ABSTRACTS

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ERYTHROCYTES


The influence of glucose-1, 6-P2 on hepatic and red blood cell pyruvate kinase was studied. This influence is quite similar to that of Fru-1, 6-P2. The hexose diphosphates can replace each other in stimulating pyruvate kinase; after maximal stimulation by one of the compounds, the other is not capable of further stimulation. It is concluded that the pyruvate kinase in the erythrocytes is fully stimulated by Glc-1,6-P2. These results are discussed in view of regulation of glycolysis by pyruvate kinase.—M.V.


A new mutant pyruvate kinase from human erythrocytes is described. The mutant enzyme shows an increased thermostability, a decreased Km value for the substrate phosphoenolpyruvate and a loss of allosteric properties during the lifespan of the erythrocytes. In comparison with previously obtained data from other patients, this is the third variant of pyruvate kinase found in the Netherlands.—M.V.

ABSTRACTS

Hb Tbingen. A New α-chain Variant (Tp10-12) with Increased Spontaneous Oxidation of hemoglobin was used to measure oral uptake, incorporation into red cells, and excretion and loss using a total body counter. Absorption of inorganic iron was low, incorporation ranged from 9% to 59% and was less than that of patients with chronic renal failure not requiring dialysis. Losses averaged 0.5%/day or more than five times that of nondialyzed patients with chronic severe renal failure. The losses would be readily explained by blood in the coil at the end of dialysis and losses incurred by the usual sample taking.—R.O.W.


The authors describe a family with congenital cyanosis due to NADH-dependent methemoglobin reductase deficiency. Two children, a 7-yr-old boy and an 18-yr-old girl, were homozygous descendent of acyanotic heterozygous parents which would imply a recessive inheritance for this deficiency. However, when the capacity of the red cells for methemoglobin reduction was measured using lactate as a substrate, inheritance of the trait appeared to be codominant with the genes acting in additive manner.—Z.R.


Congenital cyanosis caused by an abnormal hemoglobin, HbM Novi Sad, was found in a 5-yr-old girl and her mother. When all the hemoglobin was transformed to methemoglobin, the spectrophotometry revealed a new absorption maximum at 600 nm, together with irregularities in spectrum in the range between 550 nm and 630 nm. The overall appearance of the methemoglobin spectrum was that of HbM Saskatoon. Common with HbM Saskatoon was also the kinetics of the metranion of cyanide with methemoglobin: the reaction was slow with 0.66 mM cyanide concentration, but could be brought to completion with 13.3 mM cyanide concentration yielding a normal cyanmethemoglobin spectrum. These findings make HbM Novi Sad different from HbM Boston because, in the case of HbM Boston, the reaction with cyanide does not take place or is extremely slow. Other types of HbM have a normal kinetics of cyanide reaction or have abnormal cyanmethemoglobin spectrum. The fact that the described patient was cyanotic from birth raised the possibility that the abnormal hemoglobin is not identical with HbM Saskatoon. On acrylamide gel electrophoresis of the hemolysate with all hemoglobin transformed to methemoglobin, HbM Novi Sad appeared as a slow moving band, adjacent to HbA. Calculation of the kinetic data of cyanide reaction indicates that 25% of the heme groups in the patient’s blood is reacting abnormally. Abstractors’ comment: Amino acid analysis of the globin seems pertinent for the final identification of HbM Novi Sad.—Z.R.


Essential body iron amounts to 35 mg/kg; additionally up to 20 mg/kg are present in storage. Body losses are 12 μg/kg/day in the male and about 20 μg/kg/day in the female. The average male absorbs 5.5% and the average female 13% of dietary iron. In iron deficiency the upper limit of food absorption is 20%. Dietary iron averages 6 mg per 1000 calories. Iron deficiency in late infancy exceeds 25%; some 5 million adult women in the U.S. have iron deficiency anemia and five times that many have deficient iron storage. Iron deficiency in pregnancy may be as high as 25%. To improve the iron status in women, fortification of flour to 40 mg/lb is proposed. This would increase intake of iron in the male from 16.5 to 25 mg/day, but rate of absorption would decrease 5.5 to 3.6%. The author doubts that this increment in dietary iron would have a deleterious effect even on those males who are unable to regulate their absorption (hemochromatosis). In the light of the current controversy about the benefits vs. hazards of iron enrichment, this brief and lucid review article merits being read in its entirety.—R.O.W.

Hb Tübingen. A New β-chain Variant (βTp10-12) with Increased Spontaneous Oxidation of HbTübingen.
In two members of a German family suffering from a compensated hemolytic process and from mild cyanosis, an abnormal hemoglobin was detected. Methemoglobin was detectable in both patients, 8.4% and 8.5%, respectively. The abnormal hemoglobin was characterized by increased spontaneous oxidation, heat instability and normal methemoglobin spectrum. There were no Heinz bodies in the red cells. Electrophoretically the abnormal fraction migrated in a position between Hb F and Hb S; it could be separated as oxyhemoglobin and as methemoglobin but not as cyanomethemoglobin. Globin analyses showed a defect located in the core of the β-chain (β Tp 10–12, amino acid position 83–120). This anomaly does not correspond to any of the structural variants known so far.—K.B.


In a 22-yr-old German male suffering from drug-induced hemolytic crises a severely reduced activity of G-6-PD was found. The purified enzyme displayed normal electrophoretic mobility, normal pH-dependency as well as normal affinity for G-6-P and for NADP. However the turnover with Galactose-6-P and 2-deoxy-G-6-P was markedly increased; inactivation by 46°C was 70% within 1 hr.—K.B.


A 10-yr-old boy of German descent developed severe hemolysis after ingestion of fava beans. The activity of G-6-PD in his red cells was 1%–4% of normal. The enzyme was characterized by an increased affinity for G-6-PD (Km 26–28 μM), by a normal affinity for NADP, an elevated utilization of 2-deoxygalactose-6-phosphate and of galactose-6-phosphate, and by an abnor-
mal biphasic pH optimum (7.5 and 8.0-8.5). At 40°C the purified enzyme lost 80% of its activity within 60 min. Because of the normal affinity for NADP and some peculiarities of the electrophoretic properties, the authors claim that the enzyme is different from the Mediterranean type and represents a new variant.—K.B.


The method of Wachstein and Meisel was used for determination of enzymes that degrade adenosine triphosphate at pH 7.2 in smears of bone marrow and peripheral blood. A marked activity was found in reticuloendothelial cells of bone marrow, in peripheral blood eosinophils as well as in mature peripheral neutrophils. A less marked positive reaction was observed in monocytes of bone marrow and peripheral blood and in bone marrow neutrophils. A very weak and scattered reaction was present in plasma cells and lymphocytes. In blast cells a marked positive reaction was found only in six cases of leukemic lymphoreticuloses (in mature granulocytes) or atypical lymphadenoses.—L.D.


The earliest observed effect of erythropoietin in cell cultures is an increased incorporation of precursor into RNA among which are molecules with sedimentation constants ranging from 150 to 4S. This suggests that erythropoietin activates the transcription of many different genes within the nuclei of target cells. Although in the mouse the spleen is normally a minor organ of erythropoiesis, following bleeding or hemolysis it becomes a major site of red cell production. This conversion of the spleen from a lymphoid to an erythropoietic tissue follows an orderly sequence of events and is of reproducible magnitude. Erythropoietic mouse spleen was used as a model system to study RNA metabolism during erythroid cell development. The induction of hemolytic anemia with phenylhydrazine resulted in a fivefold increase in spleen weight and an increase in hemoglobin-containing nucleated cells to 70% of the splenic cell population. During this period, cytoplasmic RNA concentration increased threefold reaching a maximum 1 day prior to peak splenic erythropoiesis and 2 days before maximal splenic DNA concentration. Most of the accumulated RNA was ribosomal RNA (rRNA). In vitro measurements of cytoplasmic rRNA production revealed a rapid early increase in 28S and 18S rRNA synthesis or seven to nine-fold occurring 3 days prior to peak splenic erythropoiesis and diminishing within 24 hr. This increase in synthesis of rRNA was accompanied by an increase in nucleolar RNA content as demonstrated by histochemical staining. Nucleolar RNA content diminished in parallel with the decrease in rRNA synthesis. The synthesis of 28S and 18S rRNA was sensitive to low concentrations of actinomycin D as well as to puromycin, cyclohexamide, and chloramphenicol but not to bromodeoxyuridine. The results suggested that activation of erythroblast nucleoli and the concomitant increase in rRNA synthesis are early events in erythroid cell development.—M.S.


The administration of phenytoin to nine normal subjects did not affect the absorption of synthetic pteroylglutamate.—T.M.B.

ABSTRACTS

Fasting serum folate levels were not altered by taking oral contraceptives; if subjects were presaturated with pteroylglutamic acid, the absorption of pteroylglutamic acid and pteroylpolyglutamates were similar in control subjects and those taking oral contraceptives. However, if there was no presaturation, the rise in serum folate following administration of pteroylpolyglutamates was decreased in those taking oral contraceptives, and it is suggested that these agents may influence the rate of folate clearance from the blood.—J.M.B.


Despite the lower than normal levels of lipid seen in fetal plasma, adult erythrocytes, administered to Rh-erythroblastotic fetuses in utero, tended to increase their lipid content, particularly cholesterol. The increases approached normal neonatal red cell lipid values, particularly in one infant whose last intrauterine transfusion was 30 days prior to birth. These increases in lipid content were not accompanied by increased erythrocyte volume. Phospholipid fractionation of the transfused cells also changed, becoming more like the pattern seen in newborn infants, as did the phospholipid fatty acid distribution. Phosphatidyl choline levels exceeded those of healthy newborns, particularly in one infant with suggestive evidence of hepatic cell damage.—J.B.S.


Reticulocyte counts in normal term infants, and in term infants with significant intrauterine growth retardation were identical (4.9% and 4.5%). Reticulocyte counts for premature infants were higher (mean 8.8%) and seemed to correlate well with gestational age.—J.B.S.


An adolescent with sickle cell anemia and a compromised cardiovascular status, developed over a 1 yr period, increasing splenomegaly. Previous to this his hemoglobin ranged around 8/100 ml. With increasing spleen size, his hemoglobin dropped to 4.9/100 ml, and his red cell survival was found to be 3 days. After receiving a total of 1150 R to his spleen, spleen size decreased, and within 2 mo his hemoglobin rose to 9 g/100 ml where it remained during the following year. A liver/spleen scan indicated nonfunctioning RE tissue.—J.B.S.


The mean P50 in a group of newborn infants with jaundice was approximately 20 mm Hg, the usual figure given for newborns. Some of the infants were exchange transfused with very fresh blood, and a posttransfusion rise in P50 and in 2,3-DPG was noted. In the other infants, exchange transfusion was performed with blood 4-5 days old. In this group, there was a fall in P50 and in DPG. These differences appeared to persist for at least 24 hr. The range of values reported, and the SD of their means, are sufficiently broad, that the significance of the results is open to question.—J.B.S.


The tendency to development of severe anemia among Rh-erythroblastotic infants who are not exchange transfused in well known. When exchange transfusion is obviated by phototherapy, this risk is even greater, as evidenced by this case presentation, in which an infant became so anemic at 2 wk of age (Hct 9%) that he presented in acute heart failure.—J.B.S.
LEUKOCYTES


Enzymatic activity causing a positive Wachstein–Meisel (W.M.) reaction for ATPase (pH 7.2) in human leukocytes has been more closely specified. G-strophantine, an inhibitor of membrane ATPase, decreased the original activity by 8% to 79%. It seems therefore probable that ATPase activity on cellular membranes shares in the positivity of the Wachstein–Meisel reaction by a rather variable degree. Parachlormercuribenzoate, an inhibitor of mitochondrial ATPase decreased the reaction by 7% to 50% of original activity. The inhibited activity was not reactivated by BAL or L-cysteine and therefore could not have been considered a real mitochondrial ATPase. L-cysteine caused almost complete inhibition of the W.M. reaction. This fact proves that most probably the active enzyme is one of the alkaline phosphatases which is capable of hydrolyzing adenosine triphosphates (ATP and ADP) in conditions of the W.M. method. Final conclusions on the share of individual phosphatases or ATPase in the positivity of W.M. reaction cannot be drawn without a correlation with the biochemical assay of ATPase in subcellular fractions.—L.D.


Antilymphocyte globulin was prepared by immunization of horses with peripheral lymphocytes of patients with chronic lymphocytic leukemia or with lymphocytes from the tonsils of normal persons. Four patients with chronic lymphocytic leukemia were treated by intravenous injections of it. The titers of agglutination of their lymphocytes by the globulin were from 1:256 to 1:16,000. Daily doses were 40 ml. The administration had to be discontinued after 5, 7, 20, and 34 days, respectively because of severe reactions: Urticaria, headache, fever, generalized arthritis. In one patient thrombocytopenia with less than 1000/μm platelets occurred. No significant therapeutic effect could be detected during the time of treatment in the four patients.—K.B.


Plasma, leucocyte, erythrocyte, and total exchangeable potassium were measured in rabbits before and after induction of potassium depletion. Significant correlation was found between the change in the leucocyte potassium and the change in the total exchangeable potassium and it is postulated that leucocyte potassium may be a useful index of potassium depletion.—J.M.B.


The Y-specific fluorescent body can be identified in ordinary peripheral blood smears counterstained with a 0.5% aqueous solution of quinacrine dihydrochloride. The nuclei of large lymphocytes and monocytes were most easily read.—J.B.S.


It is still uncertain how many lymphocytes transform into blastic cells within 72 hr and what difference exists between the lymphocytes which transform and those which do not. In fact, it is difficult to distinguish the relatively small “blastic” lymphocytes from the “nonblastic” ones and there is a time difference among the various lymphocytes in commencing blastic trans-
Formation. To clarify these points, lymphocytes were cultured with PHA and small doses of colchicine (0.4 μg/ml). About 35% of lymphocytes obtained from healthy persons transformed into blastic cells within 72 hr by our method. Using the 3H-thymidine labeling method, it was confirmed that lymphocytes in conventional culture undergo mitosis once or twice within 72 hr. The addition of colchicine to the culture was to prevent mitosis of the cultured lymphocytes. Peripheral lymphocytes of chronic lymphocytic leukemia, of malignant lymphoma with or without increase in peripheral lymphocytes, and of filaria patients showed lower percentage of blastic transformation and the tendency to have a delayed reaction with PHA. It seemed preferable to count both the number of 3H-thymidine labeled cells and the cells in metaphase by our method to compare degrees to PHA blastic transformation. Peripheral lymphocytes of normal or CLL patients could survive for 28 days in culture in the presence of small (subthreshold) doses of PHA, but peripheral lymphocytes from patients with leukemic malignant lymphoma disappeared from the culture during the same interval.—K.F.


Thirty adults with active AML were randomly assigned to treatment with a sequence of cyclophosphamide (CY), 15 mg/kg i.v., vincristine (VCR) 0.025 mg/kg i.v. given 24 hr later, and a 48-hr i.v. infusion of arabinosyl cytosine (ara-C) begun 12 hr after VCR (CVC), or four doses of CY, 25 mg/kg i.v. given on successive days. Two courses of each therapy were repeated at 14-day intervals providing sufficient recovery of normal marrow elements. Both regimens induced remissions. The overall complete response rate was 50%. 3H-thymidine (3H-TdR) and 3H-arabinosylcytosine (3H-ara-C) labeling indices of tumor cells were performed before treatment. High labeling indices for both compounds correlated with clinical responsiveness, but a high index for either compound alone was insufficient. Sequential labeling studies were completed in nine patients with high density tumor (marrow replacement) treated with CVC. There was no consistent increase in 3H-TdR or 3H-ara-C labeling indices 24 hr after CY. —J.E.U.


In three-quarters of those patients with lymphoreticular tumors (reticulum cell sarcoma and lymphoreticular sarcoma) the disease begins in lymph nodes and in one-quarter of the cases of extranodular organs. If patients are suffering from primary lymph node involvement, the disease tends to be widespread. In patients with primary organ involvement, the disease remains limited to local regions for a longer time and shows a greater tendency towards a continuous rather than a discontinuous spread. Patients with primary lymph node and organ involvement have almost the same prognosis when receiving the same treatment. In stages I and II the 5 yr survival rates for primary lymph node involvement is 50%, for primary organ involvement, 59%. The corresponding percentage rates of permanent cure are 48% and 45%. Under present therapy conditions, the prognosis of both forms in the advanced stages III and IV is very unfavorable. Comparative prognosis renders it possibly advisable to classify patients with primary lymph node and organ involvement together.—J.E.U.


To investigate life shortening after partial body exposure, survival of 497 patients whose primary treatment was exclusively with x-rays and radium for squamous cell cancer of the cervix (localized and regional stages) is under continuing study. To eliminate early deaths due to cancer and to pro-
The authors report 24 cases of sarcoma Anti-AHP, a Group Specificity of Snail Origin. A. Majsky. Institute of Hematology

vide maximal follow-up time, the group includes only those who survived at least 5 yr and who were under age 55 at time of diagnosis. The patients were treated in 1932–1951 and were drawn from the University of California and Connecticut State Tumor Registries. Pelvic regional dose was calculated for each patient. Three dose groups were formed: < 23 mg-rads, 23-31 mg-rads, and 32-54 mg-rads. When dose groups were compared with one another within stage and registry, and also after pooling stages and registries, no statistically significant differences in survival were found. When uterine cancer deaths were excluded to adjust for the possibility that unsuccessful treatment might have masked the life shortening effect under study, again no association was found between radiation dosage and survival. In excess of seven cases of leukemia were predicted for the 10-yr period of 5 to 14 yr after treatment. To this time, no deaths have been attributed to leukemia.—J.E.U.


The authors report 24 cases of sarcoma of the spleen in which painful splenomegaly was often the only symptom. Blood examination showed anemia, increase of α-2 globulins and of sedimentation rate. Diagnosis was done by spleen biopsy in which sarcomatous cells were found. Splenectomy was effective only if other organs were not affected.—J.C.

**HEMOSTASIS**


Although they aggregate normally when exposed to added ADP, the platelets of newborn infants appear to have impaired ability to release endogenous ADP in response to collagen and adrenaline. The ingestion of membrane-active drugs (ASA, Phenergan, Nisentil) accentuates this defect, so that at in vitro concentrations of these drugs much below those which inhibit maternal platelet aggregation, cord blood platelets were totally inhibited from aggregating. The clinical significance of these findings is not known.—J.B.S.


The authors report the case of a girl with a hand deformity due to radial agenesis associated with a severe hemorrhagic syndrome. Beside the thrombocytopenia, they found a leukocytosis up to 68,000 WBC/cu mm eosinophilia and 10% myeloid cells among the nucleated cells. The marrow was hyperplastic without any detectable megakaryocytes. The karyotype was normal. Treatment was symptomatic. The authors report the 48 cases of the literature and believe that after 1 yr of treatment the prognosis becomes better.—J.C.

**IMMUNOHEMATOLOGY**

Anti-AHP, a Group Specificity of Snail Origin. A. Majsky. Institute of Hematology
It was proved with the help of anti-Aiip agglutinins that the group A2B is composed of two populations of red cells which differ in the content of AHP and A antigens. The mutual relation of those two populations varies from individual to individual. The incidence of atypical anti-A agglutinins is more frequent in the persons with a higher percentage of weak A and AHP antigens. Based on the different anti-AHP reaction with red cells of AiB and A2B groups a method which reliably distinguishes both groups was worked out.—L.D.


A comparative study of identification of Au-HAA in human sera using various immunoprecipitation methods was done. The results showed that the most suitable method is by using inhibitory electroimmunoprecipitation because its detection of positive results is better than that of direct electroimmunodiffusion. This is followed by radial immunodiffusion which has the disadvantage of late results caused by the necessary incubation period. Its advantage is a very high sensitivity which is very close to the sensitivity of the inhibitory method, but some of the positive results have to be further controlled. The double diffusion method of Ouchterlony has no advantage in the detection of Au-HAA in comparison with other methods mentioned.—K.F.


The techniques employed were the test tube method to study the "reaction on the cell surface," and the slide glass method to observe the "reaction around the cell." The latter reaction was thought to be more specific than the former. This method was tested in 126 cases of autoimmune hemolytic anemia (AIHA), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), chronic thyroiditis and in normal adults. Antibody-producing cells were prepared from peripheral blood, lymph nodes, and bone marrow. In a case of AIHA spleen cells were used at splenectomy. Antibody producing cells in TC-199 were incubated at 37°C for 30 min, then antigen particles were added to the cell suspension. The mixture was kept at room temperature for 30 min shaking gently. A drop of the cell layer was taken from the sediment onto a glass slide and observed with a phase contrast microscope. When latex particles covered more than one-eighth of the cell boundary or more than four coated erythrocytes adhered to the cell, these cells were counted as positive, i.e., cells in which antibody was produced. Autoantibody producing cells, by the latex method, were 10% to 32% in RA, and 0% to 12% in SLE. Higher values were obtained by the erythrocyte method. There was no direct correlation between the serum antibody titer and the number of positive cells. The positive reaction of SLE was specifically inhibited by anti-IgG serum and that of RA by anti-IgM serum. These data suggest that antibody producing cells of SLE and RA are producing IgG and IgM antibody, respectively.—K.F.


Thymic lympholysis was induced in mice by administration of cyclophosphamide. Significant increase of macrophages in the subcapsular layer of cortical zone of the thymus was observed. Some macrophages had lymphocyte debris in the cytoplasm indicating migration from elsewhere of macrophages into the thymus.—J.M.B.

The physiopathological mechanisms of blood cytopenias in rheumatoid arthritis were studied by radioisotopic techniques. "Hypersplenism" was observed in nearly all cases but was seldom the only cause of the cytopenias. A block of hemoglobin synthesis with storage of iron in the RES seemed to be the principal mechanism of anemia. Maturation abnormalities of bone marrow cells were not uncommon. Qualitative anomalies of polymorphonuclear leukocytes were noticed. Heterogeneity of findings from one case to the other can possibly explain the irregular effectiveness of corticoids and splenectomy in these patients. 

Abstracter's comment: Heterogeneity is true also for thrombocytes which can be increased and/or qualitatively abnormal.—J.C.


Serial liver scans at 3–10 day intervals are recommended in patients with CGD, for diagnosis and evaluation of treatment of liver abscesses or granulomas. Choosing the optimal time for surgical drainage, and, fairly precise localization of the abscess, can be aided by such studies.—J.B.S.


Study, to the end of 1967, of 382 patients having thymectomy during 1942-1964 provided no evidence that adult thymectomy is associated with increased incidence of neoplastic disease.—J.M.B.


Hypersplenism occurred commonly in a group of 13 patients with Wilson's disease and seven with liver cirrhosis; in these affected subjects the plasma volume and body hematocrit/peripheral hematocrit ratio were raised. The features of the hypersplenic syndrome in the two groups were similar.—J.M.B.


Three children aged 18 mo, 8 yr, 14 yr suffering from hemolytic–uremic syndrome with a lethal outcome, displayed a considerable accumulation of lipids in renal glomeruli and in the adjacent renocortical arterioles. According to the results of histochemical and electron microscopical examinations, most of these lipids appeared to be unsaturated nonpolar lipids showing a good acetone solubility and a small amount of phospholipids. In one case there was a mixture of cholesterol.—L.D.


A high value of serum muramidase in patients with refractory anemia, pancytopenia, or persistent monocytosis is a sign suggesting that the case may have a leukemic background or may be developing into leukemia.—F.W.G.