ABSTRACTS
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ERYTHROCYTES

A new hemoglobin, isolated from a 41 year old man and his daughter, was associated with a compensated hemolytic anemia, splenomegaly, and inclusion bodies in red cells after exposure to redox dyes which resembled those found with H-thalassemia. The absorption spectra of Hemoglobin Gun Hill indicated half the number of expected heme groups for the number of \( \alpha \) and \( \beta \) polypeptide chains. Recombination experiments demonstrated that heme groups were absent from \( \beta \) chains. Fingerprint analyses revealed a deletion of the 5 amino acids of the \( \beta \) chain just distal to the heme-binding histidine at B-92. Deletion of an amino acid was reported in Hemoglobin Freiburg (Jones et al., Science 154:1024, 1966) and was thought to represent an unequal crossing-over during meiosis. (Abstracter's note: The inordinate precipitation of unstable hemoglobins into Heinz bodies has also been demonstrated to involve diminished binding capacity of globin for heme in the hemolytic anemia associated with Hemoglobin Koln [J. Clin. Invest. 46: 1073, 1967].) —H.S.J.


Previous experience has indicated that malaria, folic acid deficiency and intercurrent bacterial infections contribute to the poor prognosis of sickle cell anemia in African children. In the present trial, several different forms of therapy were compared. Group 1 received a preparation containing a long-acting sulfonamide, chloroquine, folic
acid and vitamins B₁₂ and D. Group 2 was given the same preparation to which had been added an anabolic steroid. Group 3 received only routine therapy with folic acid and pyrimethamine. There were 31 children in group 1, 52 in group 2, and 52 in group 3. Treatment was continued daily for six months. Several conclusions were reached. (1) Chloroquine was at least as effective as pyrimethamine in controlling malaria. (2) Folate deficiency was effectively combated by all three regimes. (3) There was some evidence that the giving of a long-acting sulfonamide prevented infections in very young children, but there were no real differences between the groups as a whole. (4) Gain in weight was greater in the group given an anabolic steroid, but was not associated with an increase in bone mineralization. (5) The overall course of the disease, as judged by hemoglobin levels, spleen size, and painful crises, appeared to be more favorable in Group 1.—T.H.B.


Studies of 29 members of a Venetian family disclosed that this association caused a complete inhibition of the synthesis of normal beta chains and, therefore, the complete disappearance of Hb A which was replaced by the anomalous Hb D.—P.d.N.


This unusual association was characterized by a constitutional hypochromic microcytic anemia with reduced osmotic fragility of erythrocytes and a high Hb A₂ level, splenomegaly, liver cirrhosis, leukopenia and thrombocytopenia.—P.d.N.


These papers describe the results of detailed epidemiologic studies on the incidence and severity of hookworm infection in different parts of East Africa. The relative importance of malaria and of hookworm infection in the production of anemia is also discussed.—T.H.B.


One hundred subjects with hemoglobin values less than 10 Gm./100 ml. on the third postpartum day were studied. Mean age was 24 and mean parity was 3.3. Blood loss at delivery was greater than 600 ml. in 22 patients. Sixty-four percent showed no stainable iron in the marrow and only minimal amounts were present in another 26 percent. Eighty-seven percent of these 90 patients had a percentage saturation of transferrin of less than 20. Giant myeloid cells were noted in the marrows of 78 patients and megaloblastic changes also were present in 33. Half of these subjects had serum folate levels of less than 3.0 μg./ml., while FIGLU excretion was abnormal in most of the others. In contrast, serum vitamin B₁₂ levels were less than 150 μg./ml. in only one of the 78.—T.H.B.


Ascorbic acid, 100 mg./100 ml., in test tubes increased growth in folic acid standards by about 10 percent, but decreased growth in serum extracts about 4–10 percent. The authors felt that this finding may, in part, explain differences between Herbert's lower normal limit of 7.5 μg./ml., Mollin's of 5.9, Spray's of 2.1, and the author's.
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(Abstracter's comment: The difference between fasting and non-fasting subjects also may contribute).—P.G.R.

DISTURBED PROLIFERATION OF ERYTHROPOIETIC CELLS IN PERNICIOUS ANAEMIA.


Autoradiography of labeled marrow cells combined with quantitative cytochemical measurement of DNA content showed that in pernicious anemia, there was a relatively increased number of polychromatophilic megaloblasts in the G2 phase and an increased proportion of unlabeled cells with DNA content between the 2c and 4c modes. Death of these arrested cells may be responsible for ineffective erythropoiesis.—A.L.B.

ABSENCE OF AN INHIBITOR OF ERYTHROPOIESIS IN POSTNATAL PLASMA.


An inhibitor of erythropoiesis has been reported in plasma of hypertransfused animals and of human subjects with high altitude polycythemia following descent to sea level. Birth may be regarded as analogous to descent from high altitude. Cord and sixth day plasmas did not have inhibitory effects on erythropoiesis in the stimulated hypertransfused mouse.—B.R.


Myopathies have been attributed to abnormalities in muscle membrane ion transport and it has been suggested that membranes other than muscle might be affected. Previous studies (Experentia 23: 522, 1967) had indicated that myopathic muscle membranes from Pekin ducks contained a membrane ATPase which was peculiar in that it was stimulated, rather than inhibited, by ouabain. In the present study, erythrocyte ghosts from human patients with spontaneous, pseudohypertrophic (Duchenne) or limb-girdle muscular dystrophies demonstrated similar abnormalities in their ATPase activities. The significance of this finding is unclear, except in relating muscular dystrophy to a more generalized membrane defect.—H.S.J.


Hexokinase activity in human erythrocytes is associated with three distinct electrophoretic bands. In adult cells, types I and III are found. In newborns and adults with hereditary persistence of fetal hemoglobin, type II is found. The regulation of synthesis of type II hexokinase and gamma chains of hemoglobin may be linked. Since the Km for the fetal hexokinase is higher than that of the adult types and since hexokinase is probably the rate-limiting enzyme of glycolysis, the present observation may have relevance in elucidating the altered pattern of glycolysis in newborn red cells which renders them susceptible to oxidant drugs.—H.S.J.


Erythrocyte transaminases (glutamic-oxaloacetic and glutamic-pyruvic) were active parameters of dietary pyridoxine deficiency induced in rats. Levels one-fourth of normal were reached within 4–6 weeks of pyridoxine deprivation. In contrast to liver and plasma, erythrocyte GOT and GPT levels most accurately reflected B6 nutrition and remained low for several weeks after dietary repletion was begun. The apoenzyme, as well as the coenzyme, may be depleted during B6 deficiency, and was repaired only with the production of new red cells. (Abstracter’s note: Assay of erythrocyte transaminase might be a useful diagnostic test for the pyridoxine-responsive anemias; see McCurdy and Donohoe, Blood 27:352, 1966).—H. S. J.

Purification up to 2000-fold was obtained. Protein in stroma-free hemolysates was precipitated by 50 percent saturation with ammonium sulfate. The precipitate was redissolved and, after partial removal of salt ions on Sephadex C 25, was passed through CM-cellulose in a phosphate buffer, pH 6.0. Elution of the enzyme was accomplished with buffer, pH 6.4. The Michaelis constant for GSSG for the purified enzyme of defect carriers was 2-3 times that of normals. The pH optimum of the NADPH-dependent enzyme reaction was shifted from 6.8 to 6.4. The Michaelis constant for GSSG of the NADH-dependent reaction did not differ between normals and defect carriers. The pH optimum of the reaction was 6.3 in normals and 6.6 in defect carriers.—K. B.


GSH influenced the shape and the position of the dissociation curve of oxyhemoglobin. Elevated levels of GSH shifted the curve to the right (Hill’s constant was decreased). The addition of GSH to stored blood or to plasma expander solutions increased the percentage of animals surviving experimental hemorrhagic shock.—L.D.


Impressive electron photomicrographs of erythroblasts from erythroleukemia clones garnered from spleens of marrow-transplanted, irradiated mice are presented. Nuclei in late erythroblasts clearly are shown to be extruded through protrusions of the cell membrane. When exteriorized, the nucleus is surrounded by a thin rim of cytoplasm and altered plasma membrane and rapidly becomes attached to and is phagocytized by a neighboring macrophage. No evidence of nuclear disintegration within the erythroblast is noted. (Abstracter’s note: The authors raise the question of how the altered plasma membrane around the extruded nucleus differs from that of the intact reticulocyte so as to attract and attach macrophages. The equally impressive photomicrographs of LoBoglio and Jandl showing macrophages attached to presumed gamma globulin molecules of antibody-coated red cells [J. Clin. Invest. 46:1087, 1967] suggest that gamma globulin may be attracted to the altered plasma membrane and in turn may attract phagocytes).—H. S. J.


The capability of red cells to become deformed was shown to play an important role in reducing viscosity and facilitating blood flow at the high shear stresses extant in capillaries. Red cells, hardened by acetaldehyde, packed poorly in centrifuged columns and, unlike normal cells, did not show the characteristic reduction in viscosity when exposed to high shear stresses in couette viscometers. Hardened cells did not pass through 6.8 μ pores in synthetic polycarbonate sieves, whereas normal cells passed 3 μ pores with ease. (Abstracter’s note: The latter relationship between poor filterability and diminished deformability or “plasticity” of red cells has been suggested to be involved in the trapping of hereditary spherocytes and other abnormal red cells in the filter meshwork of the spleen [Jandl et al., Blood 18:133, 1961].)—H. S. J.

LEUKOCYTES


The authors described a method of electron paramagnetic resonance analysis which was applied to study the free radical con-
tent in the leukocytes of healthy persons and of patients with chronic lymphatic and myeloid leukemia. In chronic lymphatic leukemia, free radical concentrations in leukocytes proved to be 4.5 times, and in chronic myeloid leukemia, 8 times greater than in normal individuals. In remission, a regular fall of free radicals occurred. Leukocyte free radical concentrations, characteristic of metabolic activity, offer a possibility of comparing metabolic processes in normal and leukemic cells and of tracing the anaplastic dynamics of the leukemic process.—J. K.


The kinetics of granulocytopenesis in acute leukemia were analyzed and formulas were elaborated for calculating the duration of mitosis and the generation cycle of leukemic cells. The proliferative activity of leukemic cells approached that of normal myeloblasts. Leukemic hyperplasia and metaplasia of the bone marrow in acute leukemia was attributed not to intensified proliferation, but to increased numbers of mitotic cycles of the blast cells.—J. K.


Cytologic investigations pointed to a definite limit of metaplasia at different stages of leukemia. Hemocytoblastic metaplasia was revealed in a lymph node and in iliac bone marrow of 2 patients with normal splenic marrows, examined twice. Sternal metaplasia appeared 3 and 8 months after the onset of the disease.—J.K.


Sera of rabbits, immunized with saline extracts of spleens from persons who died of hemocytoblastoses or reticuloses, were adsorbed with extracts of normal human organs and with sera from healthy persons. A specific antigen was detected with antihemocytoblast sera in all leukocyte samples of patients with hemocytoblastoses. This antigen was rare in leukocytes of patients with chronic lymphatic and myeloid leukemia and was absent from leukocytes of healthy donors. Insignificant amounts of this antigen were found in normal spleens and lymph nodes.—J. K.


The Ph1 chromosome was present in the peripheral blood of two children with chronic myeloid leukemia who were 3 and 10 years old when the diagnosis was made. Chromosome damage, other than the Ph1 chromosome, was detected in one case prior to chemotherapy and radiotherapy. In the other case, a Ph1-positive aneuploid cell line with 48 chromosomes became established.—B. R.


The new type of viral murine leukemia, derived from a leukemia originally induced by X-rays, had, after repeated passage as cell-free filtrates, a short period of latency (palpable spleen 4 weeks after inoculation) and a high incidence (100 percent). It could be transmitted even to adult C57 Black, CBA, A, and BALB/c mice and to rats, but not to hamsters. This virus could be compared with the most potent mouse leukemia viruses.—L. D.


An analysis of 312 patients with leukemia was presented. When comparing the results of treatment by irradiation alone with those obtained by irradiation combined with chemotherapy, there was no sig-
significant difference in regard to the length of survival.—L. D.


Clinical material (196 patients) was subjected to analysis from the standpoint of therapeutic results and the course of the disease. Correlations were observed between therapeutic effects, age, sex and stage of the disease. Combined radio- and chemotherapy, when compared with radiotherapy alone, brought about better results with a statistically significant increase in 3 year survival rate, but not in 5 year survival. The addition of chemotherapy to radiotherapy did not influence the localization of new foci within 24 hours or 3 weeks after birth. —L. D.


One half of 7 patients with myeloid metaplasia, granulocytic and erythroblastic leukemia, of 8 with polycythemia and of 14 with lymphocytic leukemia had increased blood sugars after 100 Gm. of oral glucose. Six patients had clinical diabetes. Similar figures were found for 3 patients with Hodgkin’s sarcoma and 5 with reticulum cell sarcoma, but not in 9 with myeloma or 4 with other tumors. Of 47 controls with cardiovascular, infectious and hematologic disease, 4 had high blood sugars. (Abstracter’s comment: Disturbed metabolism of nutrients, amino and folic acids, iron, and now sugar in patients with malignant disease may explain their wasting.)—P. G. R.


Immune responses and changes in gamma globulins were studied in rabbits thymectomized and/or appendectomized within 24 hours or 3 weeks after birth and then X-irradiated. Thymectomized-irradiated rabbits had a reduced capacity for the production of humoral antibodies to sheep red blood cells and conalbumin, but not to Salmonella typhi. These findings indicated that thymectomy produced only limited impairment of immunity. Antibody levels following primary stimulation with Salmonella typhi, sheep red blood cells and conalbumin usually were much lower in appendectomy-irradiated and thymectomized-appendectomy rabbits than in irradiated controls. The cellular immune response, development of delayed tuberculin hypersensitivity and skin homograft immunity were impaired in thymectomized and thymectomized-appendectomy, but not in appendectomy, rabbits. The immune response was more strongly suppressed in thymectomized-appendectomy rabbits with very low serum gamma globulins, than in irradiated controls. These narrow bands were seen more frequently in thymectomized-appendectomy rabbits than in irradiated controls. These observations suggested that disturbed gamma globulin synthesis was caused by removal of the rabbit’s thymus and appendix.—K. F.


The cortex of lymph nodes was found to be composed of three parts: marginal islets of small lymphocytes, including primary nodules, the lymphoid matrix, and the area around the postcapillary veins. Following appendectomy and irradiation, lymphocytes were reduced in number in the matrix and medullary cords, but not in the marginal islets or around the postcapillary veins.
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The reticulo-lymphocytic system, composed mainly of lymphogonia and large basophilic lymphocytes, decreased its reactivity to antigens in the appendectomized and irradiated group and germinal centers were either unrecognizable or atrophic. In thymectomized irradiated rabbits, medium-sized and small lymphocytes were almost completely absent from the lymph nodes, especially around the postcapillary venules. The germinal centers were well developed in size and number and the reticulo-lymphocytic system was able to proliferate after the injection of antigens. Lymphocytes can, therefore, be divided into at least two groups: cells of the reticulo-lymphocytic system and small or medium-sized lymphocytes.—K. F.


Hemagglutinin-responding capacity to sheep erythrocyte antigen in aged mice was approximately 10 percent of the response in normal young adult mice. This reduction in primary antibody-forming capacity may be due to a loss of progenitor cells with aging or to a decrease in the number of uncommitted immunologically competent cells because of preoccupation with other antigens. Results of splenectomy suggested that the spleen was the major source of immunological competence in aged animals.—T. E. B.


Polythemic and phenylhydrazine-hemolytic anemia mouse bone marrow cells were incubated with H³-thymidine. In the former, a high percentage of granulocytic cells was observed and in the latter the predominant cells were erythroid. DNA was separated and H³-thymidine incorporation was measured. DNA synthesis per immature cell was identical in the two groups of mice, indicating a similar DNA synthetic activity of erythroid and granulocyte precursors.—K. F.


Lymphocytes from peripheral blood incubated with phytohemagglutinin (PHA) undergo blast transformation and cell division. When chlorpromazine was added at the start, partial suppression of cell division and blast transformation was observed. There was less inhibition when chlorpromazine was added after 24 hours. Patients who had had agranulocytosis showed depression of blast transformation when chlorpromazine was added to their PHA-stimulated peripheral blood. Addition of chlorpromazine to cultures delayed hourly accumulation of mitoses following addition of colchicine to 70 hour cultures in both drug-sensitive and random people.—T. E. B.


Peripheral lymphocytes, obtained from patients with cirrhosis and normal blood donors, were cultivated in the presence of liver antigen. There was no difference in the number of blastoid cells formed. Minimal signs of stimulation, found in three patients with cirrhosis, were occasional mitoses. No sign of stimulation was discovered in control cultures without antigen.—L. D.


Phytohemagglutinin (PHA), poke weed mitogen and streptolysin-O, all agents capable of stimulating mitosis in human lymphocytes in vitro, also stimulated the production of an antiviral substance with biological, chemical and physical properties identical to those of human interferon. Upon removal of PHA from human lymphocyte cultures, interferon production ceased within 2 hours.
Readdition of PHA resulted in reinitiation of interferon production. — T. E. B.


Homologous kidney transplantation could be performed successfully by treating dogs with anti-dog lymphocyte anti-serum from horses. Antibody production against sheep erythrocytes, as well as against horse serum proteins, was diminished. No reduction of immunoglobulins, as measured by immunoelectrophoresis or Ouchterlony technics, could be detected. Resistance against bacterial infections was not reduced. — K. B.


Data are presented which indicate that the reaction of sensitized lymph node cells with antigen results in a cytotoxic effect on innocent bystander cells. The effect is specific, has a delayed time course, and does not depend on a genetic difference between the two types of cells. The bystander cells need not be present during the primary reaction of sensitized lymphocytes with antigen. These findings provide a possible explanation for cell damage that occurs, apparently nonspecifically, in the vicinity of reacting cells in the various types of delayed hypersensitivity reactions. — T. E. B.


Histologic and histochemical studies with the α-naphthol acetate esterase reaction were performed in 33 acutely inflamed appendices removed at operation. In comparison with non-inflamed specimens, a significant increase in intravascular monocytes was found. Migration of monocytes through the wall of small vessels and morphologic and histochemical similarities between blood monocytes and inflammatory macrophages were observed. The authors concluded that inflammatory exudate macrophages developed almost exclusively from blood monocytes. — K. B.


Oral administration of ethanol depressed the clearance of micro-aggregated albumin in rats. When 0.5 ml. of 100 percent ethyl alcohol was given, the half-clearance time in the alcohol-treated rats averaged 40 percent longer than in paired glucose-treated controls. No relation was demonstrated between the degree of depression of reticuloendothelial function and the blood alcohol level. The reason for ethanol-induced delay in phagocytosis was not clear. The possible significance of these findings as an explanation for the increased susceptibility of alcoholics to infection was discussed. — T. E. B.

HEMOSTASIS


A 12 year old girl with a short history of a bruising tendency had a prolonged bleeding time, inconstantly abnormal prothrombin consumption, and platelets which failed to aggregate with collagen or adhere to collagen fibers. Release of platelet ADP by collagen was normal, as were other tests of platelet and coagulation function. — A. L. B.


Platelet adhesiveness, aggregating ability, plasma thromboplastic activity of platelet factor 3, platelet factor 4, retraction and thromboelastographic records in one day and five day old neonates were studied. There was a reduction in the percentage of adhesive platelets in both one day (9.005
percent) and five day old neonates (9.805 percent) as compared to normal children (10.905 percent). Platelet adhesiveness was determined by filtering citrated blood through a standard circular filter of glass wool. There was decreased aggregation of platelets in both groups, characterized by limited clumping in normal plasma and the retardation of formation of small clumps in plasma after recalcification. Thirty or 40 minutes after recalcification, many platelets persisted with balloon or signet ring forms. Fibrin fibers occurred later and the network formed was poorer and looser than in controls. Thromboplastic activity was significantly lower on the first than on the fifth day of life. In the thromboelastogram, the "r" value gradually increased. The mean value for "ma" was subnormal on the first day, gradually increasing to attain normal values on the fourth day. No differences were found in plasma retraction and anti-heparin activity (platelet factor 4).—L. D.


In 11 adults with idiopathic thrombocytopenic purpura, Actinomycin C alone was used for treatment, beginning with 0.2 mg. daily intravenously. Remission occurred in all patients after an average of 13 days and an average dose of 2.2 mg. The optimum effect was obtained after 52 days with an average dose of 8.1 mg.—K. B.


Preliminary observations in 18 oarsmen and 18 untrained subjects at rest, after a work load and during recovery under laboratory conditions showed that resting thromboocyte counts underwent no changes during systematic sports training. The thromboocyte count did not change significantly in the course of the all-year sports training. It was not affected by variations in the state of physical performance and preparedness obtained by training.—L. D.


Platelet antibodies were found in 17 of 53 sera from females with trophoblastic disease, mostly hydatid mole and choriocarcinoma. In 13 sera, there were incomplete antibodies, in 3, complete thromboagglutinins and in 1, thrombolsins. These antibodies were mostly monospecific. In the course of time, they often disappeared. Only one woman with antibodies had received 4 transfusions.—L. D.


In 17 patients, 10–61 years old, with thrombocytosis primary or secondary to splenectomy, mechanical platelet resistance was studied by means of the method of Storti. Increased platelet resistance was found.—P. d. N.


In 10 normal subjects, the isoenzymes I, II, III and IV (cellulose acetate, platelet extracts) were observed. In subjects with secondary thrombocytosis, 4 had the same pattern as normals and 4 had only the I, II and III isoenzymes.—P. d. N.


A case of hereditary Factor VII deficiency was described. An extensive family study confirmed previous reports that the disorder was transmitted by an autosomal gene which produces severe deficiency in the homozygote and partial deficiency in the heterozygote.—Z. R.

A circulating anticoagulant in hemophilia A was described in one case of a series of 33 cases of hemophilia A and 6 cases of hemophilia B. The modified thromboplastin generation test and the recalcification times of mixtures with normal plasma were employed for its demonstration.—L. D.


The growth of fibroblasts in vitro was enhanced by normal plasma and by plasma from a patient with a hemorrhagic disease due to the lack of Factor XIII. This finding should rule out the role of Factor XIII in determining the anomalies of scars in patients with this deficiency.—P. d. N.


Toxins from the Brazilian rattle snake, Botrops jararaca, prepared under the names Botropase and Reptilase, have a thrombin effect with a direct action on fibrinogen. The effect is rather weak and in Botropase corresponds to 0.5 U of the thrombin of Topostasin. The thrombin of this venom is not inactivated by heparin or progressive antithrombin. The fibrin clot is more easily dissolved in 30 percent urea than the clot produced by thrombin. Platelet stickiness and spreading on a slide does not change with different concentrations of Botropase. Rotated plasma with Botropase added shows increased platelet agglomeration. These changes cannot be demonstrated after intravenous injection of Botropase. In 7 of 16 thrombocytopenias and 2 of 7 hemophiliacs, partial clinical improvement has been observed. It is not certain whether this response is the result of Botropase treatment or is spontaneous remission.—L. D.


Thirty cases of idiopathic hyperlipemia were investigated. Prolongation of euglobulin fibrinolysis and decreased amounts of free plasmin were found. Plasminogen and immediately reacting antiplasmin were normal. Slow reacting antiplasmin was significantly reduced. There was no correlation between the level of lipids and fibrinolytic changes.—L. D.


Fibrinogenolysis and fibrinolysis were induced by adding streptokinase, urokinase or plasmin to fibrinogen solutions with or without thrombin. The influence of the products of lysis on the thrombin clotting time of normal human plasma, immunoelectrophoresis and simple radial immunodiffusion by the technic of Mancini et al were employed. In general, fibrinogenolysis products were labile, while fibrinolysis products were stable to heating to 56 C for 15 minutes. The prolongation effect of fibrinogenolysis products on thrombin clotting time completely disappeared after heating. Heated fibrinolysis products, however, showed the prolongation effect. Fraction D derived from fibrinogen disappeared after heating, while fraction D from fibrin and fraction E from both fibrinogen and fibrin were stable. On simple radial immunodiffusion, the diameter of the immune circle was markedly decreased with heated fibrinogenolysis products, but not with heated fibrinolysis products. The difference between fibrinogenolysis and fibrinolysis products might, therefore, depend on the presence or absence of thrombin in the digestion mixture.—K. F.