ERYTHROCYTES


This is a study of 20 patients with hepatobiliary disease and macroplania, which is defined as an increased erythrocyte surface area seen in a blood smear. The macroplania in these patients was associated with leptocytosis but without target cells. Osmotic fragility of erythrocytes labeled with Na<sub>2</sub>51CrO<sub>4</sub> was determined by measuring the radioactivity of the supernatant. Labeled normal erythrocytes were introduced into the circulation of patients with hepatobiliary diseases and leptocytosis. Thereafter a “radioactivity curve” was determined as a measure of the osmotic fragility of the injected donor erythrocytes. The following observations were made: (1) There was a positive correlation between changes in MCD and serum bilirubin. (2) There was a negative correlation between MCD and osmotic fragility. (3) There was a positive correlation between MCD and mean amount of cholesterol per sq. μm. of erythrocyte surface area. (4) The osmotic fragility of normal erythrocytes introduced into the circulation of patients with leptocytosis became similar to the (diminished) osmotic fragility of the patients’ own erythrocytes.—M. C. V.

AUTOMATIC MEASUREMENT OF ERYTHROCYTE CHOLINESTERASE USING PROPIONAL THIOCHOLINE IN VARIOUS DISEASES. H. Shibata, C. Suzuki, M. Ito and S. Shibazaki. The
Activity of cholinesterase in erythrocytes was measured routinely by automation, using propionyl thiocholine. It was found that there was a good correlation between the activity of erythrocyte cholinesterase and severity in leukemia, its measurement seems to be useful in diagnosis and treatment of this disease.—K. F.


The authors studied the metabolic properties of red cells metabolizing adenine and inosine in vitro and in vivo. An increase in lactate production, ATP/ADP ratio, glycolytic intermediates (including FDP) were found both in vivo and in vitro. Trying to prolong the life span of pyruvate kinase deficient red blood cells, an infusion therapy with inosine and adenine was applied. This therapy succeeded in case of a type B deficient patient (type B = 50-70% enzyme activity). The 1/4 of the red cells increased, the hemoglobin content rose, reticulocyte count and serum bilirubin level dropped. On the contrary, a type A deficient patient (type A = 0-40% enzyme activity) did not show a significant hematological response.—M. C. V.


Red cell glutathione peroxidase activity is significantly lower in newborns than in healthy adults. Although serum bilirubin levels were the same in 3-day-old full term and premature infants whether their erythrocyte glutathione peroxidase levels were above or below the mean, there was a tendency among full-term infants with high bilirubin levels to have lower enzyme activity. The duration of glutathione peroxidase deficiency was more prolonged in premature lasting as long as 10 months in one infant. The authors conclude that although glutathione peroxidase deficiency does not result in hyperbilirubinemia, infants with low enzyme activity may have an increased chance of developing severe degrees of jaundice.—J. B. S.


Ferrochelatase activity in rat liver mitochondria extracts was found to be stimulated by crude lipid extracts from chromatophores of Rhodopsudomonas spheroides, by neutral lipids, phosphatidylethanolamine and acidic phospholipids. The inhibitory effect of albumin on ferrochelatase could be partially overcome by lipids or long-chain fatty acids. Peroxide containing lipids did not stimulate the activity of ferrochelatase preparations of low specific activity. Oxidation of mitochondrial lipids to peroxides led to inactivation of the enzyme.—M. K.


DDTP is one of the agents commonly used to induce the experimental hepatic porphyria in animals. The following metabolic changes were demonstrated in liver mitochondria from rats treated with DDTP: enhancement of δ-aminolevulinate synthetase, decrease of catalase and ferrochelatase activity. The activity of cytochrome oxidase remained unchanged. No changes in mitochondrial respiration were observed, however, when glutamate (but not succinate)
was used as substrate; partial uncoupling of oxidative phosphorylation was found to occur. In vitro DDTP showed the same effect on the oxidative phosphorylation but did not affect the activity of the examined mitochondrial enzymes.—M. K.


Serum iron level and TIBC were examined in 43 children with rheumatic fever before and during treatment (prednisone followed by aspirin). Significant decrease of the serum iron level and slight lowering of TIBC were found only in the subgroup of children with polyarthritis dominating the clinical picture. In the cases without joint involvement both serum Fe and TIBC did not differ from the normal values irrespectively of the number of previous attacks of rheumatic fever. In the majority of cases with hyposideremia, serum iron level returned to normal values after treatment. Moderate anemia was also much more frequent in the children with polyarthritis. Accumulation of iron in inflammatory synovial changes has been recently described in rheumatoid arthritis. This mechanism is suggested to be a probable cause of sideropenia observed in the articular form of rheumatic fever.—M. K.


A group of 30 shipyard welders with pneumoconiosis was examined and compared with a control group of 14 healthy men of similar age, having no occupational exposure to inhalation of iron compounds. The mean level of serum iron was found to be increased in shipyard welders, the content of transferrin in serum was diminished and the degree of transferrin saturation was markedly increased.—M. K.


A review of the hemolytic-uremic syndrome is presented focusing on the main diagnostic points. The renal involvement with uremia; the hemolytic anemia with the presence of "Burr cells" indicating mechanical resistance to the blood flow, due to intravascular coagulation; and the platelet deficiency with hemorrhage, constitute the clinical picture. The central nervous system is involved. Ten cases are presented and discussed. They were observed in the region of Belo Horizonte, State of Minas Gerais. No correlation was found between the degree of hemolysis and that of uremia. Hyperfibrinolysis, removal of fibrinogen and of the degradation products of fibrin, would be a protection mechanism to avoid the deposition of fibrin on the capillary walls. One case, with benign evolution, presented a detectable hyperfibrinolysis. Some patients have minor lesions, with a benign course. The prognosis is severe. Since the clinical and pathophysiological features are not well known, the number of fatal cases predominates over the benign ones as pointed out by the first studies on the syndrome. Anticoagulant therapy favors clinical recovery. Some patients even overpassed the hemolytic and the hemorrhagic stages to present a picture of renal insufficiency. The author did not see any fatal case in the series of ten cases studied.—M. A. J.


The effect of a dose of 40 R of X rays on changes occurring during storage of blood taken with ACD solution were examined. Acid soluble phosphate compounds (AMP, ADP, ATP, CMP, CTP, NAD, NADP, 3,3-DPG, Pi) GSSG and potassium contents in the whole blood and in plasma did not change during 21 days of storage as compared with control unirradiated blood. The
only change observed was some increase in hemolysis in the irradiated blood.—M. K.

LEUKOCYTES


Cultures of mouse fetal liver cell suspensions were made in soft agar and compared with those of mouse marrow cells. The liver suspensions caused the growth of colonies of myeloid cells, with a linear relationship between numbers of cells explanted and colonies produced. Similar numbers of colonies grew when the same numbers of liver or marrow cells were cultured. It was concluded that in the 16½-day mouse fetus the liver contains a considerable "committed" myeloid stem cell compartment, even though initial cell counts showed only 4 per cent granulocytic cells. However, 6–8 times as many fetal liver as fetal marrow cells were needed to produce comparable numbers of spleen colonies in lethally irradiated syngeneic recipients.—F. W. G.


Three kinds of Rauscher virus vaccines were emulsified with Freund's adjuvant and used for vaccination. These vaccines almost completely protected mice against challenge inoculation of 1 per cent supernatant fluids of infected spleen homogenates sufficiently virulent to kill all the control animals, but did not protect against challenge of 10% supernatant fluids similarly prepared. Twenty days after virus inoculation, an abnormal fraction which was considered to be specific in Rauscher leukemia, not found in normal mice, was detected between α₂-globulin and β-globulin fractions and markedly increased with the progression of leukemia. Electrophoretic studies in mice which were protected against leukemia with vaccination revealed no such abnormal fraction but showed a marked increase of γ-globulin and β-globulin, probably due to a reaction of the host toward Rauscher leukemia. By electron microscopy virus particles were observed in the spleen of leukemic mice. The eclipse of Rauscher virus is considered to be within 7 days. The cells in which virus particles were formed were megakaryocytes in the initial stage of leukemia, but leukemic cells in the advanced stage of leukemia. Virus particles which were challenged in vaccinated mice were neutralized by circulating antibody. Nonneutralized particles were held in gray bodies of megakaryocytes in the form of immature C-particles.—K. F.


Resting leukocytes of newborn infants exhibit increased oxygen consumption, NBT reduction, and hexose monophosphate shunt activity. During phagocytosis newborn and maternal white cells showed normal increments in these activities, whereas leukocytes from patients with chronic granulomatous disease failed to show such responses. The newborn granulocytes also demonstrated normal staphylocidal capacity in the presence of pooled human serum.—J. B. S.


Zinc content was determined by a score technique in 30 patients with breast cancer, in 30 patients with cervical carcinoma and in 30 control healthy women. In both groups of patients, zinc content of granulocytes was lower by 23–24 per cent than in controls. The differences were not distinct enough to recommend the test for diagnostic purposes.—M. K.

NEW ASPECTS OF THE ENZYMATIC REGULATION OF THE LEUKOCYTE. A. Vanotti. From Medical Department, University of Lausanne, Cantonal Hospital, Switzerland.

The authors investigated 40 patients with chronic myelosis, 18 of them repeatedly. The studies proved that in most cases (29 patients), during nonblastic exacerbation of the leukemia, one or more (up to three) aneuploid cell clones could be found along with a clone of cells characterized by a pseudodiploid complement of chromosomes and containing the Philadelphia chromosome. Nevertheless, the presence of these “additional” clones in periods of exacerbation was extremely small (2–12%) and only in a small number of patients it reached 25–80 per cent. The karyotype of the cells in the “additional” clones was different in the different patients. In all cases, except one, these were hyperdiploid cells, usually with two Philadelphia chromosomes. In two patients, the development of blast transformation could be observed in the bone marrow with proliferation of aneuploid cells that had been seen 24 and 8 months before, respectively, during the chronic phase of the disease. In the first patient, these were cells containing 47 chromosomes and two Philadelphia chromosomes each, in the second one the cells contained 48 chromosomes (extrachromosomes of D and G groups). Thus, it is possible to draw the preliminary conclusion that formation of clones of malignant cells constituting the substrate for a blast crisis occurs long before the crisis, during the chronic phase of the disease.—M. K.


Only 11 cases of hypercalcemia associated with leukemia have been described previously. The present paper describes two adults with this complication in chronic myelogenous leukemia. The appearance of bone pain, or gastrointestinal or central-nervous-system symptoms should prompt determination of the serum calcium level.—J. E. U.


Cytogenetic studies of bone marrow and cultured blood cells in eight patients with various types of multiple myeloma and one with primary macroglobulinemia demonstrated independent abnormal cell lines characterized by presence of such marker chromosomes as MG-like, Bq–, aberrant-C, and Dq+ chromosomes, respectively. Evidence was presented that these abnormal cell lines further generate new subclones that possess new marker chromosomes in addition to the original ones. In a series of patients, associations of two marker chromosomes such as Dq+ with MG-like, Dq+ with aberrant-C, and Bq– with aberrant-C were the examples. One of the most important findings was that many of such marker chromosomes were noted in a patient with myeloma of the “no-anomaly” type. These results suggest that production of paraimmunoglobulin and chromosomal aberration are not closely related as to the cause and effect of the disease, but further studies are required to clarify such relationship.—K. F.

Mouse plasma cell tumor, MSPC-1, exhibited periodic ploidy alterations from diploid to tetraploid and vice versa during serial transplantation for two years. Pre-treatment of the host animal by 200-R gamma ray caused apparently rapid appearance of the transplanted tumor, but could not give any significant effect on the ploidy change. Using marker chromosomes and autoradiography of incorporated $^3$H-thymidine it was found that the diploid cells became tetraploid cells through the binucleate stage that resulted from failure of cytokinesis. The tetraploid cells could not survive longer than a few transplant generations. The diploid cells proliferating actively in the next diploid stage derived from a minor diploid cell population, which emerged from the previous diploid stage as a variant. Immunological significance of this periodic ploidy change was discussed.—K. F.

A theory of the pathogenetic mechanisms for leukemias is proposed. The importance of two factors is discussed on the basis of personal studies. The first is a genetic predisposition indicated by the results of anthropometric and dactyloscopic investigations. The second is a harmful ecologic factor demonstrated by epidemiological studies. The enrichment of the soil with magnesium is proposed as a possible method for leukemia prophylaxis.—M. K.

A study of 233 patients with lymphosarcoma arising primarily in the lymph nodes is reported. When microscopical examination showed fibrous banding within the lymph nodes, typical of nodular sclerotic lymphosarcoma, the prognosis was better than in the other histological groups. This improvement was more marked in patients presenting with generalized disease, when treatment can be expected to have less effect upon the outcome. With localized disease, the 5-year survival rate was similar in follicular lymphoma, diffuse lymphosarcoma and nodular sclerotic lymphosarcoma, but a greater percentage of the last type continue to survive, free from clinical evidence of disease, for periods up to 19 years.—J. E. U.

Seven patients with cardiac involvement from lymphomas and leukemias who were treated with radiotherapy are presented. Two patients who had pericardial invasion from surrounding mediastinal nodes and whose disease was otherwise confined to the lymphatic system were treated radically. They are alive without evidence of disease at 2 years and 1 year, respectively. The remaining five were treated palliatively. Of these five, three patients had excellent responses. Although significant palliation was not achieved in the other two patients because of the widespread nature of their disease, their pericardial effusions were controlled by the radiotherapy. It is felt that radiotherapy can prove valuable in the radical and palliative management of patients with cardiac involvement by lymphomas and leukemias.—J. E. U.
HEMOSTASIS


Micromeres were isolated from large volumes of freshly collected platelets. Sucrose gradient analysis of such platelet micromeres treated with sodium deoxycholate revealed the existence of two classes of heavy polyribosomes. The sedimentation coefficients of these polysomes were estimated to be 650S and 340S, respectively. The polysomes were able to incorporate 14C-labeled amino acids into polypeptide products which were characterized as myosin and actin-like proteins. Isolation and purification of the synthesized polypeptides proved that they were synthesized in a native form. This was achieved by adding pure myosin or actin to the products of the cell-free synthesis followed by repeated chemical isolation of the respective proteins until constant specific activity was reached. Analysis of the polysomal size indicated that these myosin- and actin-like proteins were synthesized on monocistronic RNA molecules.—M. G. B.

STUDIES ON LIPID PEROXIDES IN PLATELETS.


A simple method for lipid peroxide assay in platelets is described which, by the use of an ionic detergent, produced clear solutions suitable for photometric measurements. Freshly collected platelets had small quantities of lipid peroxides. The anticoagulant used in the collection of platelets had no significant effect on their subsequent peroxide content. Storage of platelets at 4°C or 20°C resulted in rapid and progressive accumulation of lipid peroxides. Platelet concentrates collected from ACD blood and stored at 20°C had a significantly higher peroxide accumulation than those collected from platelets stored at 4°C.
collected in EDTA blood and stored at 4°C. Lipid peroxide formation could be prevented by the addition of tocopherol, Tween 80 or increasing the H+ concentration in the platelet preparation. Freezing of platelets with glycerol promoted lipid peroxide accumulation in them while freezing with dimethylsulfoxide did not. The cryoprotective agents per se had no influence on lipid peroxide formation. The study of factors which can influence the formation of lipid peroxides in platelets was thought to be of practical importance because lipid peroxides are known to be able to interfere with cell function and viability.—M. S.

**The Role of Blood Platelets in the Hemorrhagic Diathesis of Scurvy in the Guinea-Pig. A. Poplawski and T. Poplawksa.** Faculty of Physiological Chemistry and Department of Hygiene, School of Medicine, Białystok, Poland. Acta Physiol. Pol. 21:363-369, 1970.

Disturbances in platelet behavior were observed in experimental scurvy in the guinea pig. The time of aggregation of platelets by ADP was markedly prolonged as compared with control animals. The aggregation induced by thrombin was normal. Impaired release of platelet factor 4 during ADP induced aggregation and prolonged coagulation time were found. Fibrinogen level and fibrinolysis in euglobulins were normal. Addition of ascorbic acid in vitro did not influence the parameters tested.—M. S.


Three brothers, 5 to 9 years of age, affected with Wiskott-Aldrich syndrome and one 10-year-old normal sibling were studied. Two striking abnormalities were found in the platelets from the patients and from their mother: (1) lack of aggregation with epinephrine; (2) decrease in the metabolic response to stimulation with polystyrene-late particles and with epinephrine. Platelets from the patients with the Wiskott-Aldrich syndrome produced less 14CO2 and 14C-lactate than did those from normals when incubated with glucose-6-14C. By referring metabolic values to the protein content rather than to the platelet number, however, only a small difference was noted in the citric acid cycle activity and fibrinolysis activation between platelets from normal controls and those from the patients. Whereas normal platelets reacted with an 8- to 12-fold increase in 14CO2 production from glucose-6-14C when incubated with latex particles, the addition of such agent to the platelets from the affected children produced only very minimal stimulation. Lactate production was also decreased compared to that in normal controls but to a lesser degree. The mother's platelets behaved similarly to the children's platelets in regard to aggregation by epinephrine, but their metabolic reaction, particularly of the citric acid cycle, to latex particles and epinephrine was greater than that of the patients and less than that of normals. It was concluded that the manifestations of the Wiskott-Aldrich syndrome may be due to a single basic defect in energy metabolism caused by failure in regulators of oxidative phosphorylation probably residing in cell granules.—M. S.


The results of systematic studies on activation of fibrinolysis in the human whole blood and in plasma, as well as in the purified system, are presented. Selective adsorption of plasminogen, activators on fibrin is demonstrated as the main mechanism of fibrinolysis activation in the clot network and as the cause of marked differences in the rate of the fibrin digestion as compared with fibrinogen in blood and plasma. It is shown that fibrin does not adsorb plasminogen.—M. K.

**The Influence of Some Proteolytic Enzymes on Serum Proteins. B. Bogdanikowa, M. Bielawiec and J. Drozdz.** First Department of Internal Medicine, School of Medicine, Białystok, Poland. Pol. Arch. Med. Wewnet. 44:311-316, 1970.

The effect of fibrinolytic enzymes and
plasminogen activators on serum proteins partially purified by Sephadex chromatography was investigated. Changes in electrophoretic mobility of immunoglobulins and of $\beta_1c$ globulin were demonstrated.—M. K.

**IMMUNOHEMATOLOGY**


The authors examined kininogens and kinins of blood plasma, acid phosphatase and acid and neutral proteases in lysosomes of granulocytes from peripheral blood during rejection of allogenic skin grafts in rabbits. The activities of both, kininogens and free kinins, were found increased. Granulocyte lysosomes showed some increase in acid phosphatase activity while that of acid neutral proteases was diminished.—M. K.


The fluctuation in immunoglobulin synthesis during the growth cycle was investigated by synchronizing the myeloma cells with excess thymidine. The cells proved to synthesize immunoglobulin maximally in S stage and minimally in mitotic stage. The synthetic pattern deviated considerably from that of total protein synthesis.—K. F.


In the present study, pathological macroglobulins were dissociated into $\mu$-chains and light chains by reduction and alkylation. The fractionated $\mu$-chains were injected into the rabbit with Freund’s complete adjuvant. Investigation of the macroglobulin producing cells by the use of the ferritin antibody technique revealed that the ferritin particles were present in both plasma cells and large lymphocytes, concentrating in the Golgi area and in the cytoplasm with abundant free ribosomes. It was to be expected that the ferritin particles would be present mainly in the cytoplasm with many free ribosomes, as the ferritin conjugate contained anti-$\mu$ chains. Similarly, the reason why the ergastoplasm of the plasma cells did not contain the ferritin particles can be well explained. The present results revealed that the macroglobulin producing cells do not stem from a single cell line, but from at least two cell lines: the large lymphocyte with broad cytoplasm and abundant free ribosomes, and the plasma cell from the immature type to the highly mature type. These cells may produce the IgM singly in some conditions or in a cooperative mode. And also, it can be said that free ribosomes in the cytoplasm of the large lymphocyte are a possible site of macroglobulin production.—K. F.


A 10-year-old epileptic boy previously treated with phenobarbital plus diphenylhydantoin, and currently on ethosuccimide (Zarontin) therapy, presented with fever, weakness, weight loss, arthralgia, joint swelling, hepatosplenomegaly, generalized adenopathy and butterfly rash. Laboratory studies revealed pancytopenia, an elevated ESR, mild proteinuria, hematuria and cylinduria, hyperglobulinemia, a positive direct antiglobulin test, and positive LE preparations. Following cessation of ethosuccimide administration some of the signs and symptoms subsided, however a week later he developed pneumonia with pleurisy. Following institution of prednisone therapy there was marked and progressive improvement. Within 2 weeks the abnormal physical findings had receded. The LE prep and positive Coombs remained positive for 3 months. Prednisone was stopped after 6 months, and 1 year later he was well.—J. B. S.
MISCELLANEOUS


The authors describe the case of a 15-year-old girl with protein losing gastroenteropathy due to lymphangiectasias in the digestive tract. Lymphangiectasia was also demonstrated as a cause of marked edema of the right upper extremity. Significant hyperperspiration on the right half of the body was observed. Absence of proteins in the sweat from the right hand was considered as the indication of protein loss into the tissues. The exudate obtained from the right hand by the Rebuck technique contained 70–80 per cent of lymphoid cells while that from the left hand only 10–30 per cent. The allogenic skin graft was rejected from the edematous right hand after 6 days, from the left hand after 72 days. According to the authors’ opinion this difference may be due to uneven distribution of immunocompetent cells in the upper extremities. In the peripheral blood, marked lymphopenia was found as well as hyperproteinemia with panhypogammaglobulinemia with deficiency of all classes of immunoglobulins. The case is an unusual variant of the protein loss syndrome accompanied by nonspecific immunotolerance due to lymph loss.—M. K.

ABSTRACTS


The present report is concerned with a female patient with acute Chagas illness and with demonstrated parasitemia, who after 20 days of being infected became pregnant. Five and one half months later she spontaneously delivered a fetus that remained alive for 15 minutes. The histological examination of the placenta and various fetal organs showed the existence of numerous nests of leishmania. These findings clearly demonstrated the transplacental transmission of the illness with intense alterations that caused fetal prematurity.—M. A. J.


Five cases of asbestosis associated with tumors of the hematopoietic system in a series of 35 cases of asbestosis proved by autopsy are reported. Two were cases of multiple myeloma; two, myeloproliferative disorders, including a case associated with reticulum-cell sarcoma; one, Waldenström’s macroglobulinemia. The incidence of this association is significantly higher than the overall incidence of such disorders in the corresponding age group of patients without asbestosis. Abstractor’s comment: This observation requires confirmation possibly with a larger number of patients than reported here.—J. E. U.