ABSTRACTS
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LEUKOCYTES


The authors presented the results of a study of rubidomycin in 38 cases of relapse of acute lymphoblastic leukemia at various stages. The overall percentage of complete remissions was 60 per cent, but if one considered only the first relapse, it was 75 per cent. The time and mode of onset of the remission were analyzed and the significance of marrow blasts in a poor bone marrow were discussed. The results and complications observed in patients when rubidomycin was also given as maintenance treatment were described and details were given of the toxicity.—G. M.


Ten patients with leukemia received methotrexate (4-MPGA) intravenously. Sensitivity of the cells depended on intracellular concentrations of the drug and of dihydrofolinic acid reductase (DHFR) and thymidine kinase. There were \(15 \times 10^{-3}\) μ moles 4-MPGA per 10^6 leukocytes in two patients; 4-MPGA was assayed with S. fecalis. Normal leukocytes did not contain DHFR. Serum concentration of 4-MPGA fell most rapidly initially and was not measurable 24 hrs after injection, except in renal failure where elimination was slower. Urinary excretion of 4-MPGA was 25 per cent of the dose on the first day and 10 per cent on the second.—P. G. R.

MEGAKARYOCYTIC HYPERPLASIA AND QUALITATIVE ABNORMALITIES OF THE MEGAKARYOCYTE-PLATELET LINE IN AN ACUTE MYELOBLASTIC LEUKEMIA. R. Zittoun, A. Bernadou and M. Samama. From

The authors reported a case of acute myeloid leukemia with abnormalities of the platelets and megakaryocytes. The platelets were normal in number but poor in quality, for there was a prolonged bleeding time, loss of platelet aggregation in the presence of ADP, and a definite fall in the liberation of factor 3. The marrow contained both excess myeloblasts and megakaryocytes. The latter were morphologically abnormal and most were PAS negative. The marrow erythroblasts were normal. This observation suggested the existence of mild involvement of the megakaryocytic series in acute myeloid leukemia.—G. M.


Transient thrombocytopenia and leukopenia were observed in the first month of life of a baby delivered of a mother suffering from acute myeloblastic leukemia. Normalization of the child's blood picture was observed after corticosteroids and blood transfusion.—M. K.


Transformed lymphocytes formed the majority of cells in stimulated cultures of leukocytes from undifferentiated leukemias, while paramyeloblasts represented the majority in cultures of leukocytes from paramyeloblastic leukemias after 4 days. The usefulness of staining for ribonucleic acids for differentiating transformed blastoid cells and leukemic blasts was stressed.—L. D.


Survival of erythrocytes labeled with Cr39 was investigated in 25 patients suffering from chronic lymphadenosis and in one female with hemoblastosis originating from the lymphoreticular system. In 13 patients, accumulation of radioactivity over the liver and spleen was studied at the same time. Sixteen of the 26 cases showed shortened survival of erythrocytes; 14 showed evidence of anemia. No correlation was established between the size of the spleen and half-time of erythrocyte survival or between size of spleen and hemoglobin concentration. A statistically significant correlation existed between half-time of erythrocyte survival and hemoglobin level.—L. D.


The authors reported 11 cases of malignant erythremia and reviewed recent data in the literature. In the last ten years, important information has been gained concerning the mechanism of the anemia, the metabolic disorders of heme and globin, and changes in the enzyme content of the erythroblasts in malignant erythremia. The clinical and hematological findings depended on whether the erythremia was acute or chronic, but the fundamental disturbance of the erythroblast was common to both forms. Erythroleukemia was the commonest hematological form of erythremia; in this form, the marrow contained an increased number of both erythroblasts and malignant myeloblasts. Apart from obvious erythroleukemia in which the erythroblasts showed definite morphological abnormalities, minimal erythroleukemia with mainly qualitative changes in the erythroblasts during acute myeloid leukemia was distinguished. These changes included excessive sideroblastosis and a positive erythroblast PAS reaction. These "minimal" erythroleukoses should be distinguished from the non-specific erythroblast changes which occur during acute leukemia.—G. M.

An unusual course in one cell-free passage of experimental virus mouse leukemia derived from radiation induced leukemia was observed. This leukemia manifested itself as lymphoreticular in C57 Black mice and as erythroleukemia in CBA mice. Instead of the 2 months latent period when the leukemia usually appears, the mice survived without signs of leukemia and were killed after 7 1/2 months. Histological findings in C57 Black mice were normal in more than half; in one-third there was massive extramedullary hemopoiesis which could not be distinguished with certainty from chronic myeloid leukemia, and in one-seventh leukemic infiltration with lymphoreticular cells was found, but not of the usual extent and more in the form of tumors. In one mouse, transition from this type to massive extramedullary hemopoiesis was found. In a parallel experiment, only temporary enlargement of the spleen was found in CBA mice which survived 1 1/2 months and were finally killed without showing signs of leukemia.


The nucleolar coefficient of lymphocytes was studied in 34 healthy subjects without professional exposure and in 73 healthy persons exposed regularly and for long terms to small doses of ionizing radiation. The mean value of the nucleolar coefficient of lymphocytes in a population of miners did not, by itself, constitute an indicator of the exposure of this population. —L. D.


The authors found two types of circulating lymphocytes differing in response to phytohemagglutinin in vitro. Both types of cells were capable of forming a new, apparently homogeneous class of cells, so called "PHA cells", but at different rates. The usefulness of cytologic criteria for the classification of lymphocytes in regard to their ability for "blastic transformation" was discussed. —M. K.


The authors described 20 cases of generalized Hodgkin's disease, most of whom had previously received other forms of treatment, in which one complete remission and 4 incomplete remissions were obtained with rubidomycin. —G. M.

HEMOSTASIS


A hemorrhagic diathesis with thrombocytopenia appeared after application of penicillin into the conjunctival sac of a 3 year old girl. The skin rash and Quincke-like edema were associated with a decreased platelet count. Symptoms regressed after cessation of penicillin treatment and administration of corticoids. —M. K.


Three cases were described. In the first, a patient of 41, there was spontaneous subarachnoid hemorrhage with left sided hemiparesis. In spite of unconsciousness for more than two days, he recovered after treatment with fresh blood plasma and AHG. The second patient, aged 18, suffered cerebral...
hemorrhages. The first time, it was probably a hemorrhage into the left hemisphere when antibodies of antithromboplastin character were temporarily found in his blood. Four years later, bleeding into the right hemisphere was confirmed by contrast X-ray examination. He also recovered completely. In the third patient, subdural hematoma and hemocephalus developed after repeated head injuries; the patient succumbed.—L. D.


A transient increase in fibrinolytic activity can be observed in blood plasma stored in glass test tubes at 0°C. Active Hageman factor activated fibrinolysis in plasma in vitro, as well as in vivo. The results suggested Hageman factor may influence fibrinolytic activity of circulating blood as the plasma plasminogen activator.—M. K.


The activity and some properties of the serum substitute produced from prothrombin are described. The active product has been purified by desalting, adsorption and elution. The full activity of the serum substitute is obtained after reaction with a purified preparation of AHG.—M. K.


Enhanced ADP-induced platelet aggregation, elevated fibrinogen and increased activity of AHG and proaccelerin were found in the blood of patients with various types of carcinomas when compared with a control group. Some abnormalities in thromboelastographic curves and moderate increases of fibrinolytic activity were also demonstrated. The role of intravascular clotting and of release of tissue activators of plasminogen in the pathogenesis of thrombosis in neoplastic disease was discussed.—M. K.

**IMMUNO-HEMATOLOGY**


An anti-human lymphocyte (ALC) horse serum was prepared using lymphocytes obtained from the thoracic duct of several patients awaiting renal transplants. Precipitation of the original serum with ammonium sulfate permitted separation of the globulin fraction. Six months after the start of immunization, the lymphoagglutinating and lymphocytic activity of this fraction was 1/1024. It had a strong stimulating activity, as measured by the percentage of cells transformed on the 7th day of culture and by the increase in DNA and RNA activity. The fine structure of the transformed lymphocytes was the same as that noted after stimulation with phytohemagglutinin. This ALC was administered to 15 patients at a dose of 80 to 120 units/Kg I.M. daily for one month and every 2 to 3 days thereafter. The lymphopenia which occurred 8 hours after the first injection was not statistically significant, contrary to the polymorph leukocytosis. Treatment over several months did not cause persistent lymphopenia. The immunodepressive effect was demonstrated by the Mantoux test becoming negative in 8 patients and a toxoplasma intradermal test becoming negative in 6 patients 8 days after the start of treatment. In 7 patients with a renal transplant, with a variable degree of histocompatibility, there were only moderate signs of rejection, enabling one to use larger doses of other immunodepressant drugs. Individual tolerance was variable, and there was no apparent toxic effect on the kidney.—G. M.

Studies were reported which suggested that specific inhibition of the migration of human white cells in vitro was a reliable indicator of cellular hypersensitivity. Brucella-positive (spontaneous and after vaccination) and brucella-negative individuals were studied. Leukocytes were isolated from the blood and put in a capillary tube which was placed in a culture chamber. After 24 hours, the area covered by cells that migrated out of the capillary tube was measured. Presence of antigen (brucella bacteria) inhibited leukocyte migration. Results were expressed in terms of a migration index (migration area in presence of antigen/control). In 38 brucella-negative (skin test) individuals, the migration index was 0.92 ± 0.07, in 38 brucella-positive 0.58 ± 0.15. Moreover, in brucella-positive persons, a definite correlation between the migration index and the strength of the intracutaneous reaction was demonstrated. There was no correlation with the brucella agglutinin titer.—S. A. K.


The induction of tolerance in adult animals by repeated injections of large amounts of protein antigens is associated with an inhibition of antibody production and of the hypersensitivity of the delayed type towards antigens not related to those employed for the induction of tolerance. This non-specific inhibition affects also the manifestations of graft immunity (homograft reaction, anti-host graft reaction). The inhibition lasts 2 to 3 weeks, but antigenic stimulation of long duration (sensitization with Freund’s adjuvant, homografts) eventually produces an immune reaction. However, a short lived stimulation (sensitization with soluble antigens, anti-host graft reaction) can be inhibited indefinitely. Consequently, a preliminary treatment of animals (donors of lymphoid cells) renders possible suppression of the cell reaction against the host in irradiated receivers, as well as the establishment of tolerance for donor tissues, so that skin grafts survive for some considerable period or even permanently.—G. M.


Antinuclear antibodies appeared after neonatal thymectomy in more than 70 per cent of Swiss/Gif mouse survivors, whereas the incidence in normal controls or partially thymectomized mice was less than 10 per cent. The first positive results occurred in the 15th week. The maximum percentage of animals with antinuclear antibodies and the highest titers were encountered in the 20th and 30th weeks. The authors were unable to demonstrate a close relationship between the presence of antinuclear antibodies and the onset of a “wasting disease”. Neonatal thymectomy caused the appearance of the stigmata of auto-immunization but this did not prove the theory of an autoimmune etiology in “wasting disease”. Various theories were put forward to explain the mechanism including the possibility of antigenic stimulation by the intestinal flora of the experimental animals.—G. M.


Neither removal of Fabricius’ bursa in the newborn chick nor thymectomy prevent the production of normal antibodies. Antigen phagocytosis in immunized chicks enables one to detect traces of antibody. By this method it has been found that neonatal bursectomy causes a considerable fall in the production of anti-bacterial antibodies without total inhibition. The response to secondary immunization is almost identical in bursectomized chicks and in controls.—G. M.

The authors described 12 cases of ICG or IGA paraproteinemia without apparent myeloma. In 3 cases, the protein abnormality was secondary to lymphoid hyperplasia and in one or two other cases secondary to carcinoma. In the others, it seemed to be an incidental discovery during various disorders not necessarily hematological. In the idiopathic forms, the distinction from multiple myeloma may be difficult, as the absence of bony lesions and plasma cells in the marrow may not be sufficient to exclude the latter. Serological and immunological tests have been of definite but limited value. The course of the disease was definitely the most important factor enabling one to distinguish multiple myeloma from other forms of paraproteinemia. Even so, there may be exceptional cases difficult to classify, an example of which was reported here. The pathophysiology of the paraproteinemia was discussed in the light of recent reports in the literature. — G. M.


The presence of Rh(D) antigen in the epithelium of the amniotic membrane was demonstrated by an immunofluorescent technic. The usefulness of this technic for early detection of serologic conflict between fetus and mother, as well as the immunological role of the amniotic membrane were discussed. — M. K.

**ERYTHROCYTES**


Megakaryoblasts, like rapidly proliferating cells, have high concentrations of thymidine kinase and other thymidine synthesizing enzymes. These concentrations are reduced 12 hrs after B12 treatment; B12 requirements are 0.5 μg per mg total-body-B12. To refill total body stores in p.a., 62 injections of 500 μg CN-B12 or 22 of OH-B12 are required. Later, 4 OH-B12 injections yearly suffice. (Abstracter's comment: Not only are there indications that leukemic cells grow slowly, but also that those in "aregenerative" p.a. grow rapidly.) — P. G. R.


Intrinsic factor antibody was looked for by means of a charcoal technic in 1600 individuals. In 1100 hospital patients (none with known pernicious anemia, thyroid disease, "idiopathic" adrenal insufficiency, diabetes, hypochromic anemia, gastric carcinoma, postgastrectomy syndromes, myelomatosis or polycythemia vera), intrinsic factor antibodies were found in 0.7 per cent of 700 aged over 50, but in none of 400 aged less than 50. No antibodies were found in 500 unselected blood donors below 60 years of age. The incidence of intrinsic factor antibodies suggested that latent pernicious anemia may be present in about 1 per cent of hospital patients over 50. — F. W. G.


Eighteen patients with pernicious anemia in remission were treated with prednisone, 20–15 mg daily for six weeks. Gastric acidity, secretory volume, and non-IF-B12-binding capacity did not change, neither unstimulated nor with maximal histamine stimulation. Prednisone caused a significant increase in intrinsic factor secretion. In nine of the patients, small islands of relatively normal gastric glands containing parietal cells were noted in gastric biopsies after prednisone. The Schilling test became normal in seven cases. Circulating IF-antibody and parietal
cell antibody were also studied. A decrease in antibody concentration was observed in some cases. However, a clearcut association between antibody titer changes and changes in the other parameters studied could not be established; thus, a response to prednisone was seen in 4 of 8 patients without change in gastric antibody titer. It was, therefore, questioned whether or not the finding of gastric antibodies supports the auto-immune concept of pernicious anemia.

---S. A. K.


Chromosome studies on bone marrow cells in a case of classical pernicious anemia in relapse disclosed structural abnormalities in 38 per cent of the metaphases analyzed. They were promptly corrected after vitamin B₁₂ administration. No quantitative anomalies were detected. On the basis of these findings, the nature of the megaloblastosis and the possible role played by folic acid and vitamin B₁₂ deficiencies in malignant transformation were discussed. Chromosome abnormalities found in pernicious anemia and in pyrimethamine intoxication were similar to those produced by X-rays and might explain the finding of megaloblastic marrowls and cellular gigantism following radiation injury. On the other hand, considering the role played by chromosome breakage and rearrangements in neoplastic transformation, the occurrence of pernicious anemia and leukemia in the same patient may not be a fortuitous event. In this respect the development of acute myeloblastic leukemia in a patient with megaloblastic anemia secondary to sulfanilamide therapy was of special interest (Reisner, 1958). These concepts, if correct, may imply that the megaloblastic proliferation due to folic acid and vitamin B₁₂ deficiencies are not to be considered, in spite of the similarities, as malignant growth. It would be more reasonable to assume that the deficiencies of these vitamins actually predispose to the development of malignancy by way of the chromosome aberrations they can induce.—M. J.


T 1/2 of intravenous radio-B₁₂ bound to TC I was 9–10 days, and was 1.5 hours when bound to TC II.—P. G. R.


The loss of 1.5 ml blood, administration of hemolsate of their own red cells, Pyrifer and feeding raw beef liver in the food enhanced the renewal of hemopoiesis in irradiated rats when a small part of the bone marrow had been protected. This renewal was further enhanced by administration of vitamin B₁₂, tetrahydrofolic acid and vitamin C.—L. D.


Four well-studied patients with PNH (one Coombs-positive) were treated with prednisone after other forms of therapy had been unsuccessful. They remained in substantial remission for 2–4 years on initial doses of 40 mg and maintenance doses of 5.0–12.5 mg per day, with occasional increases. (Abstractor's comment: The use of steroids in PNH is controversial, failures and some successes having been previously reported. In view of the authors' clear evidence that occasional patients derive substantial benefit, a trial of steroid therapy appears indicated in cases refractory to other methods of treatment, and especially those requiring frequent transfusions.)—F. W. G.

THE HEMOLYTIC ACTIVITY OF SILICA AND SOME OTHER DUSTS. K. Zajusz, Z. Para-

The influence of various dusts contaminating the atmosphere of Silesian mines on the structure of the erythrocyte membrane was studied. Silica dust had most potent hemolytic activity which appeared to be proportional to the content of crystals. The significance of the determination of the hemolytic activity of dusts for the examination of miners was pointed out.—M. K.


Fe59-labeled "stress" macrocytes from phenylhydrazine-treated rabbits were compared with Fe59-normocytes from untreated animals as to stability during incubation for 24 hours. Autohemolysis was somewhat higher in the macrocytes when glucose was supplemented and pH controlled. Under conditions of glucose deprivation and/or low pH, the autohemolysis of stress macrocytes was much greater (28 per cent) than of normocytes (7 per cent). The results were of interest in light of the observations of Stohman (Proc. Soc. Exp. Biol. Med. 107: 884, 1961) that stress macrocytes were short lived in vivo and that splenic destruction was especially involved. It was thought likely that glucose deprivation and low pH in the present studies were also extant in the splenic microvasculature.—H. S. J.


Target cells, found in patients with obstructive forms of jaundice (including hepatitis), were demonstrated to contain excessive cholesterol and to be of diminished osmotic fragility. These attributes were closely related and both could be induced in normal red cells by their circulation in jaundiced patients or simply by their prolonged incubation in jaundiced sera. Bile salts added to normal sera inhibited cholesterol esterification and shifted free cholesterol from sera to red cells. The resulting increase in surface area (without alteration in cell volume) was reflected in flattening (targeting) and increased distensibility (decreased osmotic fragility) of the affected red cells.—H. S. J.


The rates of utilization of glucose by human and dog erythrocytes were studied simultaneously using an apparatus in which temperature, partial gas pressures and pH could be kept constant. When pH was kept between 7.10-7.25 or 7.50-7.70, Pc02 constant, the rate of glycolysis was significantly lower with dog erythrocytes than with human cells. No significant difference was found with pH between 7.20 and 7.45. When pH was kept constant, the rate of glycolysis of dog erythrocytes was significantly lower than for human cells, if Pc02 was kept between 70 and 100 mm Hg; under the same conditions, no significant difference was found if Pc02 was maintained in the 38-44 mm Hg range. In both species, the rate of glycolysis was related to Pc02: the higher the Pc02, the lower the rate of glycolysis. No change in ATP content of erythrocytes (always lower in dogs than in man) was observed during 28 hours.—G. M.


A method for purification and crystallization of D-glyceraldehyde-3-phosphate dehydrogenase from human erythrocytes was described. The crystalline enzyme was found to be homogeneous on starch gel electrophoresis and by ultracentrifugation, and was found to be completely inhibited by sulphydryl reagents. Some properties of the enzyme were determined.—M. K.

A laboratory trial was undertaken to measure serum transferrin on the assumption that Rivanol at pH 8.0 coagulates all serum proteins, with the exception of gammaglobulin, transferrin and hemopexin. Two ml of 0.75 per cent Rivanol solution in TRIS buffer, pH 8.0, 0.005 M will coagulate 1 ml of serum. The concentrated filtrate, after Rivanol has been discarded, was used for paper electrophoresis. At the same time, the original serum was also examined by paper electrophoresis. The amount of serum transferrin was calculated from the absolute value of gammaglobulin and from the relation of the gamma and beta fractions in the Rivanol filtrate.—L. D.


When 5-hydroxytryptamine was injected intramuscularly into rabbits in daily doses of 3 mg per kg for 4 weeks, a 15 per cent increase in red cell count, but a decrease in hematocrit value (13 per cent) were observed. Mean red cell volume decreased from 71.15 μ₃ to 54.9 μ₃ and the Cr⁵¹ erythrocyte life span was prolonged by 21 per cent.—M. K.


The authors investigated their important observation (Biochem. Biophys. Res. Comm. 26:162, 1967) that 2,3, diphosphoglycerate decreases the oxygen affinity of hemoglobin about 30-fold. Their previous data indicating that 2,3 DPG provides for heme-heme interaction and the usual sigmoidal oxygen affinity curve of hemoglobin must now be modified. The present study indicates that 2,3 DPG in physiological concentration displaces the hemoglobin oxygen dissociation curve markedly (in the direction of decreased oxygen affinity), perhaps providing for efficient oxygen unloading at tissue sites. The results also suggest, as have Edwards and Rigas (J. Clin. Invest. 46:1579, 1967), that the increased oxygen affinity of red cells aged in vivo may reflect lowered 2,3 DPG contents of senescent cells.—H. S. J.


Human hemoglobin dissociates into αβ dimers when small quantities are dissolved in concentrated salt solutions. By a variety of technics measuring ligand (mainly CO) binding to dimer hemoglobin, it could be concluded that such αβ subunits exhibit all the characteristic features of the kinetics of the reaction of tetramer hemoglobin with ligands. This finding suggests that a reinterpretation of hemoglobin kinetics in terms of models based on dimers, rather than tetramers, might be fruitful.—H. S. J.


The interaction between human hemoglobin and haptoglobin was followed by measurement of the quenching of haptoglobin fluorescence which occurs with complex formation. A variety of data indicated that hemoglobin tetramers did not react with haptoglobin, but that subunits, either αβ dimers or monomers, were bound. Separated α chains, but not β chains, reacted with haptoglobin. However, β chains would attach to haptoglobin which had been preincubated with α chains. The authors con-
cluded that the normal reaction between hemoglobin and haptoglobin proceeds either by consecutive binding of α and β monomers or by attachment of αβ dimers through the α chain. (Abstractor's note: It would seem important that haptoglobin binds dimers of hemoglobin, rather than tetramers, since considerable evidence exists that in the low concentrations in which hemoglobin is found in plasma, appreciable dissociation of the molecule occurs. Recently Bunn and Jandl, Clin. Res. 15:272, 1967, have presented evidence that hemoglobin leaks into renal tissue as dimers, rather than as the intact hemoglobin tetramer. To prevent renal damage, therefore, haptoglobin must be, and evidently is, capable of binding such dimers).—H. S. J.


Rabbit reticulocytes (induced by administration of acetylphenylhydrazine) failed to synthesize heme from C14-glycine if globin generation was inhibited by cycloheximide or puromycin. Heme synthesis with delta aminolevulinic acid as precursor continued at normal rates. The authors interpreted the findings to indicate that the lack of globin to act as an acceptor for heme allowed free heme to accumulate within the cell and to reach a concentration which was inhibitory to ALA formation and heme synthesis. This interpretation favored the view that synthesis of globin plays a role in regulating the synthesis of heme.—H. S. J.

MISCELLANEOUS


Between 1959 and 1966, 61 cases were treated with Pelentan. In the first group, average duration of treatment was 50.8 months. The second group was composed of 42 patients not treated with Pelentan; 20 patients interrupted treatment. Distribution of age, sex, and severity of cardiac infarction was similar in both groups. A statistically significant difference was found in mortality, relapse and incidence of more serious symptoms in favor of the treated group. Even the length of survival was greater in this group. No statistical significance was found in thromboembolic complications which occurred three times in the untreated group. There were three instances of bleeding during anticoagulant treatment; all being successfully mastered.—L. D.


In granulation tissue produced by skin excision in hyposideremic rats, synthesis of collagen proteins was inhibited, while the formation of non-collagen protein was significantly enhanced. The same finding was obtained in the heart which in anemic rats was characterized by compensatory hypertrophy. Addition of inorganic or colloid iron to skin slices incubated for two hours with C14-proline did not influence the formation of the two types of proteins. Inorganic copper inhibited protein synthesis in general. —L. D.