance of clot retraction in hemostasis is not clear, particularly since it has been held by some that the force of clot retraction is so weak that clot retraction does not occur at all in vivo. In this study, the force of blood clot retraction was measured and found to be at least $10^4$ dynes/cm². This is sufficient to deform many soft tissues and to allow clots to retract in vivo.—R. G.


The amount of factor IX for different genotypic classes was determined by means of a variant of the thromboplastin generation test. The mean value for factor IX in carrier females was about half of the mean value for normal males and normal females; the mean values for the latter two groups were about equal. This dosage compensation is interpreted as evidence to support the hypothesis that one X chromosome is inactive in mammalian females.—R. G.

Proteolysis, Fibrinolysis, and Coagulation—Significance in Thrombolytic Therapy. B.
ABSTRACTS


This paper summarizes a considerable amount of basic research in coagulation and fibrinolysis done in the authors’ laboratory. The fibrinolytic mechanism is considered as a fourth phase of clotting which helps maintain coagulation in delicate balance. “Shwartzman”-induced in vivo coagulation, combined with fibrinolysin, produces profound clotting defects with severe hemostatic failure, in contrast to the relatively innocuousness of either alone. The same defects are obtainable in vitro. The thrombin-fibrinogen interaction is blocked, simulating the in vivo “afibrinogenemia.” These observations are pertinent to thrombolytic therapy invoked for conditions where intravascular clotting may be occurring. Fibrinogen clottability is also blocked by trypsin. Trypsin markedly activates factors II, VII, and X, resembling the action of thromboplastin. In contrast, fibrinolysin and thrombin are inert on these clotting factors, whereas on factor V they first activate and then destroy it. The activating effect of trypsin on factors II, VII and X precludes its use in thrombolytic therapy.—R. G.


Twenty-two rabbits were sensitized to horse serum, and following induction of anaphylactic shock, blood coagulation studies were performed at 10 minutes, 24, 48, and 72 hours. Decreased platelets, hypocoagulability (as demonstrated by thrombelastographic examination), increased inhibitors, and fibrinolytic activity (fibrin platelet method; euglobulin precipitate with streptokinase) were observed.—P. d. N.


Case report in a newborn. The following coagulation defects were manifest: factor V, factor VII, factor IX, platelet factor 3, prothrombin and fibrinogen, increased fibrinolytic activity. Postmortem findings are reported.—P. d. N.


Prothrombin and recalcification times, thrombelastogram and fibrinolysis (fibrin plate method) were studied in experimental carbon disulfide poisoning (6 mg./Kg./day intramuscularly for 30, 60 and 90 days), and in the course of diet-induced hypercholesterolemia (1.5 Gm. cholesterol daily for 60 and 90 days) in the rabbit. In carbon disulfide poisoning, marked hypocoagulability and increased fibrinolytic activity were observed. In experimental hypercholesterolemia, signs of hypocoagulability and marked diminution of fibrinolytic activity developed. The latter represents the differential pattern between the two experimental conditions, and is similar to the analogous finding in the thrombophilic states due to atherosclerosis in human pathology.—P. d. N.


In 86 patients (18 aged between 45 and 65, 68 older than 65 years), with vascular, arteriosclerotic, and thrombotic diseases, the degree of bleeding was evaluated by means of the method of de Nicola and Candura. (Hemostase 1:113, 1961). In a high percentage of cases a reduction of total bleeding, mean intensity as compared with normal mean values was observed, without significant variations of bleeding time. In certain individuals an increase of total blood loss was evident, even in the presence of a normal, or “apparently” normal bleeding time. This finding was used to select patients for anticoagulant therapy.—P. d. N.


The administration of 2 mg. vitamin K1 for 3 days favorably influences prothrombin time and factor VII in the full-term newborn but not in the premature newborn.—P. d. N.
MISCELLANEOUS


Univalent 3.5S fragments of rabbit 7S antihuman red cell agglutinating antibodies prepared with papain or pepsin and mercaptoethylamine reacted specifically with their homologous antigens, but failed to produce agglutination of erythrocytes. Prior treatment of the cells with proteolytic enzymes or suspension in 30 per cent albumin still failed to produce agglutination. Combination of the fragments with the homologous antigen was demonstrable by agglutination upon subsequent addition of chicken or goat antibody specific for the univalent fragments of rabbit γ-globulin. Similar results were obtained with univalent fragments of rabbit antibody to human γ-globulin by use of human cells sensitized with human γ-globulin. The results are consistent with current theories as to the mechanism of action of bivalent and univalent antibodies, and provide another example of the properties of univalent subunits of antibody.—H. H. F.


The incidence of rheumatoid agglutinating activity (RAA) in parents and siblings of patients with acquired agammaglobulinemia was found to be considerably higher than the incidence of RAA in the normal population. Elevated γ-globulin levels were also occasionally present, as well as alterations in the β2 and β2M proteins in such relatives. Laboratory findings suggestive of lupus erythematosus were observed in two instances. The high incidence of serum γ-globulin abnormalities in relatives of patients with idiopathic "acquired" agammaglobulinemia suggests that this is a genetically determined disorder.—H. H. F.


Yoshida tumor cells were detected in smears of blood and imprints of lymph nodes and adrenal of rats soon after injection of these sarcoma cells. Studies were also performed on organs taken from tumor-bearing animals. The following conclusions were reached: (1) Yoshida sarcoma cells enter the circulation soon after tumor transplantation; (2) Yoshida sarcoma cells are also promptly observed in imprints of organs, their number being proportional to that observed in the blood; (3) the ability to reproduce a secondary tumor (metastasis) is partially determined by the number of neoplastic cells in the blood and consequently in the organs.—M. I.

NEWS AND VIEWS

BLOOD CLUB MEETS APRIL 28 AT ATLANTIC CITY

The Blood Club will meet at 8:00 P.M., Sunday, April 28, in the Vernon Room of Haddon Hall, Atlantic City, New Jersey. (Chairman: Dr. Charles P. Emerson, Boston University Medical Center, Boston, Massachusetts.) The topic of discussion will be "Phagocytosis." The program: "Metabolic Basis of Phagocytic Activity," Dr. Manfred Karnovsky, Harvard Medical School, Boston; "Cinephotomicrographic Studies of Phagocytosis," Dr. James Hirsch, Rockefeller Institute, New York City; "Erythroclasia," Dr. James Jandl, Thorndike Memorial Laboratory, Boston.