ERYTHROCYTES


A Filipino family is described in which the proband, his father, four of his six brothers and sisters, and a paternal aunt had abnormal-looking red cells, without anemia or evidence of hemolysis. The erythrocytes were elliptical, and many had slitlike areas of central pallor. The most distinctive finding was the presence within some of the elliptocytes of two or three transverse slits of decreased density, which the authors suggest could have resulted from multiple infoldings of the cell membrane. Increased erythrocyte autohemolysis after 48 hr of incubation, preventable by the addition of glucose, was seen in affected family members. Intraerythrocyte sodium concentration was normal; intracellular potassium was moderately decreased. A 60% increase in red cell glucose consumption was found, and incubation of washed cells in a glucose-free medium caused increased sodium gain and potassium loss. Despite increased cation permeability, erythrocyte osmotic fragility was decreased, and a lesser than normal degree of cell swelling in hypotonic saline was noted.

—J.B.S.


Erythrocytes of infants weighing less than 1200 g at birth contain increased total lipids, lipid phosphorus, and cholesterol, when compared with full-term infants. These increases suggest a continuum, with low birth weight infants having the greatest erythrocyte lipid content, full-term infants less, and adults the least. The increased lipid per cell may be accounted for by a greater cell volume as in the smaller infants. The lipid composition of the premature infants was similar to that of term babies.
ABSTRACTS

infants. Both groups of infants demonstrated increased amounts of phosphatidyl serine and phosphatidyl inositol in comparison to red cells of normal adults. In comparison to term infants, the red cells of the smallest premature infants demonstrated increased levels of phosphatidyl choline. The significance of the differences in phospholipid fractionation is not known.

—J.B.S.


A high incidence of chromosome breakage and endoreduplicated polyploidy was found in cultures of peripheral blood from three brothers with Fanconi's anemia. In contrast, a normal cytogenetic pattern was found in the bone marrow cells studied by a direct method. No gross abnormalities were disclosed in bone marrow or peripheral blood cells from both parents. In the children, chromosome breaks were randomly distributed both among and within the chromosomes. It is assumed that a susceptibility to chromosome breakage is present in patients with Fanconi's anemia and is manifested when cells are exposed to culture conditions.—M.J.


The principle dietary and circulating folate compound, 5-methyltetrahydrofolic acid, was absorbed in unaltered form equally well in all segments of rat small intestine. There was no accumulation against a concentration gradient and no alteration in transfer by metabolic inhibitors. These observations indicate that 5-MTHF transfer across the intestine is by passive diffusion.—F.A.K.


Biologically active folate appeared in the mesenteric vein following instillation of increasingly long, glutamyl chain lengths from pteroylemono- to heptaglutamate. The absorption rate appeared to be inversely proportional to the length of the gamma-glutamyl side chain. Evidence was obtained indicating that the mono and diglutamate forms can cross the mucosal barrier directly and that reduction and methylation are not necessary for their absorption.—F.A.K.


Equilibrium of spinal fluid tritium levels with those in the serum was rapid (3 hr) after intravenous administration of reduced folates but not of folic acid. 5-Methyltetrahydrofolate was taken up preferentially by the spinal fluid, and other folate congeners were converted into this compound before uptake. Diphenyhydantoin (DPH) did not alter uptake into the spinal fluid. Abstractor's comment: Observations reported in Trans. Amer. Neurol. Assoc. 95:196, 1970 showed depression of CSF but not of brain folate concentrations after long-term administration of DPH to cats.—F.A.K.


Nearly 90% of an oral dose of radioisotopically labeled formyltetrahydrofolate was absorbed. Peak serum levels of the isotopic labels occurred at 60 min, and the principle serum form was shown by chromatographic analysis to be 5-methyltetrahydrofolate.
ABSTRACTS


Two infants, who presented at 3 and 5 wk of age with a megaloblastic anemia, were found to have normal serum levels of vitamin B12 but a deficiency of the B12 transport protein transcobalamin II. Complete hematologic remission was achieved by weekly parenteral administration of massive amounts of B12. The finding of reduced absorption of B12 intrinsic factor in one sibling suggests that transcobalamin II plays a role in the absorption of this vitamin.—F.A.K.


Mean serum and red cell folate concentrations were lower, and urinary FIGLU excretion was higher among 176 women receiving combination-type oral contraceptives than in 140 normal control subjects. Serum folate values fell progressively with continuation of contraceptive intake, and all three determinations improved within 3 mo after stopping the pill. Abstracter’s comment: Similar results concerning serum folate levels were also described Amer. J. Clin. Nutr. 24:603, 1971.—F.A.K.


A six-yr-old boy with pancytopenia, multiple skeletal and renal anomalies, increased chromosomal breakage, increased fetal hemoglobin levels, and marked growth retardation is described. Endocrinologic studies revealed normal thyroid function, apparently normal ACTH and TSH production, but marked deficiency of growth hormone. Although short stature is characteristic of Fanconi’s anemia, this represents the first instance of its association with isolated growth hormone deficiency.—J.B.S.


A new observation of Hb-Porto Alegre is reported. This rare abnormal hemoglobin, which manifests in the fresh hemolysates as a “fast” component, was identified in two of three members of an Argentinian family studied: the father (53-yr-old, white, Spanish origin, clinically and hematologically normal, normal Hb A2), in whom it was present in the heterozygous state. The abnormal Hb was present in the son in association with beta thalassemia (20-yr-old; clinically normal with normal hemoglobin concentration, erythrocytosis, and the features of a thalassemic trait, including elevated Hb A2). The mother (47-yr-old, white, Italian ancestry) had the characteristics of a typical beta-A2 thalassemic trait (elevation of Hb-A2 similar to the son). Hb-Porto Alegre was identified by fingerprinting and subsequent amino acid analysis of the carboxymethylated globin, which showed the structure of the abnormal fraction to be 2 29 (A6) ser cys, as previously described. This is the second family in which Hb-Porto Alegre has been identified and the first instance of its association with beta thalassemia. Lack of interaction with the latter is stressed.—E.S.S.

Sideroblastosis as an Indicator of Disturbed Iron Metabolism in Hypoplastic and Aplastic Anemia. V. V. Felisor, N. C. Turbina, and Yu. G. Soboleva. Central Institute of Hematology and Blood Trans-
ABSTRACTS

An increase in bone marrow sideroblastosis was noted in 23 patients with hypoplastic or aplastic anemia, and this appears to be a useful indicator of abnormal iron metabolism in this disease and perhaps in other disorders.—J.V.

LEUKOCYTES


In 1130 patients with lymphoma seen at the Stanford Medical Center between 1959 and 1969, there were 129 cases of Herpes zoster-Varicella infections, an incidence of 11.4%, including 21 disseminated cases. Ninety-one of the 592 patients with Hodgkin’s disease (15.4%) had Herpes zoster-Varicella infections at some time during the course of their disease. These cases are analyzed according to age, clinical status, stage of lymphoma when infection occurred, and for survival after either localized or disseminated zoster. In addition, the effects of antitumor chemotherapy, including combination chemotherapy, are presented. A significantly increased incidence of herpes zoster-varicella infections was found in the 150 patients with Hodgkin’s disease who underwent splenectomy either as part of their initial evaluation and staging or during the course of their disease, although survival of this group was apparently unchanged by the infectious complication of their lymphoma.—J.E.U.


Liver biopsy sections of 127 patients (89 without previous therapy) were classified for the presence of Reed-Sternberg cells and other cellular infiltrates and abnormalities. These histologic findings were then correlated with the clinical and laboratory findings in each patient. In seven of eight biopsies with atypical reticulum cell infiltrates, Reed-Sternberg cells could be found if sufficient sections were examined. All other cell infiltrates and pathologic changes showed no correlation with Reed-Sternberg cells or clinical findings. The frequency of positive biopsies was approximately doubled by performing laparotomy or peritoneoscopy after a percutaneous biopsy was negative. Clinical examination of the liver and liver function tests, including alkaline response. During remission he reacted to a mumps skin test, SKSD antigen, DNFB, a skin homograft, and to PHA. Following relapse, these cellular responses were again absent or very subnormal. From these studies the authors infer that the immunologic defect seen in Hodgkin’s disease is the result of the disease, and that the disease is not, in and of itself, the result of an immune disorder.—J.B.S.


Twin brothers, vaccinated with BCG in the newborn nursery, died at ages 4 1/2 and 9 mo of multiple infections. At postmortem widespread acid-fast infection, with Langhans cells, and caseous necrosis, was found. The nature of the underlying disorder was unknown at the time, but a nephew was later found to have CGD, and the mother of the twins had abnormal granulocyte bactericidal activity and decreased reduction of tetrazolium.—J.B.S.
ABSTRACTS

165,000 and 10,600 for RNA and control of ence was significant with maximal values of 845 X 10\(^6\) cells in the RNA-treated animals compared to 254 mg and 265 X 10\(^6\) cells in the controls. As for the WBC, the differences were observed on days 4 and 7, and in patients with high initial white blood cell count. No differences were found between acute lymphatic and acute myelogenous leukemia patients.—E.S.S.


The previous observation that BALB mice injected with syngeneic normal RNA and subsequently challenged with LD50 inocula of a syngeneic leukemic line, H110, had 100% mortality rate compared to 52% in the control group led to a study of the effect of normal RNA on the dynamics of the splenic colonization of leukemic cells H110, in animals previously treated with this RNA. A group of 24 BALB mice received 1 mg of normal liver RNA i.p. and 5 days later were challenged with 100 leukemic cells, H110, along with a control group of 24 animals. The mice were bled and sacrificed in groups of three on days 4, 7, 10 and 14. The white blood cell counts, spleen weights and splenic cell counts were determined. No differences were observed on days 4 and 7; on day 10 the spleen of the RNA-treated animals averaged 419 mg with a cell count of 416 X 10\(^6\) as compared to 150 mg and 142 X 10\(^6\) in the control group. Maximal differences were observed on day 14 with an average of 670 mg of spleen weight and 845 X 10\(^6\) cells in the RNA-treated animals as compared to 254 mg and 265 X 10\(^6\) cells in the controls. As for the WBC, the difference was significant with maximal values of 165,000 and 10,600 for RNA and control groups, respectively, on day 14. As for the survival rate, all treated animals died on day 14 while the controls died within 21 days. These results suggest that normal RNA has an immunodepressing effect which would favor the proliferation of syngeneic leukemic cells.—E.S.S.


By means of a study performed in 117 acute lymphatic and 103 acute myelogenous leukemia patients, it was concluded that the incidence of central nervous system infiltration is directly related to the length of survival; the risk being higher in children and in patients with high initial white blood cell count. No differences were found between acute lymphatic and acute myelogenous leukemia patients.—E.S.S.


Thirty-five patients with advanced lymphosarcoma (32 without previous therapy) were treated with intensive 5 day courses of cyclophosphamide and prednisone, with vincristine given on the first day; this was repeated every 3 wk until complete remission was attained or tumor resistance appeared. There were 20 (57%) complete and 12 (34%) partial responses. Eighty-nine per cent of the complete remissions lasted more than 1 yr. Of all 35 patients, 79% lived more than 1 yr. Survival of complete responders was significantly better than that of partial responders. Patients with more extensive disease had significantly shorter survival. Toxicity, chiefly leukopenia, was tolerable, reversible, and not cumulative. In inducing frequent and long-lasting complete remissions this regimen (CVP) appears superior to those previously reported.—J.E.U.

HEMOSTASIS


Phosphatase and sulfobromophthalein were of little value in predicting positive findings; abnormalities in these tests may be indicators of a nonspecific hepatic reaction to the presence of Hodgkin's disease elsewhere in the patient. However, patients with splenomegaly had a high risk of liver involvement.—J.E.U.
**ABSTRACTS**


A thrombin-like enzyme was purified to homogeneity from the venom of *Crotalus adamanteus* (Eastern diamond-back rattlesnake). The enzyme is a glycoprotein with a molecular weight of 32,700 and acts directly on fibrinogen without affecting any other proteins involved in blood coagulation. Both clotting and esterase activity were inhibited by diisopropyl phosphorfluoridate, showing that the enzyme, like thrombin, is a serine esterase.-H.J.W.


The authors transplanted livers, spleens, and kidneys into hemophilic and normal dogs. Transplantation of a normal liver into a hemophilic dog resulted in a complete and long-lasting cure. Splenic transplantation resulted in only a temporary increase in AHF activity. The authors conclude that the liver is the major site of AHF synthesis in the dog and that the spleen may be a storage site of AHF. Since normal dogs that had received livers from hemophilic animals still produced some AHF, extrahepatic sites of AHF synthesis also exist.—H.J.W.


The authors describe various types of hemorrhagic defects developing as a result of operations on the open heart with the use of extracorporeal circulation. The causative factors are discussed which cause an increased frequency of pathological bleeding many times higher than in other surgical cases. The most important are, beyond doubt, activation of intravascular coagulation and fibrinolysis because of their frequency and severity as well. Interconnection between these two pathological states and the differential diagnostic methods are analyzed. It is stressed that during extracorporeal circulation the diagnosis is far more difficult than in other clinical conditions. The prophylactic methods described in the literature as well as those based on authors’ own experience are discussed.—M.K.


The growth and embolization of a thrombus can be analyzed by the interaction of two antagonistic hemodynamic forces: a cohesive force (Fc) that binds the different elements of the thrombus within themselves and to the vascular wall, and a dispersive force (Fd) given by the strength of the blood flow. These forces were studied in a Teflon tubing extension of the common carotid artery of rats. Coagulation time (CT) was modified by i.v. heparin. Thrombi were induced by connective tissue extract (CTE) or by altering the flow (AF) in sites of different cross-sectional areas (A) with diameters of 40–900 μ. Differential pressure (△P) was measured through micropipettes. Fd = △PA; Fc was considered as the mean of two Fd values where in one an occlusive thrombus can form and not in the other. The results of the Fc of five experiments with different heparin concentrations and thrombotic stimuli were: (1) with no heparin, CT of 2–10 min, and CTE of 100%, Fc was > 464 dynes; (2) with no heparin, CT of 2–10 min, and CTE of 50%, Fc was 384 dynes; (3) with heparin 100 U/kg, CT of 3–6 hr, and CTE 50%, Fc was 197 dynes; (4) with heparin 100 U/kg and CT of 3–6 hr, AF:Fc was 84 dynes; (5) with heparin 500 U/kg, CT > 48 hr, AF:Fc...
was 10.4 dynes. Increasing heparin to 1500 U/kg, no further decrease in Fc was found. In 40% of the cases a further decrease in Fc was observed with doses of heparin of 3000 U/kg. The amount of fibrin surrounding platelet aggregates observed by electron microscopy was related directly to CTE concentration and inversely to CT. Only when CT was > 48 hr, fibrin was absent in the thrombus. In spite of "therapeutic" levels of heparinization, Fc remained high. The antithrombotic effectiveness of heparin is determined by vessel diameter and differential pressure. This concept could explain certain limitations and failures of therapy.—E.S.S.


Platelet acid phosphatases were investigated in 48 normal samples of platelet rich plasma. Platelets were lysed with Triton X-100, digitonin, freezing and thawing and sonication. ADP, adrenaline, collagen, thrombin and serotonin were used as aggregating agents in both stirred and unstirred systems. High speed centrifugation allowed to differentiate between release and availability. The most effective of the lytic agents was Triton X-100 which also released most of the activity in the soluble fraction. Collagen was the only aggregating agent which released small amounts of acid phosphatase in the extracellular milieu. However, platelet-bound acid phosphatase became activated under the influence of all aggregating agents in a fashion similar to PF3 (platelet factor 3). Further, the strong similarity between acid phosphatase and PF3 activation at several periods of time, under the influence of the various aggregating agents, suggests that they are activated or unmasked on the platelet membranes or other structures during platelet aggregation, following specific patterns of activation different for each aggregating agent. No increase in activity was detected when either unstirred incubation with the aggregating agents or stirring with isotonic saline were performed.—E.S.S.


In thrombasthenia, group I, in which platelet fibrinogen is low and ATP normal, the platelet sialic acid was slightly decreased while l-fucose was increased. In different, acquired megakaryocytopenies, mainly in preleukemic states in which heavy platelets are not found, the authors noticed a decrease of platelet sialic acid which is half the normal range.—J.C.


The authors describe a factor in normal plasma which inhibits collagen-induced platelet aggregation. The factor has a molecular weight in the range of 330,000 is destroyed at 56°C, and migrates with the alpha-globulins on starch block electrophoresis at pH 8.6. The authors suggest that the inhibitory effects observed were owing to the adsorption to collagen of a plasma protein and that plasma proteins may regulate platelet adhesion to collagen and other vessel wall materials.—H.J.W.


Evaluation of 20 children with chronic ITP revealed diffusely abnormal electroencephalograms in ten. Six of these ten demonstrated the behavior pattern characteristic of minimal cerebral dysfunction—hyperactivity, short attention span, poor memory, and visuomotor deficiency. The authors conjecture that chronic ITP may
be associated with multiple minute capillary bleeds which produce cerebral damage. Not discussed or evaluated is the possibility that such damage occurred during the acute phase, when the children had widespread cutaneous and mucous membrane bleeding.—J.B.S.

IMMUNOHEMATOLOGY


The distribution of HL-A antigens has been compared in 112 patients with Hodgkin’s disease with that in 122 controls. There is an increased frequency of A1, A5, and A8 in the Hodgkin’s disease population taken as a whole. There is a frequent association of A1 and A8 with the mixed-cellularity and lymphocytic-predominance types of disease. In contrast, the frequencies of these specificities are not increased in patients with nodular sclerosis. Thus, the excess frequencies of A1 and A8 present in the whole Hodgkin’s disease population are accounted for entirely by patients having the mixed-cellularity or lymphocytic-predominance patterns. Patients with nodular sclerosis have an increased frequency of A5 only. These results suggest that the histological pattern of disease may be influenced by factors related to the HL-A phenotype of the host. A decreased frequency of A3, as compared with the control population, is found only in patients with recent onset of disease. A8 is increased in frequency only in those having the disease for more than 5 yr. The frequencies of A1 and of A5 are high regardless of the duration of disease.—J.E.U.


Sarcoma 180 (S180) is a nonspecific murine tumor which kills 100% of BALB mice. In spite of this, by means of repeated excisions and reimplantations it has been possible to obtain BALB mice immune to S180. Some of these animals developed leukemia, termed L180; two cell lines are maintained within the strain, L370-II and L370-III. Previous experiments confirmed cross immunity between S180 and L370-II; mice bearing S180 showed inhibition of migration of peritoneal cells (IMCP) in the presence of partially purified saline extracts (ESP) of both S180 and L370-II. Consequently it was considered worthwhile to attempt isolation of the cellular fraction responsible for the antigenic effect and to study the presence of cross-reaction between S180 and BALB leukemias of different origin. Cell membrane separation was carried out by differential centrifugation and controlled by ultramicroscopy. These extracts led to 39% IMCP in mice bearing S180. Extracts were also prepared from spleen and lymph nodes of mice bearing different leukemias which was spontaneous in the BALB, AKR and A strains and L180, induced by S180, in the BALB and C57/B1 strains. All these leukemic extracts gave positive IMCP in mice bearing S180 except those of strain A and similar extracts obtained from normal tissues or normal cell membranes. The results suggest that there is cross antigenicity between S180 and the leukemias developing in the BALB, AKR and C57/B1 strains, which may be related to the presence of the G antigen.—E.S.S.


Ribonucleic acid (RNA) extracted from normal liver of inbred G rats was added to syngeneic peripheral lymphocyte cultures both before and after stimulation with phytohemagglutinin, P-Difco (PHA). The cultures were carried out in 0.22 μ Millipore chambers introduced into the peritoneal cavity of syngeneic rats; these were removed 3 days later, opened up, slides were prepared and stained with May Grünwald-Giemsa. Both blastic transformation
and mitotic index were determined. All the experiments were carried out simultaneously on the same pool of rat peripheral lymphocytes (L). The following groups were studied: (1) RNA (a) 20 µg, (b) 100 µg, (c) 20 µg previously incubated with 2 µg RNase, added 5 min after PHA, and (d) PHA alone, as control; (2) the same RNA groups a, b, and c but adding the RNA 75 min before PHA, (d) 20 µg RNA (e) 100 µg RNA, without PHA, as controls. It was observed that when either 20 or 100 µg RNA were added to lymphocyte cultures 5 min after PHA, blast transformation was increased to 30%; when 20 µg RNA were added 75 min before PHA, the results were similar, whereas 100 µg gave the highest values, 50%, as compared to 20% in the PHA controls and 5% in the RNA controls. The mitotic index ranged from 0 to 4%. The effect of RNA was not inhibited by RNase. It was concluded that normal synthetic RNA has a stimulating effect on PHA-pretreated lymphocyte cultures. This does not seem to be an additive response since RNA alone has a very poor effect. RNA might, however, modify cell biology in such a way that PHA transformation would be favored. The fact that RNase does not influence the RNA effect indicates that degradation products may also be active.—E.S.S.

MISCELLANEOUS


Although blood glucose concentrations rose significantly in newborns, following exchange transfusion with ACD blood, the concomitant rise in serum insulin levels led to significant hypoglycemia 1–3 hr post-exchange in 10 of 13 infants. The authors caution that apnea or convulsions due to hypoglycemia may occur following exchange transfusion with ACD blood and suggest consideration of the routine administration of parenteral glucose for several hours posttransfusion.—J.B.S.


Of 66 infants who received intrauterine transfusions for severe erythroblastosis fetalis, 24 survived the neonatal period. Serial follow-up studies between 1 and 5 yr of age revealed that one infant with neonatal hypoxia and acidosis had severe CNS damage; another had a speech handicap, and a third suffered hearing loss, which may have been related to excessive kanamycin therapy. The other 21 fell into the normal range for the parameters studied. Of interest was the finding that improvement in achievement levels frequently continued through the age of 3 yr.—J.B.S.

ABSTRACTS


Over a 24-mo period, among 419 patients with cancer, zoster occurred in 25% of patients with Hodgkin’s disease, 8.7% of other lymphoma patients, but in only 1.2% of patients with acute leukemia and 1.8% of patients with solid tumors. A disseminated or generalized form of zoster occurred in 12 of the 37 patients with zoster; these patients were more frequently DNCB-negative, but they were not necessarily receiving continuing cancer chemotherapy. Localization of zoster was frequently related to a site of prior radiation therapy. Recurrence of zoster occurred in eight patients. Patients with advanced Hodgkin’s disease, cutaneous anergy, and recent nodal radiotherapy were inordinately predisposed to zoster. Absent varicella-zoster complement fixation titers in exposed patients with lymphoma but not leukemia also predisposed to zoster development. Zoster was an exogenously acquired reinfection in many patients, with a prolonged incubation period. No exposed staff member developed clinical infection, and only two had an antibody rise.—J.E.U.


Determinations of plasma ceruloplasmin were made in 127 patients with various clinical conditions. In cirrhosis, ceruloplasmin was increased in 60 of 92 patients; there was a correlation between these levels and the extent, severity, and activity of the hepatic process; the increase also correlated with the degree of anemia. Ceruloplasmin levels were reduced in posthemorrhagic hypochromic anemia and increased in active rheumatic disease despite an anemia.—J.V.


In preserving bone marrow in PVP contact between cells and PVP solutions should not exceed 30 min before freezing; otherwise cells will shrink; this effect is also seen on incubating cells in PVP solution. Autologous marrow, preserved in this way, was returned to patients with advanced malignant disease after high doses of cytotoxic drugs. The results suggested that preservation by this technique had been satisfactory.—J.V.


In a study of 51 patients with severe trauma and shock, death from complications was due to acute renal or pulmonary insufficiency in 45.9%. Acute renal insufficiency was always due to prolonged hypotension or lengthy periods of peripheral vascular failure following unresored blood loss. When hypotension or hypovolemia was corrected the patient recovered. Acute respiratory failure was characterized by altered hemodynamics and, later, by ensuing inflammatory disease. Hypercoagulation was a feature and the use of direct-acting anticoagulants (heparin, fibrinolyisin) with artificial respiration was of great value; artificial respiration alone was only of temporary benefit. These changes are considered the result of disturbed microcirculation during shock; treatment by direct anticoagulants should be a routine procedure in the management of trauma and shock.—J.V.