ERYTHROCYTES


In the population of the Skolt Lapps in Finland, a significant frequency of low red cell glutathione reductase activity was found. There was no indication of an altered enzyme. Half saturation constants of GSSG and FAD were normal, as was the capability of the enzyme to be stimulated by FAD. The deficiency can be attributed to riboflavin deficiency, since the intake of this vitamin with the diet is low in this population. It is interesting that NADH-dependent methemoglobin reductase, which contains FAD, was not lowered in the glutathione reductase-deficient subjects. - K.B.

Hemolytic Anemia in Wilson’s Disease (Hepato-lenticular Degeneration). B. Willms, K. G. Blume, and G. W. Löhr. Departments of Internal Medicine, Universities of Göt- tingen and Freiburg, Germany. Klin Wochenschr 50:995, 1972

Four cases of Wilson’s disease are described in which hemolytic attacks preceded the diagnosis of the disease. It could be shown in vitro that copper ions, in a concentration of \(3 \times 10^{-5} M\), reversibly inhibit the activity of pyruvate kinase. This was true in a cell-free system, as well as in intact red cells. Fructose-1,6-diphosphate was able to correct the copper effect. The authors claim that this effect is a more probable explanation for hemolytic at- tacks in Wilson’s disease than is an inhibition of glucose-6-phosphate dehydrogenase or of glutathione reductase, as reported in recent papers. To be effective on these two enzymes, copper concentration must be higher than can be reached in vivo. - K.B.

Oxymetholone Therapy in Aplastic and Other Refractory Anaemias. P. Sacks, Diana Gale, T. H. Bothwell, and K. Stevens. Department of Medicine and Department of Haema- tology, School of Pathology, University of the Witwatersrand and the South African Institute for Medical Research, Johannes-
The value of oxymetholone in the treatment of refractory anemias was studied in a group of 19 patients. Thirteen patients had aplastic anemia of whom five died soon after treatment had been started. Seven of the remaining eight patients who received oxymetholone for more than 2 mo responded. Three of the responders had previously failed to respond to testosterone. In four patients all blood elements rose, while in three patients a response was noted in the hemoglobin alone. In the latter three cases, bleeding manifestations were greatly reduced, although thrombocytopenia persisted. Six of the seven patients who responded to oxymetholone have remained in remission for 22-33 mo. The drug may also benefit some patients with advanced myelofibrosis requiring regular blood transfusions. In two such patients, transfusion requirements were reduced by oxymetholone therapy. No response was noted in the four cases whose refractory anemia was due to causes other than marrow hypoplasia. Two patients who had received oxymetholone developed acute myeloblastic leukemia, and one developed a lymphocytic lymphoma. The relationship of the drug therapy to the malignant transformation is not clear.-T. H. B.


Electronic determination of red cell MCV is proposed as a valid screening test for thalassemia trait. Using the Model S Coulter Counter, all members of a selected high-frequency population who had β-thalassemia trait by other techniques, including quantitative hemoglobin analysis, had microcytosis with MCV less than 17 cu. False positive results due to iron deficiency occur and depend on affluence and age of populations studied. α-Thalassemia is also detected by this method, although confirming studies are required. The simplicity and availability of such a screening procedure, particularly in high risk populations where thalassemia trait is often not diagnosed or is misdiagnosed, should lend itself to greater use. Evaluation of iron status must be used as an adjunct to this technique where microcytosis is detected.—T. F. N.

Studies of joint effusions occurring in seven patients with sickle cell anemia were carried out. These effusions generally were acute, of short duration, and appeared coincident with painful crises. Common causes of arthropathy were ruled out, and synovial fluid analysis was typical of noninflammatory effusions. Adjacent periosteal elevation and bone infarcts were seen occasionally but were not felt to be etiologically significant. Synovial needle biopsies performed in five of the patients revealed small vessel occlusion, which the authors relate to sickling and postulate as the major pathogenetic factor in such effusions. —T.F.N.


Oral urea, 2 g/kg/day, administered to 11 patients with sickle cell anemia, failed to influence favorably hematologic parameters, painful crises, or hospitalizations. During treatment the average number of hospitalizations doubled. In 8 of the 11 patients no increase in blood urea nitrogen could be achieved with this therapy, whereas at least 30 times the normal blood level is required to inhibit in vitro sickling. —T.F.N.


A new hemoglobin variant with increased oxygen affinity is described in a family with erythrocytosis. Chromatographic and electrophoretic techniques failed to demonstrate the abnormality. Fingerprinting studies disclosed an unexpected sulfur-positive peptide, βT3.

Amino acid analysis and peptide mapping of cyanogen bromide cleavage isolates demonstrated that β20 valine is replaced by methionine in 40% of the chains. The functional effects of a substitution involving a residue on the surface of the peptide chain, not implicated in allosteric interactions, is discussed. This hemoglobinopathy is yet another example of hereditary erythrocytosis. It is of interest in that it was impossible to identify the presence of this abnormality except by oxygen dissociation studies. —T.F.N.


Further studies of a previously reported high oxygen affinity hemoglobin with substitution at the β145 penultimate tyrosine are reported. As in Hb Ranier, with a similar substitution, carriers exhibit erythrocytosis with high oxygen affinity, and isolated hemoglobin preparations demonstrate abnormal heme-heme interaction, decreased DPG effect, diminished Bohr effect, and, presumably, decreased deoxyhemoglobin stability with equilibrium shifted in favor of the oxy form. For unknown reasons, whole blood from carriers of HB Bethesda exhibits a normal Bohr effect, in contrast to carriers of Hb Ranier. Erythropoietic and iron kinetic studies confirmed the erythropoietic dependance of such hemoglobinopathies. The estimated half-normal tissue oxygen supply in these individuals has not yet been shown to be associated with symptomatology. —T.F.N.

LEUKOCYES


In chronic granulocytic leukemia, storage
cells can be seen in the bone marrow resembling the storage cells in Gaucher’s disease. High resolution electron microscopy revealed a lamellar system of 40-A dark layers and 30-A light layers within the stored material, in contrast to the tubular system in Gaucher's disease. Cytochemical studies showed that the material consists of glucolipids. The presence of peroxi-
dase-positive constituents and a positive Prus-
sian blue reaction suggests that the content of these cells derives from phagocytosed granulo-
cytes and erythrocytes or erythroblasts.—K.B.

The Outlook for the Adult With Acute Leu-
kaemia in 1972. F. W. Gun., J. A. Levi, and
P. C. Vincent. Kanematsu Memorial Insti-
tute, Sydney, Australia. Med J Aust
2:403-408, 1972

Forty-one adult patients with acute leukemia were treated by combination therapy of two primary doses of hydroxyurea followed by cytosine arabinoside and thioguanine. Main-
tenance was with intermittent courses of the latter two drugs. The remission rate was 68%, with 46% of the remissions being complete. The median survival in those responding was 44 wk. Four of six patients aged over 60 yr achieved a remission.—A.A.M.

Studies of Muramidase in Haematological Dis-
orders: Serum and Marrow Muramidase in
Leukaemia. J. A. Levi, J. B. Speden, P. C. Vin-
cent, and F. W. Gun.: Medical Research
Department, Sydney Hospital, Sydney, Aus-
tralia. Pathology 5:59, 1973

Bone marrow muramidase concentration in 13 normal individuals was 10.285 μg/ml, and marrow/serum muramidase ratio was 1.5:3:1. In 33 untreated patients with various leukemias, the marrow muramidase levels paralleled those in the blood and reflected the type of leukemia (high in acute myelomonocytic and chronic granulocytic, variable in acute myeloblastic, normal in acute promyelocytic and undifferen-
tiated and chronic lymphocytic, and low in lymphoblastic leukemia). Most leukemias had normal marrow/serum muramidase ratios, but two had excessively high ratios probably because of intramedullary cell death. Serial studies of treated patients showed parallel fluctuations in marrow and serum levels of muramidase in most cases, but in some patients the ratios became temporarily elevated during onset of remissions, either because of rises or of delays in the fall of marrow muramidase levels. Thus, estimations of marrow muramidase levels in leukemia occasionally present information on marrow granulopoiesis not pro-
vided by serum estimations alone.—F.W.G.

Hodgkin’s Disease—A Clinicopathological
Study of 250 Cases With a Five Year Follow-
Up. K. A. Newton, D. H. Mackenzie,
M. F. Spittle, and A. Mikolajczuk. West-
Cancer 27:80, 1973

Both the Rye Conference histologic classi-
fication and the Cross classification were reasonably effective in predicting the prognosis in 250 cases of histologically proven Hodgkin’s disease followed up for at least 5 yr. The over-
all survival was 54%, at 5 yr and 23% at 10 yr
The presence of clinical symptoms adversely affected prognosis. At 5 yr the percentage sur-
vival with different histologic types was:
lymphocytic predominance 69%, nodular scler-
osis 57%, mixed cellularity 41%, and lympho-
cytic depletion 40%.—J. M. B.

Experimental Studies and Clinical Observa-
tions on the Combination Chemotherapy of
Leukemia Using Vincristine and Cytosine
Arabinoside. G. Cardinali, G. Cardinali,
G. Turlontano, and P. Ballatore. Laboratorio
di Ematologia, Instituto di Genetica Medica,
University of Rome, Italy. Haematologica
(Pavia) 57:107, 1972

The antitumor effect of cytosine arabinosi-
de (ARA-C) used alone or in combination
with vincristine (VCR) was studied in C3H
mice carrying the lymphosarcoma 6C3HED.
The percentage of survival was significantly higher in the animals treated with both agents as compared to the animals treated with ARA-C or VCR alone. The effect of the combined treatment with the two compounds was also studied in six cases of acute leukemia and two cases of blastic transformation of chronic
myeloid leukemia. Partial remissions were ob-
tained in four cases of acute leukemia and in
one case of blastic transformation of chronic
myeloid leukemia.—G.L.

Bleomycin, an Antitumor Antibiotic: Clinical
Experience in 274 Patients. A. Yagoda, B.
Mukherji, C. Young, E. Ectuba nos, C La-
monte, J. Smith, C. Tan, and I. Krakoff.
Sloan-Kettering Institute and Memorial Hos-
Bleomycin, a mixture of antibiotic polypeptides, was evaluated for therapeutic activity and clinical toxicology in 274 patients with far-advanced, nonresectable neoplastic disease. Therapeutic effect was most marked in advanced Hodgkin's disease, in which 50% of patients had significant objective and subjective improvement, in some cases for periods now approaching 2 yr. Scattered responses of brief duration were seen in other neoplastic diseases. The clinical toxicity of bleomycin appears to be unique among antitumor agents: it produces no important effects on the blood-forming organs, gastrointestinal tract, liver, kidneys, or central nervous system. Pulmonary functional impairment, however, is common; irreversible pulmonary fibrosis, although rare, is a serious and sometimes lethal manifestation of bleomycin toxicity that may limit its use in early neoplastic disease.


Patients with acute nonlymphocytic leukemia frequently die of infection before cytotoxic therapy has had an opportunity to be effective. Prevention of infection might lead to increased remissions and, consequently, prolonged survival time. To establish the types and causes of infection, for 2 1/2 yr 48 patients have had extensive microbiologic surveillance cultures taken repetitively, beginning at admission. Each organism was defined as either part of the baseline flora or hospital acquired. All patients acquired multiple potential pathogens during hospitalization. These cultural data, in conjunction with observation of all infectious episodes, indicate that most infections arise from the patient's own flora. In 47% of these microbiologically documented infections, however, the pathogens had become part of the patient's resident flora after being acquired from the hospital environment. Measures to prevent infections in these patients must include reducing the acquisition of potential pathogens, especially Pseudomonas aeruginosa.

Change in Leukocyte Ascorbic Acid During the Common Cold. R. Hume and F. Wevers. Southern General Hospital, Glasgow, Scotland. Scot Med J 18:3, 1973

In seven subjects within 24 hr of the onset of symptoms of the common cold, the level of ascorbic acid in the leukocytes had fallen significantly: the return to normal levels at 5 days coincided with the end of clinical illness. It was possible to modify these changes by oral ascorbic acid therapy. — J. M. B.

IMMUNOHematology

Successful Treatment of an Infant With Severe Combined Immunodeficiency by Transplantation of Bone Marrow Cells From an Uncle. J. M. Vossen, J. de Koning, D. W. van Bek-
ABSTRACTS


Lymphocytes from normal adult blood contained some 53, T cells, as estimated by using rabbit anti-human T-lymphocyte antisera in the cytotoxic assay. Blood from patients with Bruton-type agammaglobulinemia contained normal T-lymphocytes, whereas with Hodgkin's disease there was a significant reduction in percentage of T cells in the peripheral blood. — J.M.B.


Clinical, pathologic, and immunocytochemical studies were performed in three patients with α-chain disease. All three patients were female, aged 21, 44, and 23 yr, respectively. Malabsorption, diarrhea, abdominal pain, and progressive general deterioration were the main clinical features; the liver and spleen were normal or only slightly enlarged, and the skeleton appeared roentgenologically unaffected. Biopsy and/or autopsy evidence was obtained of a malignant lymphoma extensively involving the small intestine and the mesenteric lymph nodes. The histopathologic picture consisted of a dense cellular infiltration that was pleomorphic, although composed mainly of plasma cells. The serum electrophoretic pattern showed an abnormal band in one patient and only minor changes in the other two. A peculiar protein, immunologically related to the heavy polypeptide chains of IgA but devoid of light chains, was detected by immunoelectrophoresis in serum and concentrated urine samples; trace amounts were also detected in the saliva but not in the tears of one patient. It was also demonstrated in the cell extract of an intestinal biopsy specimen. The peculiar IgA was antigenically deficient as compared with some IgA myeloma proteins and sedimented more slowly in the ultracentrifuge than both IgA myeloma protein and albumin. In addition, it showed striking electrophoretic heterogeneity, a tendency to form polymers, and belonged to the α1-subclass in all three cases. All three patients were from the south of Italy, and in none of them could an Arab or Jewish ancestry be definitely established. — J.E.U.

A 4½-mo-old boy, the child of first degree cousins, was studied; three siblings had died in infancy from diarrhea, failure to thrive, and infection. At age 2 wk the patient showed oral candidiasis, and lymphocytopenia was found. A diagnosis of severe combined immunodeficiency was made. By albumin gradient separation, a stem cell-rich fraction of bone marrow from an HL-A genotypically identical uncle was prepared and transfused. Full immunologic reconstitution followed. The only allotypic marker of his IgG different from the donor allotype remained in the serum after transplantation. — J.M.B.

Function and Distribution Pattern of Human "T" Lymphocytes. I. Production of Anti-T Lymphocyte Specific Sera as Estimated by Cytotoxicity and Elimination of Function of Lymphocytes. F. Aiuti and H. Wigzell. First Department of Infectious Diseases, Rome University, Italy and Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden. Clin Exp Immunol 13:183 189, 1973

Lymphoid cells from a patient with Bruton-type agammaglobulinemia were injected into rabbit, and antisera were prepared that, in the presence of complement, showed two plateau levels of cytotoxicity against human peripheral lymphocytes. It is suggested that these antisera selectively kill human T-lymphocytes, while not affecting function of B-type cells. — J.M.B.

Function and Distribution Pattern of Human T Lymphocytes. II. Presence of T Lymphocytes in Normal Humans and in Humans With Various Immunodeficiency Disorders. F. Aiuti and H. Wigzell. First Department of Infectious Diseases, Rome University, Italy and Department of Tumor Biology,
ABSTRACTS

MISCELLANEOUS

Biochemical Diagnosis of Cystinosis by an Increased Content of \( \beta \)-Alanine in Lymphocytes and Thrombocytes. G. Wenske and F. Linneweh. University Children's Hospital, Marburg, Germany. Klin Wochenschr 50: 1082, 1972

In lymphocytes and thrombocytes of six patients with cystinosis, a marked elevation of an amino acid was found that could be identified as \( \beta \)-alanine by column chromatography. The mean values were 4.5 nmoles/mg of protein and 7 nmoles/mg, respectively, as compared with below 1 nmole/mg in normal controls. The heterozygous parents did not differ from the controls. Up to now, the elevated content of \( \beta \)-alanine cannot be explained. — K.B.

HEMOSTASIS


Studies on the isolated and purified subunits of thrombosthenin, actinlike thrombosthenin A and myosinlike thrombosthenin M, leave little doubt about the close relationship of thrombosthenin to muscle actomyosin. Evidence for the presence of regulatory proteins has also been presented, and the "relaxing factor" (i.e., calcium storage vesicles) have been described. This makes it most likely that the induction of contraction in platelets is also governed by the availability of \( \mathrm{Ca}^{2+} \) ions. On the other hand, the major contractile functions of the platelets, such as contraction of aggregates and clot retraction, are one-way processes and can be inhibited by the removal of \( \mathrm{Ca}^{2+} \) ions from the surrounding medium. The possibility of an altered permeability for cations of the platelet membrane in the course of irreversible aggregation as well as the available evidence for a surface-localization of thrombosthenin are discussed. Thrombosthenin A shows a close relationship to the subunits of microtubular protein and the appearance of morphological evidence for contractile filaments within the cytoplasm coincides with the progressive breakdown of the microtubules. Studies on other cell types have shown that it is cyclic AMP which seems to influence motility of the cell and, most likely, the equilibrium between microtubules and contractile protein. An attempt in applying these findings to the situation encountered in blood platelets has been made; it has been suggested that a slight decrease in intracellular c-AMP, most likely in combination with a release of calcium, induces the formation of the contractile complex, whereas in situations with a c-AMP level above normal, the platelet is stabilized in the resting state. The functional importance of thrombosthenin is primarily seen in the consolidation, by contraction, of platelet aggregates in hemostasis and thrombosis. Other manifestations of contractility include the release reaction, clot retraction, and morphological changes. It is not likely that there is direct and essential involvement of thrombosthenin in primary, reversible aggregation. — J.C.

The Mechanism of Adenosine Diphosphate Induced Platelet Aggregation: Binding to Platelet Receptors and Inhibition of Binding and Aggregation by Prostaglandin E1. D. J. Boulin, A. R. Green, and K. S. Price. Laboratory of Preclinical Pharmacology, National Institute of Mental Health, St. Elizabeth Hospital, Washington, D.C. J. Physiol (Lond) 221: 415-426, 1972

Normal human platelets were incubated with \( ^{14} \mathrm{C} \)-ADP for 10-360 sec and the aggregation responses were correlated with the platelet bound radioactivity recovered from platelets separated from the plasma within 25 sec of the end of the experiment. The platelet aggregation response was related to the plasma \( ^{14} \mathrm{C} \)-ADP concentration and related linearly to the log of the platelet bound \( ^{14} \mathrm{C} \)-ADP 60 to 120 sec after addition of the nucleotide to the plasma. By thin layer chromatography of the platelet bound radioactivity it could be shown that 78\% to 90\% of the labeled ADP was unmetabolized, the remainder was converted to AMP. Radioactivity in the plasma was found in ADP, AMP and adenosine. There was no detectable radioactive cyclic AMP in either platelets or plasma. Prostaglandin \( \mathrm{E}_1 \) inhibited aggregation and platelet \( ^{14} \mathrm{C} \)-ADP accumulation when added to platelet rich plasma 60 sec before the labeled nucleotide. A significant correlation was found to exist between inhibition of aggregation and inhibition of \( ^{14} \mathrm{C} \)-ADP
accumulation. Prostaglandin E₁ was able to reverse ADP aggregation also when added to platelet rich plasma after the nucleotide (within 60 sec) with an accompanying decrease in the platelet bound ¹⁴C-ADP. The authors conclude that ADP induces platelet aggregation by binding to specific receptors probably located in the plasma membrane and that prostaglandin E₁ inhibits this effect by interfering with the ADP binding.

M.S.


The duration of the inhibitory effect on the platelet release reaction of a single dose of aspirin varies according to the individuals: atheromatous patients show a statistically significant tendency towards hyperaggregability (Born's method). The authors studied the variation in the duration of the inhibitory effect after absorption of a single dose of aspirin (1 g) as a function of the degree of hyperaggregability and of the lifespan of platelets (¹⁸Cr method). These three parameters correlated closely. The results obtained in vitro confirmed these results. At the conclusion of their work, the authors suggest that there exists, in the atheromatous, a qualitative anomaly in the platelets, which is the cause of the hyperaggregability phenomenon. The nature of this anomaly is still undetermined.—J.C.
aldehyde), adenosine becomes an inducter of platelet aggregation, but it still also preserves some action as an inhibitor. The corresponding mono- and ditetrazolium derivatives show a slight inhibition in ADP induced aggregation. A mode of action of these sugar derivatives of adenine on platelet aggregation is suggested. — J.C.


Platelet's stickiness to perivascular connective structures and collagen fibers plays an important role in the early stages of hemostasis and thrombosis. After their binding to collagen fibers, platelets undergo morphological changes. They spread along the fibers, unite together and, without addition of new platelets, form homogeneous clumps supported by the fibers. Very few techniques can give a quantitative measure of platelet stickiness. Their results raise different hypotheses concerning the mechanism of stickiness. Neither the coagulation process nor ADP intervene in this phenomenon. The role of total electric charges or of mucopolysaccharides on the platelet's surface is not proved. On the other hand, the formation of a complex between the platelet's glucosyltransferase and unsaturated polysaccharide chains of collagen should be a very important factor. — J.C.


The mechanism of the platelet release reaction induced by collagen is in certain respects different from that of adrenaline or ADP, because besides other facts, platelet factor 4 (PF₄) release and the availability of platelet factor 3 (PF₃) and acid phosphatase (APh) are not influenced by the concentrations of alpha or beta receptor blocking agents which clearly interfere with adrenaline or ADP-induced release. It has been stated that aggregation is not always necessary for a release of platelet compounds and that there was a dissociation even between PF₄ release and the availability of PF₃ and of APh because under certain experimental conditions the release is blocked, whereas availability is quite normal. On the basis of previous papers and of present experiments the authors point to a close connection between APh and PF₃. They assume that APh is probably an enzymatic marker of the lipoprotein complex which shows the activity of PF₃, and that APh and PF₃ are "liberated" from platelets by a mechanism other than the platelet-release reaction. This phenomenon was denoted by the term "availability reaction." — J.C.

Thrombokinetic Studies in Alcohol Related Thrombocytopenia. D. H. Cowan. Department of Medicine, Case Western Reserve University School of Medicine, Cleveland Metropolitan General Hospital, Cleveland, Ohio. J Lab Clin Med 81:64-76, 1973.

Thrombokinetic studies were done in five folate deficient and three folate replete alcoholic subjects. Measurements were done during abstinence and during a period of supervised alcohol ingestion on a metabolic ward. Three of the five folate deficient and two of the three folate replete subjects developed thrombocytopenia during alcohol ingestion. Platelet life span during alcohol related thrombocytopenia was decreased (2.2 to 3.8 days compared to eight to nine days during abstinence). Although total thrombopoiesis determined from the total mass of megakaryocytes increased one and a half to three fold in folate deficient thrombocytopenic patients with alcohol ingestion, effective thrombopoiesis determined from the platelet turnover decreased 30% to 50%. In folate replete thrombocytopenic patients, total thrombopoiesis varied from no change to mild increase. In three patients in whom alcohol ingestion was unaccompanied by thrombocytopenia, changes in the megakaryocytes were variable, platelet life spans ranged from 50" to 100", of controls, effective thrombopoiesis was increased 1.1 to 1.5 fold and the ratio of total to effective thrombopoiesis was unchanged or decreased. The results indicate that alcohol related thrombocytopenia is characterized by a reduced platelet life span and an inability of the marrow to compensate appropriately for the premature removal of platelets from the blood stream. — M.S.

In 26 leukemic patients, clot-promoting activity of white cells, isolated from blood and bone marrow, was studied in vitro, using suspensions of either intact or sonicated cells 5.10^7/ml. In addition to a common cephalin-like activity, in 13 cases of acute leukemia (5/7 promyelocytic, 6/8 myeloblastic, 2/5 “undifferentiated”) the blast cells showed a heat labile tissue factor activity, demonstrable only in the presence of Factor VII and increased by cell destruction. In the two other cases of promyelocytic leukemia the cell suspensions acted as a factor X activator simulating Russell Viper venom activity. As demonstrated by cell treatment with anti-human Factor VII antibodies, this activity was supported by a complex of “tissue factor” and plasma Factor VII. In seven of these patients, including the two aforementioned cases, severe diffuse intravascular clotting occurred during the course of the illness (6/7 promyelocytic and 1/5 “undifferentiated” leukemias). In contrast, abnormal fibrinolysis was observed in none of these patients, despite the presence of plasminogen-activator activity in all cases of granulocytic leukemia, greatly increased by cell sonication.—J.C.