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ERYTHROCYTES

A STUDY OF THE ERYTHROPOIETIN CONTENT OF PLASMA AND URINE IN POLYCYTHEMIA VERA BY GORDON'S H-R METHOD. F. Gréc, L. Takácsi-Nagy. From the II. Department of Medicine, University Medical School, Budapest, Hungary. Orv. Hetil. 8:399–401, 1968.

Alterations of the hematocrit value and reticulocyte number of the rat were used for the determination of the erythropoietic activity of plasma extracts and urine. The degree of activity was given in so-called H-R units (Gordon). In the normal plasma there was no activity demonstrable in H-R units (mean values = 0.26 H-R units). The H-R values in plasma and urine from subjects with polycythemia were significantly increased (mean values = 4.7, resp. 5.5 units). Blood letting increased the erythropoietic activity of both the normal and polycythemic plasma.—S. R. H.


The visible absorption spectrum of acid ferrihemoglobin A2 differs from those of Hb A1 and Hb F by having a distinct absorption band instead of a slight inflexion at 530 mμ and also by higher extinction coefficient in the region below 600 mμ. Hypothetical absorption spectrum of pure ferri- δ-chains has been calculated on an assumption of identical spectral characteristics of oxidized α and β chains.—S. R. H.


Red cell survival in rabbits was deter-
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mined by labeling in vitro with $^{51}$Cr or in vivo with $^{32}$DFP. Eight to ten days later, hyperthermia was created by injection of pyrogen or milk or by external heating in a climate chamber. The hyperthermia was maintained for five consecutive days. During this febrile period definite anemia developed. The red cell survival data strongly suggest that this was due to hemolysis. Arguments are presented that this increased hemolysis was due only to the elevation of body temperature.—S. A. K.


Eight cases of PNH are reported. Only one patient fitted the classic description, the remaining seven cases presented as aplastic anemia, initially with a negative Ham's test or a longstanding pancytopenia. Signs and symptoms of PNH were encountered in two patients with myelofibrosis. An interesting aspect is that the majority of the patients had an abnormal EEG. The possible significance of this could not be determined. Abstractor's note: The occurrence of the PNH-defect seems to be considerably more frequent than the classic PNH-disease. It is, therefore, not merely an academic exercise to look for the PNH-defect in many patients. Demonstration of the PNH-defect is also of definite practical importance in the management of the patient.—S. A. K.


Serum vitamin $B_{12}$ was determined in healthy subjects and in 120 patients with various hematologic disorders using the method of radioisotopic dilution and coated charcoal. The results obtained by the radioisotopic technic were compared with the results obtained by the microbiologic method employing Euglena gracilis Z strain. The radioisotopic determination of vitamin $B_{12}$ was found to be much simpler and very accurate.—M. K.


Twenty patients with characteristic clinical and laboratory evidence of porphyria cutanea tarda were treated by phlebotomy of 2500-8500 ml. of blood over a period of 3-8 months. All showed dramatic remission of clinical manifestations and reduction of uroporphyrin excretion at the end of the course of therapy. The clinical response persisted, and in fact a progressive reduction in porphyrin excretion continued in 18 of the patients for periods up to 4 years after phlebotomies had ceased. The rearrangement in porphyrin synthesis from “useless” hepatic uroporphyrins to “useful” protoporphyrins by iron deprivation remains obscure. Nevertheless, the data seems sound and convincing.—H. S. J.


Hemin stimulates synthesis of globin and promotes and stabilizes the formation of polyribosomes in the intact reticulocyte in vitro. This action is thought to be of importance in maintaining synchrony of heme and globin production in the maturing erythroid cell. The present studies indicate that hemin concentrations which stimulate globin synthesis and stabilize polyribosomes also is a potent inhibitor of ribonuclease activity in the erythroid cell. It is suggested that hemin by inhibiting ribosomal disaggregation might act to stimulate globin production thus synchronizing hemin and globin availability.—H. S. J.


Utilizing short-term incubation of reticulocytes with tritiated leucine, followed by
trypsin digestion and peptide analysis of radioactivity, the authors investigated the kinetics of beta chain synthesis in beta-thalassemia. Normal rates of beta chain synthesis, and therefore messenger RNA translation were found in this disorder. The evidence indicates that beta-thalassemia reflects defective messenger RNA production. A less likely possibility, not ruled out by the present studies, is a defect in beta-chain initiation by otherwise normal messenger RNA.—H. S. J.


The recent important observations by Benesch and Yu (Proc. Natl. Acad. Sci. 59:526, 1968) that the oxygen affinity of hemoglobin is decreased by its interaction with organic phosphates, particularly 2,3-diphosphoglycerate, provoked these studies of the possible relationship of altered organic phosphate levels in red cells and oxygen binding in subjects at high altitude. Within 24 hours following change in altitude in either direction, a corresponding alteration in cellular 2,3-DPG and hemoglobin affinity for oxygen were recorded. For instance, significant increases in cellular DPG and decreases in hemoglobin affinity for oxygen occurred within the first 24 hours of climbing from sea level to 13,000 feet. Reciprocal changes occurred upon descent. Previous studies have indicated that increased glycolysis and organic phosphate production occur in red cells under hypoxic or anaerobic conditions. A compensatory mechanism is therefore suggested which would automatically control the effects of anoxia by decreasing hemoglobin affinity for oxygen and thus delivering increased amounts of oxygen to the tissue during anoxic states.—H. S. J.


Seven premature infants developed widespread edema and anemia with decreased DF32P erythrocyte survival about the second month of life. All the infants had been fed commercial formulas containing iron and high levels of polyunsaturated fatty acids, but low levels of Vitamin E. Serum tocopherol levels were diminished in all and prompt correction of anemia and clearing of edema followed Vitamin E supplementation. The mechanism of hemolysis was not elucidated although it was suggested that the catalysis of lipid peroxidation by iron might underlie red cell membrane disruption in these patients. Ab-stractor’s note: It is of interest that no pyknocytosis was demonstrable in these patients, unlike those reported by Oski and Barness, Journal of Pediatrics 70:211, 1967. —H. S. J.


Plasma hemoglobin concentration was studied in seven patients with PNH. The results confirmed that increases in plasma hemoglobin level were related to sleep and not the time of the day. They further demonstrated that this finding was not constant, an increase being present only in five of the seven cases. Contrary to previous reports, a sleep-related increase in plasma hemoglobin was also observed in a splenectomized patient. It has been suggested that the diurnal variations in hemoglobin in PNH may be inversely related to the plasma cortisol level; however, in the present study, fluctuations in plasma hemoglobin concentration could be observed also in patients on prednisone.—S. A. K.


Lactate dehydrogenase (LDH) activity and isozymes distribution was studied in peripheral plasma, bone marrow plasma, and lysates of bone marrow cells. Studies of thirteen hematologically normal individ-
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...uals confirmed previous findings that LDH in marrow plasma is markedly higher than in peripheral plasma. This LDH activity is dominated by anodic isozymes which probably are derived from erythroid precursors. In thirteen patients with a variety of hemolytic anemias, peripheral LDH was elevated in nine; among these were six of seven patients with intravascular hemolysis (paroxysmal nocturnal hemoglobinuria and microangiopathic hemolytic anemia). In contrast, the LDH concentration in marrow plasma did not differ from normal. This suggests that no significant hemolysis took place within the marrow. Finally, one patient with regional enteritis and granulocytic hyperplasia of the marrow was studied. LDH activity was markedly increased in marrow plasma but normal in peripheral plasma. In the marrow plasma the isozyme pattern showed a predominance of cathodic enzymes which are prominent in granulocytic cells. This suggests intramedullary destruction of late myeloid precursors or of mature granulocytes in this case. Abstractor's comment: it has been claimed that normal granulocytepoiesis, at least in the dog, is partially ineffective, with a large fraction of cells dying at the myelocyte-metamyelocyte stage. The present observation of high concentration of cathodic LDH in granulocytic hyperplasia suggests that under pathologic conditions, some of the late granulocyte precursors and/or mature neutrophiles die in the marrow.—S. A. K.


Reported experience with the sucrose hemolysis test (hemolysis after exposure of red cells to 10 per cent sucrose) as a diagnostic tool in PNH is still scanty. In the present study, the test was positive in all seven patients with PNH studied and negative in 20 normal controls. Among 50 patients with a large variety of hemolytic anemias, only three had slightly positive tests. In one of these, appropriate controls suggested that hemolysis was due to a potent red cell autoantibody. In the remaining two cases, the red cell concentration of acetylcholine esterase was lower than normal, and it is thus possible that they had the PNH-defect to a mild degree, in spite of a negative Ham's test. Some evidence is reported which suggests that the sucrose hemolysis test may be somewhat more sensitive than the Ham's test. It is concluded that the sucrose hemolysis test has definite value in the diagnosis of PNH. In contrast, the simplified version of the test, the sugar water test, was found to be too insensitive. Exposure of nucleated bone marrow cells from PNH-patients to sucrose did not result in lysis of the cells. It appears therefore that PNH bone marrow cells are insensitive to the factors which will lyse the denucleated red cells.—S. A. K.


The intensity of erythrocyte destruction was estimated by radiochromium used as a tracer of the patients' own erythrocytes. The results in eleven patients indicated that the life-span of erythrocytes and the radiochromium accumulation in the spleen and the liver were normal. When the disease was complicated by secondary hemolytic anemia the survival of erythrocytes was drastically reduced. The authors failed to establish a relationship between reduction of the erythrocyte life-span and extent of the spleen enlargement. In the aforementioned complication augmented accumulation of radio-chromium took place either in the spleen or in the liver or in both organs simultaneously. There were also individual instances showing prevalence of intravascular hemolysis. In such cases the isotope accumulation in said organs did not exceed normal values.—J. K.

Disturbances of Porphyrin Metabolism in the Clinic. L. I. Idelson. From the Central Postgraduated Institute, Moscow, U.S.S.R. Leningrad, 1968.

The book is devoted to disturbances of porphyrin metabolism in the clinic. It deals with modern concepts of biochemistry of
porphyrins and describes in detail iron metabolism and research methods for the study of porphyrins in the urine, feces and erythrocytes and also methods for the determination of some enzyme activities participating in the biosynthesis of porphyrins. Disturbances of porphyrin metabolism in patients with different forms of anemia are also described. These include iron deficiency anemia, anemia of infection and inflammation, hemolytic anemia, as well as hereditary sideroachrestic anemia. Methods of treatment for these anemias are discussed. Also reported are cases of idiopathic sideroblastic anemia and cases of lead poisoning. Particular attention is paid to clinical methods and treatment of the different forms. The book is illustrated with 28 tables and 20 figures. Bibliography contains 468 entries.—M. J.


Based on Herbert’s technic, which uses the radiodilution of a standard B₁₂⁵⁷Co vitamin mixed with vitamins from blood serum or other biologic fluids, the authors have standardized a method which increases the accuracy of the method originally described by Herbert and co-workers, mainly when the vitamin level in the serum are within or under their normal minimum. The modification consists in hydrolyzing the serum before the test in greater volumes than those used by Herbert and co-workers. During the second stage of the test, two dilutions of the hydrolized serum are used which allow greater accuracy. Standards are introduced in the experiment which permit evaluation of the validity of the result. A method to obtain a vitamin B₁₂⁵⁷Co standard is also described.—M. J.


An artificial standard for the colorimetric determination of hemoglobin pigments is prepared by suitable mixture of inorganic ions, giving a solution of constant optical density between 530 and 580 μ. This standard serves for the calibration of spectrophotometers as well as of less sensitive filter colorimeters. The authors describe its application for measuring the concentrations of oxyhemoglobin, cyanmethemoglobin, and methemalbumin, as well as for determining the percentage of blood saturation with methemoglobin, carboxyhemoglobin and sulfhemoglobin.—M. J.


The occurrence of acute hemolytic anemia during the course of infectious hepatitis appears to be an unusual complication, with only a few cases reported in the literature. The purpose of this communication is to report two examples of this association and the results of a review of 338 consecutive case records of infectious hepatitis studied in a 10 year period in the Hospital of the Instituto de Nutritión, México.—M. J.

**LEUKOCYTES**


The possible diagnostic value of the so-called atypical monocytes found in the peripheral blood was studied in malignancy. It was concluded that their presence can not be evaluated as a tumor-specific sign. There is, however, a correlation between their appearance, the decrease of serum iron level, the increase of serum copper level, and alterations of the serum protein
fractions. Based on cytochemical studies it may be assumed that atypical monocytes are of lympho-reticular origin, although according to most recent data the monocytes originate from the myeloid system.—S. R. H.


In experimental chloroma, protein synthesis in the cell nucleus is very active and application of DNase to the nucleus results in marked lowering of this activity. This fact suggests that protein synthesis in the nucleus is regulated by DNA or histone liberated by DNase. In the present series of studies, chemical properties of nuclear ribosomes were compared with those of the cytoplasm, correlation among histone, DNA, and ribosomes was examined, and the mechanism of information transfer from DNA to protein synthesis was discussed.—K. F.


In an electron microscopic survey of buffy coat cells from acute leukemia patients three of the patients displayed large numbers of 100 nm particles in some of the blast cells. The particles were exclusively cytoplasmic. Their general features were similar to those of viruses; they had an internal “shell,” a core and a cytoplasmic “envelope.” They resembled substantially the immature forms of murine leukemia viruses, but marked discrepancies were observed. Whether these particles are viruses remains to be proved by further experiments.—G. M.


A fatal case of agranulocytosis after a short term Pernasine treatment of schizophrenia and one case of recurrent agranulocytosis after chlorpromazine therapy are described. It is to be noted that in the second case the administration of phenothiazine derivatives other than chlorpromazine (for instance Sparine) did not evoke granulocytopenia. Susceptibility of the hematopoietic system to Pernasine has not been as yet described.—M. K.


Pretreatment of AkR and (CBA-T6 × AkR) F1 mice with Corynebacterium parvum (C parvum) vaccine induced a considerable increase in resistance to the growth of transplanted AkR leukemia cells. This beneficial effect was more pronounced in F1 hybrids than in the syngeneic recipients. Whereas all control animals inoculated intraperitoneally with 100 AkR leukemia cells were dead within 24 days, 50% of AkR mice and 83% of F1 hybrid mice survived the disease when they had been treated 7 days previously with 1mg. C. parvum intraperitoneally. This protective effect was reduced if the number of leukemia cells transferred was increased from 100 to 1000, or if the C. parvum was injected intravenously rather than intraperitoneally. The necessity of injecting both C. parvum and leukemia cells by the same route suggests that local factors were important in this phenomenon of induced resistance. Although the administration of C. parvum increased the phagocytic activity of the R. E. S. and diminished the level of liver catalase activity in parallel with the increased resistance to leukemia, it is not considered that either of these factors played
a determinant role. The authors suggest that the mechanism involved may be an adjuvant effect in both syngeneic and F1 hybrids hosts against leukemia specific antigens in the transplanted cells, which may be supplemented in F1 hybrids only by the phenomenon of allogeneic inhibition. —G. M.


Three in vitro established cell lines (JLS-V5, JLS-V6 and T5) all derived from mixed thymus-spleen cultures from normal Balb/c mice, were studied for the production of hyaluronic acid. In experiments extending over a period of several months, the production of hyaluronic acid accumulating in the medium regularly, was 3 to 5 times higher per 24 hours in the JLS-V5 line chronically infected with the Rauscher leukemia virus than in the JLS-V6 line non infected with this virus. The T5 line, developed in vivo passages from the JLS-V5 and carrying a highly attenuated non leukemogenic variant of the Rauscher virus, showed a level of hyaluronic acid production similar to that of the JLS-V5 line. These data are discussed in connection with a similar phenomenon observed in cells infected and transformed by another RNA virus, the Rous sarcoma virus. —G. M.

**HEMOSTASIS**


This paper describes a twenty-year old man with congenital deficiency of Factor XIII (FSF) and a life long hemorrhagic disorder. Although the hemorrhagic manifestations were in accordance with the now well recognized symptomatology of this deficiency syndrome, intramuscular bleeding was a predominant symptom in this patient. All bleeding manifestations were controlled by transfusion of fresh or stored plasma. The “usual” clotting tests were normal. The diagnosis was made with the 5 M urea solubility test and confirmed by crossmatching with another factor XIII deficient patient. Relevant data on the role and the mode of action of factor XIII were presented.—Z. R.

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**HEMOSTASIS**

**FIBRINOLYTIC ACTIVITY OF BLOOD IN PATIENTS WITH CHRONIC COR PULMONALE.** M. Bielawiec, H. Łukjan, A. Poniecki. From the 1st Department of Internal Medicine, School of Medicine, Białystok, Poland. Pol. Arch. Med. Wewn. 41:325–330, 1968.

Fibrinolytic activity of blood was examined in 63 patients with chronic cor pulmonale. The inhibition of blood fibrinolytic activity was demonstrated and it could be related to the degree of cardiac insufficiency. In patients without circulatory failure the fibrinolytic activity was elevated and it decreased in parallel to the severity of the cardiac failure. Oxygen breathing enhanced the fibrinolytic activity in patients from pathologic clones to mature granulocytes was confirmed. It was shown that 6-mercaptopurine would paradoxically increase the proliferation of the pathologic cells. Location as well as maturation and proliferation of each clone were compared. It was shown that accelerated proliferation in combination with a relatively rapid differentiation serve as one of the causes for the predominance of the pathologic clone. —J. K.
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with chronic cor pulmonale as well as in healthy subjects. This effect of oxygen breathing may be important for the prophylactic therapy of thromboembolic complications in chronic cor pulmonale.—M. K.


A decrease in platelet count and shortening of prothrombin and clotting time were observed together with a statistically significant increase in the level of plasma factor V after artificial abortion during the first trimester of pregnancy. All examined parameters were within normal limits in the pregnant women before the interruption of pregnancy.—M. K.


Seven patients with PNH were studied. "Laboratory hypercoagulability" was found in six, as indicated by a short kaolin-cephalin time, accelerated thromboplastin generation screening test and/or increased activity of factors II and VII and VIII. Serial studies in individual patients showed that "hypercoagulability" increased during periods of increased hemolysis which were usually accompanied by abdominal pain, possibly caused by small mesenteric thromboses.—S. A. K.

IMMUNOHEMATOLOGY


The cold agglutinin titre of the examined serum with "O" Rh negative red blood cells was 1:4,000,000 at +4 °C. By agar gel electrophoresis a paraprotein was detected between the beta-2 and gamma globulins. By immunoelctrophoresis a gamma-M specific, kappa type paraprotein was demonstrated. The isolated cold agglutinins were gamma-M paraproteins. The patient had a secondary antibody deficiency syndrome. Cold agglutinin disease is considered a primary paraproteinemia similar to Waldenström macroglobulinemia.—S. R. H.


The authors recommend administration of packed erythrocytes and albumin instead of whole stored blood or concentrated blood for exchange transfusion in newborns. Administration of blood with high hematocrit values removes symptoms of hypoxemia in the newborn and has a good therapeutic significance. The transfusions of whole blood were followed by symptoms of overloading of the circulatory system and cardiac or brain hypoxemia. These observations were made on carefully studied groups of 20 newborns each and they seem to be of practical importance for the treatment of the hemolytic syndrome by the method of exchange transfusion.—M. K.


Transformation of lymphocytes cultured with phytohemagglutinin (PHA) in vitro was assessed using an autoradiographic method in eight patients with chronic lymphatic leukemia, before and at various times after therapeutic splenic irradiation. In all except one case, irradiation of the spleen resulted in a marked fall in the PHA-insensitive lymphocyte population. In 3 cases, all of which showed evidence of impaired marrow function, there was a con-
comitant depression of the PHA-sensitive population. In one patient it was possible to characterize the PHA-insensitive lymphocyte population morphologically. Microspectrophotometric determinations of the nuclear DNA content showed that the abnormal lymphocytes constituted a tetraploid mutant population coexisting in the peripheral blood with a morphologically normal diploid small lymphocyte population. Cytogenetic analysis confirmed that only the diploid lymphocytes proceeded to mitosis when incubated in vitro in the presence of PHA. Splenic irradiation in this case exerted a greater effect on the PHA-sensitive lymphocytes. The significance of the findings in relation to the known immunological defects which occur in chronic lymphatic leukemia is briefly discussed.—G. M.


Autoantibodies against intrinsic factor, gastric parietal cells, thyroglobulin, thyroid cell cytoplasm, pancreas, adrenals and nuclear substances were found in some sera of Addison-Biermer patients associated or not with thyroid disease. In non addisonian megaloblastic anemias, the association of various antibodies was quite rare. The various antibodies seemed to have a narrow specificity.—J. C.


Lymph node cells with an intensely basophilic cytoplasm can easily be demonstrated on histologic sections using stains such as Mann-Dominici or slow Giemsa. Their morphology and distribution enable one to distinguish lymphocytes of 4 types: pulp or follicular basophilic cells which give birth to lymphocytes in the resting or hyperplastic lymph node, to plasmocytes and “irritative” basophilic cells in response to antigenic stimulation. Recent experimental work, e.g., lymphocyte culture and drainage of stimulated nodes, seems to prove the lymphocytic origin of the “irritative” cells. The latter give rise to plasmocytes and diffuse throughout the lymphatic system to increase the immune response. Cytoplasmic basophilia and pyroninophilia which are found in all these cells are due to the presence of increased RNA and correspond under the electron microscope to the appearance of numerous ribosomes and/or ergastoplasm which are all signs of increased protein synthesis. In human pathology, these basophilic cells are stimulated by various types of agents and lead to hyperplastic reactions. Their localization is of help in diagnosis.—G. M.

MISCELLANEOUS


An infant born after an unsuccessful attempt to terminate the pregnancy with methotrexate, demonstrated multiple anomalies, including dextrocardia, oxycephaly, low-set abnormal ears, hypertelorism, micronathia, simian creases, and only a single toe. Similarity to other infants born after maternal ingestion of folic-acid antagonists can be seen in the review of the literature.—J. B. S.


Repeated injections of tritiated thymidine into pregnant rats resulted in complete labeling of all cells in the bone marrow of
newborn offspring. This model was used for studying the development of primitive bone marrow cells after birth. The histologic and autoradiographic studies showed that the cartilage cells of the endochondral bones disintegrated focally, producing empty lagoons which were then populated by 100 per cent labelled cells from the primitive bone marrow cavity. From here, the pattern of thymidine labeling studied as a function of time, indicated that there are two types of cytokinetic behavior: (1) cells belonging to the matrix (osteoblasts, reticulum cells, endothelial cells) remained labelled for a prolonged period of time (labeling index 80-90 per cent at 38 days after birth), (2) other cell categories had a rapid decrease of the labeling indicating rapid renewal (erythopoietic and myelo-poietic cell types). The method used should prove to be a useful tool for future quantitative evaluation of cell systems with different renewal rates.—H. J. H.


Exploration of occult gastrointestinal hemorrhage done with $^{51}$Cr-labeled erythrocytes is a valuable diagnostic aid. It helps to recognize not only the blood loss, but also to estimate it quantitatively. In cancer, occult bleeding was found to be of a continuous character, while in gastric and duodenal ulcers it proved to be intermittent. The method can be successfully used in patients with iron deficiency anemias of obscure origin and in those suspected of suffering from cancer of the gastrointestinal tract in whom no clearcut roentgenologic signs are available.—J. K.


Granulocyte alkaline phosphatase activity was low in viral hepatitis, whereas in obstructive jaundice produced by gall stones high values were observed. Increased activity in malignant obstructions could not be regularly detected. In viral hepatitis following the administration of prednisolone, leukocytosis and increase of granulocyte alkaline phosphatase activity generally did not develop.—S. R. H.

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Cytogenetic Findings in Erythroleukemia by Heath et al. Page 453—Peter H. Wiernik’s name was misspelled.
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