ERYTHROCYTES

On the Molecular Basis of Pyruvate Kinase Deficiency. I. Primary Defect or Consequence of Increased Glutathione Disulfide Concentration. T. J. C. van Berkel, J. F. Koster, and G. E. J. Stool. Department of Biochemistry I, Faculty of Medicine, Erasmus University, Rotterdam and Hematological Department, State University Hospital, Utrecht, The Netherlands. Biochim Biophys Acta 321:496-502, 1973

Human erythrocyte pyruvate kinase can be converted into an oxidized form by incubation with oxidized glutathione. The oxidized enzyme can be reduced again by incubation with mercaptoethanol. With reduced glutathione a partial reduction of the enzyme is obtained. The oxidized enzyme shows a lower affinity for the substrate PEP and for the allosteric effector fructose 1,6-diphosphate. The thermostability of the oxidized enzyme is markedly increased, compared with the freshly isolated or reduced enzyme. The data obtained with the oxidized enzyme are discussed in relation to the data obtained with pyruvate kinase from pyruvate kinase-deficient patients. It is concluded that erythrocyte pyruvate kinase deficiency can be the consequence of an increased oxidized glutathione concentration in the red blood cell.—K.P.


Failure to identify the patient with sickle cell hemoglobin prior to induction of anesthesia, which subsequently is complicated by hypoxia or dehydration can result in sudden unexpected death. Five cases which more or less indicate this danger, are briefly described. It is rather surprising to find that black youngsters are still undergoing elective surgery with general anesthesia, prior to testing for Hb S. The potential danger is obvious!—J.B.S.

Oral Urea Therapy in Children With Sickle Cell Anemia. B. H. Lubin and F. A. Oski. Children's...
Hemoglobin O Arab in Gypsy Family of South Macedonia, Yugoslavia. G. Efremov, S. Apostolova, K. Duma, J. B. Wilson, and H. J. Huisman. Faculty of Agriculture, Division of Biochemistry, Faculty of Medicine, Department of Internal Medicine and Medical College of Georgia, Department of Cell and Molecular Biology, Augusta, Ga. Acta Med. Jugosl. 27:359-368, 1973

In a survey of different patients, a 49-yr-old Gypsy housewife was found to be heterozygous for Hb-O Arab (Alpha2, Beta2 121 Glu-Lys). The same hemoglobin variant was found in four family members through three generations. The propositus and relatives were not anemic. The literature relevant to the significance of the Hb-O Arab is also reviewed. — Z.R.


Some immunochernical difference between human urinary and serum erythropoietin are described in this paper. Antisera against erythropoietin were obtained by immunizing rabbits with human urinary erythropoietin. Neutralization of the biological activity of erythropoietin was used as a proof of the specificity of antibodies to erythropoietin. When human serum and urinary erythropoietin were tested by using the method of micro-hemagglutination inhibition no differences between erythropoietin from the two sources were observed. When these erythropoietins were allowed to react in immunoelectrophoresis with erythropoietin antisera, the precipitation line in the alpha region was obtained with erythropoietin from normal human urine. This line was not observed from the urine of patients with chronic renal insufficiency or with normal or erythropoietically active human serum. It is concluded that although these findings implicate a difference between erythropoietin molecule from various sources, the possibility that antibodies react to impurities cannot be ruled out at the moment. — Z.R.


A boy with sickle cell anemia (SCA), beginning at the age of 7 yr, demonstrated a chronic inability to maintain normal serum and whole blood folate levels and normal weight gain, while on an adequate diet. In the initial episode, bone marrow and peripheral blood cells indicated megaloblastic changes, which reverted quickly with folic acid therapy. Serum B12 levels were always normal, and no evidence could be found to indicate subnormal folate absorption, or increased folate excretion. Whenever folic acid was given, blood folate levels and urinary FIGLU excretion returned to normal, and weight gain likewise became normal. The explanation for the folate deficiency in this youngster is not apparent. — J.B.S.


It is well known that plasma obtained from heparinized blood has a raised B12 binding capacity, as assayed by the Charcoal method, compared with plasma separated after the use of alternative anticoagulants. In the present experiments heparin used in normal anticoagulant quantities did not raise B12 binding when this was assayed by a dialysis method. The presence of stored leukocytes did not influence B12 binding capacity. Thus the effect of heparin is a function of the method employed for estimating plasma B12. Methodology may be less important if serum rather than plasma is assayed. — F.W.G.


Twenty-one patients with Crohn's disease in whom routine blood picture suggested iron deficiency were further studied. In the bone marrow, stainable iron could be demonstrated in all but nine of the patients. It is suggested that the basic disease process may be associated with iron-deficient erythropoiesis in the absence of true deficiency of iron. — J.M.B.

Oral urea therapy given to 11 children with sickle cell anemia over a study period ranging up to 16 mo was without apparent clinical or hematologic benefit. — J.B.S.
ABSTRACTS


Among premature infants neither agar (125 mg/q. 3 hr p.o.) nor intermittent phototherapy (12 hr/day x 4 days) had any significant effect on neonatal bilirubinemia. Infants so treated were indistinguishable from untreated infants and all three groups had serum bilirubin values significantly higher than seen in the fourth group treated with continuous phototherapy. — J. B. S.

Significance of the Circulating Blood Volume and Central Venous Pressure Indices in the Determination of Transfusion Volumes in Patients With Blood Loss and Trauma. E. S. Zolotokrylina, and V. V. Ivleva. Botkin Hospital, Moscow, USSR. Probl Gematol Pereliv Krovi 18:51--56, September 1973

Measurements of circulating blood volume and central venous pressure (CVP) were made in 115 patients with postoperative and obstetrical hemorrhage and severe trauma. During the first three days of treatment of uncomplicated blood loss by transfusion, blood volume and CVP correlated well in 60%, 70%, of cases. The following observations were made: Low CVP associated with hypovolemia requires further blood transfusion. Low CVP with normal blood volume and satisfactory general condition requires no emergency transfusion therapy. Normal or increased CVP with poor clinical condition is usually associated with hypovolemia and indicates disturbance of pulmonary circulation or the development of acute cardiac weakness. — J. V.

LEUKOCYTES


Marrow from 15 patients without known hematologic disease was grown in soft agar cultures on feeder layers of normal leukocytes. A linear relationship between numbers of cells plated and colonies grown was found. Radioactive thymidine suicide experiments showed an average of 32% of the in vitro colony forming cells to be in the DNA synthetic phase. Thus the colonies must have been derived from a proliferating precursor compartment. Eighty-five to ninety-five percent of cells in colonies were granulocytes; the remainder, mostly macrophages. Thus the precursors were probably committed stem cells as in the mouse. The culture system has obvious value for the quantitation of granulopoiesis and the study of neutropenia. — F. W. G.

Successive Ultrafiltrations of Type C Virus of Murine Leukemia. Biological and Ultrastructural Studies. J. E. Correa, Z. de Tkaczewski, and C. D. Pasqualini. Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, and Department of Microbiology, Faculty of Medicine, University of Buenos Aires, Buenos Aires, Argentina. Medicina (B Aires) 32:705, 1972

In murine leukemia, the number of viral particles morphologically identifiable is not necessarily related to the leukemogenic potency of an extract: there may be abundant viral particles and a low incidence of leukemia or vice-versa. The object of this paper is to try to elucidate the role played by the virus, as a morphologic entity, in the mechanism of leukemogenesis. The serial acellular line of leukemia PLLV (T2) maintained in BALB mice was used. It is characterized by the presence of type C viral particles in leukemic spleen, a marked splenomegaly and a short latency period averaging 30 days. Acellular extracts obtained from leukemic spleens were successively filtered through Millipores of 1.2, 0.8, 0.45, 0.22, 0.10 and 0.05 μ in diameter. These filtrates were ultracentrifuged at 35,000 rpm for 1 hr to obtain a pellet. All filtrates and their respective pellets were inoculated in 1-mo-old BALB mice, using a total of 250 animals. The leukemogenic effect was 100% with all filtrates down to 0.22 μ diameter of the filter. These values were predictable, but a 52% incidence of leukemia after filtration through a 0.10-μ diameter filter was unexpected, since the diameter of these viruses under the electron microscope ranges from 80 to 90 nm. According to Black’s coefficient these particles should not pass through a 100-nm filter. The examination of these preparations in both filtrates and pellets did not reveal the presence of viral particles. In all cases, a granular material, spherical electron dense elements (15--20 nm) were observed. However, type C particles were always present in the spleen of animals developing leukemia upon inoculation of any of these preparations. It can be concluded that a sub-

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viral element of smaller size and different structure may be capable of transmitting viral information or of triggering the leukemogenic process in mice. - E.S.S.


This is a fascinating case report of a girl with onset of repeated bacterial infections of skin, respiratory tract, mastoids, and soft tissues, beginning at 3 mo of age, followed by the appearance in childhood of pancytopenia, and progressive appearance in bone marrow and blood of histiocytes capable of phagocytosis but apparently not of bacterial lysis. Immunoglobulin levels and NBT reduction by her polymorphs were normal. At age 9, the disease culminated in rapidly fatal histiocytic leukemia. The leukemic process appeared shortly after splenectomy was performed for thrombocytopenic bleeding. Although this disease was somewhat similar to LRE and histiocytic medullary reticulosis, it could not be defined as either. - J.B.S.


Diffuse interstitial pneumonitis similar to that previously described in children receiving maintenance therapy with Mtx, was seen in four such leukemic youngsters who presented with fever and chronic cough with diminished breath sounds but infrequent rales. Three children also had a fine truncal erythematous rash and two had evidence of Mtx toxicity. Lung biopsy in two patients showed a mixed interstitial and exudative pneumonitis with variable mononuclear infiltration, focal necrosis, occasional giant cells, and/or epithelial proliferation with increased mitotic figures. No microscopic evidence of pneumocystic or tuberculosis infection was seen. In two patients there was serologic evidence suggesting respiratory syncytial virus infection, and in one, complement fixation titers to PPLO rose significantly. From these observations, the authors suggest that the pneumonitis associated with maintenance Mtx therapy may be viral in origin. J.B.S.


Thirty children with non-Burkitt’s undifferentiated lymphoma were surveyed retrospectively. Overall, 70%, eventually showed bone marrow involvement, and of this group, half developed CNS involvement. Marrow involvement occurred in almost all (91%) of children with mediastinal presentation and rarely with abdominal presentation. None of the six with primary small bowel tumor developed marrow changes or menigitis, and the latter was limited to patients with abnormal bone marrow. The median duration of disease at time of marrow conversion was 3.4 mo. The median survival for patients with marrow and meningeal involvement was 47 wk; for children without leukemic changes, median survival was only 14 wk. Median survival after appearance of menigitis was usually 4 mo, but several long-term survivors were seen indicating that meningeal involvement need not confer a grave prognosis. - J.B.S.


The case is reported of a woman who, 12 yr after the diagnosis of multiple myeloma had been made, developed an acute leukemia of the myelomonocytic type. The myeloma had been treated initially with urethane; years later, with melphalan for 27 mo. The increasing frequency of acute leukemia in patients with myeloma may be due to the leukemogenic effect of cytostatic agents as well as to the longer survival of these patients. K.P.


In two patients of 194 with thyroid carcinoma treated with 131I in Denmark from 1948 to 1972, myeloid leukemia developed. Expected cases were 0.097 (0.05 > p > 0.01). These findings support those from other centers that the use of 131I is associated with increased incidence of leukemia. J.M.B.

Immediately following exchange transfusion, all peripheral blood white cell elements fall below 50% of the pretransfusion values. Within 24 hr they return to preexchange levels, and over the next 5-6 days, polymorphs and eosinophils rise significantly above the levels seen in normal newborns. Lymphocyte counts remain unchanged, and monocyte levels are somewhat subnormal. All levels returned to normal by day 8. - J.B.S.


Four youngsters receiving VCR developed generalized seizures 5 or 6 days following infusion. The episode did not recur despite continued injections of VCR, and was seen as early as after the first dose and as late as after the eleventh dose. In no instance was there evidence of electrolyte disturbance or underlying CNS disease. The precise mechanism is unclear, but the authors view these episodes as due to VCR neurotoxicity. - J.B.S.

HEMOSTASIS


The technique developed by Harada and Zucker for the measurement of platelet F4 (PF4) activity in various conditions was evaluated. It was found that platelet-rich plasma (PRP) had a slightly higher PF4 activity than the corresponding platelet-poor plasma (PPP). Triton X-100 released more PF4 activity than freezing and thawing (x 3). PF4 was released at 37°C by ADP and adrenaline only when the general release reaction occurred. Aspirin completely inhibited such release. No release was detected at room temperature. Collagen released significant amounts of PF4 at both temperatures and was partially inhibited by aspirin. In contrast, thrombin and serotonin released PF4 activity without the production of the second wave of aggregation (indicative of the release reaction) and were not inhibited by aspirin. Cyclonamine inhibited the development of PF4 activity. "Storage-pool disease" patients had normal amounts of PF4 activity but defective release. The results suggest that PF4 participates in the release reaction induced by ADP, adrenaline, and collagen, but that it might be located in different platelet granules or structures than the nonmetabolic nucleotide pool and serotonin. Also, PF4 activity is closely related to its electric charge. Finally, the test developed by Harada and Zucker appears suitable for studying PF4 in a variety of conditions and seems to offer advantages over the methods employing fixed heparin concentrations. - E.S.S.


The aim of this study was to test whether the serum of hypofibrinogenemic animals induces increased concentration of fibrinogen in the blood. Hypofibrinogenemia in rabbits was produced by intravenous infusion of 500-550 I.U. of thrombin solution. The infusion of thrombin led to markedly decreased fibrinogen in rabbit blood which reached its peak immediately after infusion. In the next few hours fibrinogen gradually increased and reached preinfusion levels after 11 hr. An overshooting of the fibrinogen level following thrombin infusion was also observed. The serum of hypofibrinogenemic rabbits taken between 7 and 8 hr after thrombin infusion had a significant effect in increasing the fibrinogen level in injected mice. The sera of rabbits taken at other intervals did not bring about any significant changes of the fibrinogen level in the injected mice. From this experiment the existence of a humoral factor regulating fibrinogenesis is postulated. - Z.R.


The estimation of prothrombin time is of prognostic value in patients with acute hepatic
necrosis but otherwise estimations of the levels of clotting factors are not of significant benefit in the management of patients with liver disease. Impaired coagulation mechanisms are of only minor importance in determining the onset of bleeding. — J.M.B.


Isotope studies demonstrated that the thrombocytopenia which follows injection of Walker 256 tumor cells into rats results from trapping of platelets in the lung. This thrombocytopenia was prevented by heparin injections but not by Dipyridamole or Ancrod therapy. It is suggested that embolic tumor cells cause endothelial damage going on to local thrombin formation and irreversible platelet aggregation. — J.M.B.


Among seven patients with “idiopathic” thrombocythaemia, three had hemorrhage as main symptom and four thromboembolism with or without hemorrhage. One case terminated as acute granulocytic leukemia. In another, a “marker” chromosome was found in the marrow. Mean survival at date of writing was 7.5 yr from onset of symptoms and 3.0 yr from diagnosis. Treatment with 32P or busulfen kept platelet counts at manageable levels and decreased severity of symptoms. — F.W.G.


Home care of hemophilic bleeding episodes by nurse-practitioners was evaluated and compared to in-hospital treatment. Significantly more bleeding episodes were reported among patients on home care than among those receiving in-hospital care. This difference was due primarily to greater reporting of mild to moderate bleeds. Severe bleeding episodes were significantly more common among the group receiving in-hospital treatment, suggesting that parents may have delayed seeking treatment when they knew it would require hospitalization. At the end of the 2-yr period, 27 of the 28 families in the study preferred home care to hospitalization. The study indicates that where families were unable to administer home care to themselves, a modified program using a nurse-practitioner can be effective in decreasing days of hospitalization and limiting days of absence from school. — J.B.S.


A newborn with classical hemophilia developed evidence of subarachnoid hemorrhage at 5 days of age. Treated promptly with AHS, he developed progressive hydrocephalus with clinical evidence of increasing intracranial pressure. At 3 wk of age a ventriculoperitoneal shunt was inserted successfully and at 6 mo of age the infant appears to be neurologically normal. J.B.S.


A 6-yr-old boy with sickle cell anemia (SCA) presented with fever, progressive dyspnea, and lethargy. Left side pneumonia was noted by both physical examination and chest x-ray. Lumbar tap was unremarkable. Several hours later he had two seizures and died. At autopsy, massive subdural hemorrhage over brain and spinal cord was found. This type of hemorrhage is a very rare cause of death in SCA. — J.B.S.

IMMUNOHEMATOLOGY

The presence of specific antibodies for native DNA (Ab-Anti-DNA) was investigated in sera of patients with malignant lymphopathies. A radioimmunological method was used with tritiated DNA (DNA-3H) and ammonium sul-
ABSTRACTS


We have demonstrated that the subcutaneous implantation of plastic cylinders (PC) in BALB mice and the inoculation of AKR neoplastic cells (S15) histoincompatible with BALB, led to the development of tumors (lymphoma P) with a latency of 37 days and an incidence of 57% (33/59). Simultaneous controls receiving either S15 or CP alone, did not develop tumors. Lymphoma P could be transplanted both to the host strain, BALB, and to the donor strain. AKR and cellular lines could be established in the latter. It was postulated that neoplastic AKR cells had colonized in a host which normally rejects them, because of alterations brought about by the PC, the mechanism of which involved the production of serum blocking factors. In order to confirm this hypothesis, PC + S15, "progressor serum" from animals bearing a lymphoma P (P+) serum) was added to the original model as well as "regressor serum" from animals which had failed to develop lymphoma P (P- serum). The addition of P+ serum increased the incidence of tumors to 82% (89/48), significantly different from 56%, 58%, and 49% obtained in controls with normal BALB serum, normal AKR serum, or saline, respectively. On the other hand, P- serum dropped the tumor incidence to 10% (3/29). Comparatively, when the sera were replaced by lymphocytes obtained from normal AKR or BALB lymph nodes, it was observed that no tumors appeared with the former (0/36) while BALB lymphocytes increased the incidence of lymphoma P to 80% (23/29). It can be concluded that P+ serum carries a factor, possibly blocking antibodies, capable of increasing the tumor incidence. As for P- serum, its protection against tumor growth could involve antibodies capable of rejecting the tumor (cytotoxic) or antibodies against unblocking factors. The lack of tumor development or rejection observed with AKR lymphocytes can be explained on the basis of strong stimuli of histoincompatible antigens. The enhancement associated with BALB lymphocytes is more difficult to explain and needs confirmation; it may favor an increased production of blocking factors. —E.S.S.


The inoculation of AKR neoplastic cells (S15) in a plastic cylinder (PC) implanted subcutaneously in BALB mice led to the development of an allogeneic tumor, lymphoma P, while simultaneous controls either with S15 or PC, alone, never developed tumors. The object of this paper is to study the effect of an AKR skin graft, donor strain of S15, on the incidence of lymphoma P. A group of 23 BALB mice were grafted with a 1-sq-cm piece of AKR.
skin. After 11 days, coinciding with the rejection of the graft, a PC was implanted, followed 2 days later by the inoculation of S19. None of these animals developed a tumor, while the control group of 18 animals, grafted with syngeneic BALB skin gave a 45% incidence of lymphoma P. In order to determine whether this rejection effect was specific for AKR skin graft, the experiments were repeated with skin grafts from C57Bl and A mice, obtaining similar results, that is, absence of tumors. This led us to replace the allogeneic skin grafts for xenogeneic ones, using rat and hamster skin. Out of 34 BALB mice grafted with skin from G rats, inbred strain, only two (6%) developed lymphoma P. Contrarily, skin from inbred albino hamsters permitted the development of lymphoma P in 70% (16/23) of the animals, a value not significantly different from that of the controls. It can be concluded that pretreatment with an AKR skin graft protects against the development of lymphoma P as well as skin grafts from other mouse strains histoincompatible with the BALB host. This could be explained on the basis of cross-immunity to histocompatibility antigens and would also be the explanation for the lack of tumors after pretreatment with rat skin grafts. No common antigens would be present in hamster skin.-E.S.S.


“Immunoresistant” ascites tumor cells transplantable to many mouse strains have “masked” antigens which can become exposed on cell rupture, and a cell-membrane carbohydrate composition differing from that in nonimmunoresistant, nontransplantable tumor cells. When fused with normal fibroblasts, the transplantable tumor cells lose transplantability, and acquire cell-membrane characteristics of nontransplantable cells.—P.G.R.


Following therapy with daunorubicin and cytosine arabinoside, 45 of 107 patients with acute myelogenous leukemia came into remission. One group received chemotherapy with 5-day courses of cytosine arabinoside and daunorubicin alternating with 5-day courses of cytosine arabinoside and thioguanine, while the other group received this chemotherapy and in addition immunotherapy consisting of weekly injections of B.C.G. and of stored irradiated AML cells. Of 19 patients in the chemotherapy group, only five are still in first remission (median remission length 186 days), whereas eight of the 23 getting immunotherapy are in first remission (median duration 312 days). Comparison of median survivals after attaining remission shows significant effect for addition of immunotherapy. —J.M.B.


The ABO blood groups were determined in 864 patients with occlusive disease and 246 with ectatic disease. Blood group O seemed to confer some degree of immunity to occlusive disease but not to ectatic disease. These findings would support the hypothesis that occlusive and ectatic arterial disease are essentially different entities. —J.M.B.


Lymphocytes from the peripheral blood and lymph nodes of five patients with well differentiated lymphocytic lymphoma were studied at frequent intervals for their reactivity to phytohemagglutinin (PHA). In almost all instances the response was lower than normal and no correlation could be derived between the extent of clinical disease and the responsiveness of peripheral blood lymphocytes. Chemotherapy did not affect the degree of response to PHA. It is suggested that disorder of the relationship between lymphocytes and vascular endothelium may contribute to the random
pattern of lymph node and organ involvement seen in this disease.—J. M. B.

MISCELLANEOUS

Tumor Development in Mice Bearing a Plastic Cylinder and Inoculated With Human Neoplastic Cells. II. Histologic and Ultrastructural Studies. L. Schwartz, Z. de Tkoczevski, and C. D. Pasqualini. Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina and Department of Microbiology, Faculty of Medicine, University of Buenos Aires, Buenos Aires, Argentina. Medicina (B Aires) 32:703, 1972

Human neoplastic cells, GH7 and LDLT, originating from a lymphosarcoma and established in vitro, were inoculated in plastic cylinders (PC) subcutaneously implanted in BALB mice. Tumors developed with an average latency of 9 mo, killing the animals in an interval of 3 wk after the first symptoms of local growth. Tumor incidence averaged 62% and fell to 0% in the absence of PC. Histologically these tumors were classified as anaplastic sarcoma, richly vacuolarized in spite of extensive zones of necrosis. The tumor cells were markedly pleomorphic with elongated forms and primitive reticular types. The latter had large nuclei, regularly dispersed chromatin, and prominent nucleoli, with a pyroninophilic and PAS negative cytoplasm. Atypical multinucleated giant cells and abnormal mitoses were observed. Reticulin fibers were abundant. Ultrastructural studies with the electron microscope confirmed the presence of reticular and elongated cells. The latter were characterized by large nuclei with lax chromatin and prominent nucleoli, and in the cytoplasm a well-developed reticuloendothelial system, with dilated cisternae, numerous ribosomes, and mitochondria with edema. Images of contact between macrophages and/or lymphoid cells with tumoral elongated cells were observed. Type A viral particles were found within the cisternae, both in reticular and elongated cells, while no type C particles or budding processes could be seen. There was no morphologic difference between these tumors and those obtained from animals which had merely been implanted with a plastic cylinder or with PC and allogeneic AKR neoplastic cells. These tumors were structurally different from the human cell lines, GH7 and LDLT. These results indicate that the cylinder is an indispensable factor for the appearance of these tumors and that both human and AKR neoplastic cells are capable of potentiating the tumorigenic effect of this plastic implant.—E. S. S.


The paper describes an adult patient with gout and without any demonstrable activity of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HG-PRT). Few of the neurologic abnormalities generally associated with severe HG-PRT deficiency were exhibited by this patient. Psychologic studies, however, disclosed that some characteristics of the behavior anomaly in the Lesch-Nyhan syndrome are present, but only in a concealed form.—K. P.


In a study of peripheral blood of 646 blood donors at various times of the year, it was noted that during the summer season there occurred an increase in segmented leukocytes and a decrease in lymphocytes and eosinophils (absolute values). An additional reduction of leukocytes and eosinophils was noted during magnetic storms.—J. V.