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THE ERYTHRON


Sixty-six cases of pernicious anemia were treated exclusively with a B12-intrinsic factor preparation. Twenty-two of them became refractory to the treatment, i.e., 33 per cent. The refractoriness developed on an average after 12 months, with a range from primarily suboptimal response to 29 months. In 17 of the refractory cases, the Schilling test was performed with B12 alone, then with B12 together with 10,000 cci units of intrinsic factor concentrate, and finally together with 50 ml. neutralized human gastric juice. A low excretion was detected after the oral administration of B12 alone or together with the intrinsic factor preparation, but a normal or considerably increased excretion was observed in all cases but five tested with human gastric juice. A crude preparation of intrinsic factor from human gastric mucosa was prepared and tested together with radioactive B12 vitamin in 9 of the refractory cases. The excretion was significantly increased in 5 cases, but no change was observed in 4 of them.—C. W.

APLASTIC ANEMIA: AN ANALYSIS OF 50 CASES. D. N. Mohler and B. S. Leavell. From the Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Va. Ann.Int.Med. 49:326-362, 1958.

This paper is a careful analysis of 50 patients suffering from a “morphologic or functional inadequacy of the bone marrow.” Patients were excluded from this study if they had chronic infection, malignancy, malnutrition, renal disease or liver disease. Toxic exposure was suspected in 7 cases (benzol, mesantoin, phenylbutazone, arsenic and chloramphenicol). The authors tabulated 12 previously reported series of patients with aplastic anemia and found a toxic exposure in 21.5 per cent of 362 cases. In one-third of their own cases, lymphadenopathy, splenomegaly and hepatomegaly were found, presumably related to the greater use of transfusions in recent years. Testicular atrophy was present in 14 per cent, but testosterone in physiologic dosage was without therapeutic
effect. The anemia was macrocytic in 64 per cent with an M.C.V. greater than 115 cubic microns in 14 per cent. Lymphocytosis (>40 per cent) was found in 86 per cent. Reticulocytes were not infrequently elevated. In 74 per cent the bone marrow was hypoplastic, but 10 per cent had hypercellular marrow. In most cases more than 20 per cent of the bone marrow cells were lymphocytes. Leukocyte alkaline phosphatase was generally subnormal. The possible relationship to hemochromatosis and leukemia is discussed, as well as prognosis and treatment. A complete remission occurred in 6 patients and partial and temporary remission in another 6 patients.—A. J. E.


The authors propose a method of quantitative estimation of granulopoiesis based on the release of erythrocytes (C.R.Acad.Sc. 242:2663, 1956.) and the ratio of granulocytes to erythroblasts in the bone marrow.

This method could not, of course, be applied to pathologic states unless it could be demonstrated that the duration of various mitotic and intermitotic phases are not modified for erythroblasts and granulocytes. (The latter could be indeed modified by certain toxic agents, e.g., alkylating agents and by radiation.)—G. M.


In this short note, the authors state the possibility of measuring the production (or release) of erythrocytes by the bone marrow, proceeding from the following data: life span of erythrocytes as measured by Cr51, changes accruing to the hematocrit and the blood volume during the test.—G. M.


This paper is basically identical with that of Combrisson-Le Bolloch et al. (C.R.Acad.Sc. 242:2663, 1956.) Erythropoiesis is quantitatively estimated from the same data: life span of erythrocytes as measured by Cr51, changes occurring in the hematocrit and the blood volume during the test. The meaning of the curvilinear curve of disappearance of Cr51 is discussed; the index of chromium elution is measured in normal subjects.—G. M.


Since sedoheptulose is formed in erythrocytes as a product of glucose-6-phosphate oxidation during incubation with D-glucose, the existence of this process in erythrocytes has been proved. The original level of the sedoheptulose in the incubates (8 mg. per cent) following the incubation with glucose rose from the basic value by 37 per cent; after adding methylene blue to the medium it rose by 69 per cent. Following incubation with D-ribose, the increase was not significant. This paper presents a comparative review of current methods of sedoheptulose estimation, and the possibilities of utilizing this enzyme system in erythrocytes for clinical research.—L. D.

For many years, mammalian erythrocytes other than those of man and ape were considered to be impermeable to glucose. However, species differences in glucose permeability hitherto unknown have been established, although the reasons underlying these differences are not known.—O. P. J.


Erythrocytes of rats were incubated with glucose, and the increase of sedoheptulose and pentose in the incubate was checked.

The rats were divided into four groups: (I) control animals, (II) animals after administration of cortisone, (III) animals after adrenalectomy, and (IV) animals after adrenalectomy and treated with cortisone.

The data support the authors’ opinion that the glucocorticoids activate the oxidative cycle of glucose-6-phosphate. An increase after adrenalectomy is explained as a consequence of removing the inhibitory effect of cortical androgens. The effects could be partially explained in terms of higher enzymatic activity of younger erythrocytes (post-operative reticulocytosis).—L. D.


Adenylate kinase is an enzyme which catalyzes the conversion of ADP to ATP and AMP. In the present paper, an earlier observation that adenylate kinase is present in erythrocytes is confirmed. The ATP, formed by the action of this enzyme, unlike externally added ATP, can pass either into the interior of the cell or into the external medium. This paper thus suggests another means by which external additives may increase intracellular high energy phosphate compounds in the red cell.—E. B.


Glucose-6-phosphate dehydrogenase activity in erythrocytes is significantly higher in newborn infants than in adults, and is higher in premature than in full-term infants. After 36 weeks of age all infants tested showed levels within the adult range. 6-phosphogluconic dehydrogenase and aldolase were similarly increased in the newborn. Newborn infants, however, demonstrate marked glutathione instability of erythrocytes incubated with acetylmethylhydrazine. This instability, obviously unrelated to the levels of G6PD, continues for at least 52 hours after birth, but not long after 95 hours. The reason for the transient glutathione instability in the newborn is as yet not known.—I. S.


Hemolytic anemia occurs in only a small percentage of children known to have ingested naphthalene (moth balls). The majority of cases of naphthalene hemolytic anemia has occurred in Negroes. The racial incidence and individual susceptibility is similar to that found in primarquine-induced hemolytic anemia and suggested the possibility of a common mechanism, namely a congenital defect in glutathione metabolism related to a deficiency of glucose-6-phosphate dehydrogenase in the erythrocytes.

Four Negro patients with naphthalene-induced hemolytic anemia were studied. Two of these consisted of a mother who had ingested naphthalene, and her newborn infant, both of whom manifested hemolytic disease. The whole blood glutathione levels were normal in all four patients and remained so for several months after the hemolytic episode.
The glutathione sensitivity test, after incubation of blood with acetylphenylhydrazine, was consistently abnormal in all.

In vitro tests indicated that naphthalene itself was innocuous but that its metabolic products, alpha and beta naphthols, and naphthoquinones, produced marked decreases in glutathione concentration in sensitive erythrocytes. The alpha group was the more active in this respect.—J. S.


Mature human erythrocytes possess the enzyme potential to recycle five carbon fragments produced in the hexose monophosphate pathway to glucose-6-phosphate. One of the reactions in this recycling is the transketolase reaction in which two molecules of pentose phosphate form one molecule of heptulose phosphate and one molecule of triose phosphate. The transaldolase reaction which follows the transketolase reaction results in the formation of a hexose phosphate molecule in which the 2-carbon of the originally oxidized glucose molecule now occupies the one-position.

When red blood cells are incubated with glucose specifically labeled in the C2 position, the recovery of C14O2 may be considered indicative of the efficiency of the recycling mechanism. Thiamine pyrophosphate is known to be an essential co-factor in the transketolase reaction. In the present study, the recovery of C1 and C2 as CO2 from erythrocytes incubated in the presence of methylene blue has been compared for normal subjects and subjects with Wernicke's encephalopathy. The effect of thiamine therapy has also been observed. Erythrocytes of patients with Wernicke's encephalopathy showed a failure to utilize the second carbon of glucose in CO2 formation at a normal rate. This is interpreted as indicating impairment of the transketolation mechanism. The defect in transketolation was partially reversed after treatment of the patient with thiamine. In vitro addition of thiamine or cocarboxylase did not significantly alter the defect. Patients exhibiting clinical signs of thiamine deficiency who did not have ophthalmoplegia showed no abnormality of glucose metabolism. This paper represents an excellent example of successful exploitation of the human erythrocyte as a metabolic model in which to study disease states.—E. B.

**HEMOLYTIC ANEMIA DUE TO THE ABSENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN THE ERYTHROCYTES AS A DOMINANT HEREDITARY DISEASE. G. W. Lohr and H. D. Waller. From the Medical University Clinic, Marburg/Lahn, Germany. Klin.Wschr. 36:865–869, 1958.**

One year ago the authors described hemolytic anemia in a 30 year old Persian man whose erythrocytes showed absent glucose-6-phosphate dehydrogenase. Erythrocytes of his half brother (same mother, fathers were brothers) had the same defect, but a paternal first cousin was normal. Additional family history suggested six other cases of hemolytic anemia, all in the maternal family. Since the defect is similar to that reported in favism and certain drug-induced hemolytic anemias, a common mechanism is suspected.—H. M.

**HEMOGLOBIN CATABOLISM. I. GLUTATHIONE PEROXIDASE, AN ERYTHROCYTE ENZYME WHICH PROTECTS HEMOGLOBIN FROM OXIDATIVE BREAKDOWN. G. C. Mills. From the University of Texas Medical Branch, Galveston, Tex. J.Biol.Chem. 229:189–197, 1957.**

Ascorbic acid undergoes coupled oxidation with hemoglobin to form choleglobin. The author shows that GSH inhibits choleglobin formation when the hemoglobin is supplied as a red cell hemolysate, but not when crystalline oxyhemoglobin is used. It is demonstrated that the hemolysate contains a heat-labile, nondialyzable substance which catalyzes the oxidation of GSH by H2O2. This enzyme is shown to differ from catalase and is named glutathione peroxidase.—E. B.

Choleglobin and methemoglobin formation has been studied in repeatedly washed intact red cells exposed to ascorbic acid. The greatest choleglobin formation occurred when azide was added to the cells to inhibit catalase activity and when no glucose was present. When glucose was added, choleglobin formation was largely prevented, even in the presence of azide. Choleglobin formation appears to correlate well inversely with maintenance of GSH levels. Similarly, in hemolysates, substrates known to keep GSH in the reduced form were effective in preventing choleglobin formation. The author concludes that the GSH-GSSG peroxidase system may play an important physiologic role in the protection of hemoglobin.—E. B.


The experiments reported in these two papers were undertaken to study the separate inward and outward rates of K movement with the aid of K at various NaF concentrations and the effect of pyruvate on the NaF effect. It was possible to show, by using the technic of differential osmotic hemolysis, that the rapidly exchanging K represents all the K in a fraction of the cells, rather than a fraction of K in all the cells. Metabolic studies on these cells show that the only co-factor of glycolysis reduced in the NaF-poisoned system is adenosine triphosphate.—O. P. J.


The properties displayed by ghost systems are generally considered to represent properties characteristic of the plasma membrane of intact cells. In this investigation the diffusion of hemoglobin during the initial hemolysis of intact cells, the rehemolysis of the resulting ghosts, the relation of these and other properties to intact cells were studied. Although the ghost suspended in its hemolysate will revert to its original volume upon reversal, this volume subsequently changes to a new stable level when the ghosts are washed or resuspended in an isotonic solution of different ionic composition. The reconstituted ghosts were of smaller volume than the original intact cells.—O. P. J.


Red cells of differing ages were separated by centrifugation in 30 per cent bovine albumin. By labeling with Fe it was confirmed that newly formed red cells are less dense than old cells. Young cells were found to contain more potassium and lipid, and less sodium than old cells, but no difference was found in the phosphate ester or glutathione content. The difference in lipid content appeared to be mainly in the phospholipid fraction.

The osmotic fragility of young and old cells is similar but old cells show a greater increase in fragility on incubation in their own plasma.—R. M. H.


Seventeen persons with blood group A1, Rh received each within three days 1500 ml blood of the group O, Rh+. One to seven days later blood was withdrawn and further
Blood specimens were taken off until the 94th day. The transfused erythrocytes were separated by differential agglutination and the activity of nine different enzymes was estimated.

Significant diminution was only found in the content of phosphoglyceraldehyde dehydrogenase (PGADH) and of glucose-6-phosphate dehydrogenase (G-6-PDH). When 70 per cent of PGADH is lost there occurs a diminution of ATP, and the loss of G-6-PDH leads to deficient synthesis of nucleotides. Since in bank blood too the activity of PGADH and of G-6-PDH show the greatest lability it should be determined whether these observations are of importance in blood storage.—H. M.


The resolving power of starch gel is higher than that of paper and suggests that new hemoglobins may be discovered. The case of hemoglobin isolated from blood samples collected in South Vietnam, with a mobility slower than that of hemoglobin A demonstrates the usefulness of the technic.—G. M.


These methods have demonstrated three to four different antigenic fractions in hemoglobins A, S, E, C and F. At least three of them are common to hemoglobins A, S, E and C. Hemoglobin F has no definite antigenic specificity: its solutions contain at least two fractions, the antigenicity of which is identical to those of the constituents of hemoglobins A, S, E and C. One of them is the “slow fraction” as obtained by paper electrophoresis.—G. M.


The blood of a young Englishman was found to contain 27 per cent of a new hemoglobin variant, which moved slightly faster than hemoglobin J on paper electrophoresis at pH 8.6. It could also be distinguished from hemoglobin J by its partial separation from hemoglobin A on paper electrophoresis at pH 6.5 and pH 7. Other properties of the new hemoglobin are listed. It was present in the same proportion in the blood of the father and brother of the propositus. None of the affected subjects have any clinical or hematologic abnormality.

A new scheme for the naming of hemoglobins is put forward as a basis for discussion. In this scheme, the fetal hemoglobins are divided from the adult variants, and the latter are grouped according to their electrophoretic mobilities on paper at pH 8.6, as follows:

**Fetal**
- F, “Fessas and Papaspyrou,” “Bart’a.”

**Adult**
- Group I or C group: C, A1, E-A2, O.
- Group II or S group: S-D and its variants.
- Group III or G group: L-P, G, Q.
- Group IV or A group: A-M.
- Group V or J group: K, J, “Norfolk,” N.
- Group VI or I group: I-B.

It is suggested that the hemoglobins within each group could be numbered according
to their mobility, and that those with the same mobility could be given the additional letters a, b, c, etc., according to the time of their discovery.—R. M. H.


Hemoglobin J was found in two unrelated women of Gujarati-speaking Lohana stock. One of them also carried a thalassaemia gene. There was no evidence for any detrimental interaction between the two genes.—R. M. H.


Electrophoresis of hemoglobin in starch gels permits the separation of the A\(_2\) fraction and a chromoprotein from the A hemoglobin. With this technic, an elevation of the A\(_2\) fraction in cases of thalassemic trait can be observed.—G. M.


The Eti-Turks form a small Arabic-speaking population living near Mersin, in Southern Turkey. In this survey, just over 100 specimens of blood from Eti-Turks attending a hospital out-patient department, and a similar number from an unselected group of Turks from the same region, were examined for blood groups. It was found that the ABO, MNS, Rh, Lutheran, Kell and Duffy blood group frequencies of both groups resembled those of southern and eastern Europe rather than those of Asia, the Turks tending a little more towards Asiatic features than the Eti-Turks, by virtue of their slightly higher frequencies of B and Fy*.

Sickling is common in the Eti-Turk population, but no sickle cell hemoglobin was found in 240 samples from Turks. Hemoglobin E was found in two of 138 Eti-Turks examined, and hemoglobin D in one of the 240 Turks. The pedigree of an Eti-Turk family with hemoglobins A, S and E is shown. Thalassaemia occurs in both populations.—R. M. H.


After a description of the methods used to establish a blood bank from a somewhat reluctant donor population in Cyprus, the distribution of ABO groups in 10,000 Cypriots is presented. This is very similar in the Greek-speaking and Turkish-speaking Cypriot communities, both of which show low O and high A frequencies. In this they resemble the inhabitants of the neighboring Asiatic mainland rather than those of Greece. The ethnologic significance of this finding is illustrated by a thumbnail sketch of the history of Cyprus since the days of ancient Greece.

In a series of 2000 tests, only 2.5 per cent of Greek-speaking and 3.2 per cent of Turkish-speaking Cypriots were found to be Rh(D) negative. No explanation is offered for these frequencies, which are the lowest in the Mediterranean basin.—R. M. H.


A transitory polyagglutinability of the erythrocytes is recorded, lasting approximately

Some new aspects of the agglutination of tanin-treated red blood cells have been described. The factor inducing the hemagglutination appeared to exist in pathologic as well as normal sera. The hemagglutinating activity was, however, more powerful in some pathologic states, either as a result of increased hemagglutinating factor or decreased inhibitory factors.—L. D.


The authors recommend, on the basis of new observations on the properties of bilirubin, that quantitative estimation of direct and indirect bilirubin in the serum of newborns with hemolytic disease should be regularly carried out. From the viewpoint of the metabolism of bilirubin, hemolytic disease can be divided into two forms: the first with primary fault in the conjugation of glucuronic acid, and a second form with simultaneous abnormality of excretion of direct bilirubin from the liver. Conclusions are presented in the discussion which are derived from these observations and observations on therapy. First experiences with the use of thiocaprylylic acid in the treatment of hemolytic disease of the newborn are presented.—L. D.


This study concludes that citrated plasma is not suitable for transfusion purposes. Citric acid is a strong acid which removes cations from bicarbonate binding, thus producing acidosis and decreasing the rate of CO₂ loss from the body. Since it is not rapidly filtered in the capillary bed and is not oxidized, it can result in severe metabolic disadvantage to the patient. The unfavorable conditions for citrate removal from the blood are still worse with hemorrhagic shock because of low blood pressure and tissue anoxia, precisely when large quantities of transfusion fluids are administered. Moreover, in higher concentration citrate binds cations and leads to cardiac failure. Other anticoagulants should be sought for blood preservation.—L. D.


Thirty per cent of the blood volume of dogs was withdrawn under narcosis in 15 minutes. After a 30 minute interval, this amount was transfused in 5 minutes (6 ml./Kg./min.).

All dogs died with a citrate concentration of 0.76 Gm. per cent. With citrate level at 0.38 Gm. per cent cardiac failure occurred, but all dogs survived. There were no toxic signs at 0.25 Gm. per cent, the course being the same as transfusion with heparinized blood. On the basis of these experiments, it is suggested that the citrate in preserved blood be lowered to 0.25 Gm. per cent of undiluted blood, which will increase the safety of transfusion from the viewpoint of citrate toxicity.—L. D.