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ERYTHROCYTE PHYSIOLOGY


Normal rabbit erythrocytes were suspended for four hours at 37 C. in plasma from normal rabbits and from rabbits poisoned with litharge. At the end of this time reticuocytes and stippled cells were counted. The cells in the abnormal plasma showed the presence of 56 to 139 stippled cells per 1,000 red cells in contrast to those in normal plasma where there were only 3 to 7 per 1,000. There was no apparent effect on the number of reticuocytes.—S.T.C.


Smears from embryonal mouse and from human umbilical cord blood have been exposed to a solution of ribonuclease at 60 C. for from ten to ninety minutes and afterwards treated with Giemsa’s stain. Basophil granulation, substantia reticulofilamentosa and polychromatic plasma have been fermented in a similar way with ribonuclease. The author therefore considers these substances to be of identical constitution chemically.—C.M.

The Erythrocyte Coproporphyrin and Its Variation in Respect to Protoporphyrin and Reticuocytes in Certain of the Anemias. C. J. Watson. From the Department of Medicine, University of Minnesota Hospital, Minneapolis, Minn. Tr. A. Am. Physicians 65: 219-229, 1950.

Detailed studies are described of the erythrocyte coproporphyrin (ECP) which is correlated in amount with the reticuocyte percentage. The consistent lag between ECP and reticuocytes, especially in pernicious anemia in relapse, indicates that these are sequential phenomena in the same process. The ECP was studied in the common anemias. The conclusion is reached that ECP is a sensitive index of actual or attempted hemoglobin synthesis.—T.R.T., Jr.


This is a preliminary report of the effect of glycerol in preventing hemolysis due to freezing and thawing. Heparinized rabbit or guinea pig blood diluted with equal volumes
of glycerol in Ringer's solution or normal NaCl was apparently well preserved after freezing quickly to -79°C, storing at this temperature for periods up to three months and thawing at +40°C. The most satisfactory concentration of glycerol was 10 to 15 per cent. One sample of human blood treated in this way showed only slight hemolysis after eight weeks.—S.T.C.

**SINGLE CELL AUTOGRAPHS OF BONE MARROW AND BLOOD FROM RATS USING RADIOACTIVE PHOSPHORUS.** W. J. Morse, II. From the Department of Medicine, McGill University, Montreal, Canada. Am. J. M. Sc. 220: 522-529, 1950.

A technic for single cell autographs is presented and some of its potential uses discussed. Data are given which show that the youngest erythropoietic and granulopoietic forms from the marrow have the most intense autographs, whereas lymphocytes and megakaryocytes showed very faint autographs.

Faint autographs of lymphocytes were seen in preparations of the marrow, and none from the peripheral blood. The author suggests that this may indicate some advantage of radioactive phosphorus over x-ray therapy in myelogenous leukemia.—T.R.T., Jr.

**ERYTHROCYTE DESTRUCTION and HEMOLYTIC ANEMIAS**


The fact that hemolytic transfusion reactions may occur in the absence of a specific hemolysin has been well known for many years, but direct experimental evidence has not previously provided an explanation of in vivo hemolysis when in vitro tests were positive only for specific agglutination. Consequently, the authors have studied the secondary effects of red cell agglutination, and have presented in beautifully concise form the results of these studies.

A patient of blood group O received 500 ml. of group A blood and suffered a nonfatal hemolytic reaction. The first sample of blood three hours after transfusion revealed a fall in titer of anti-A agglutinin to 1:4, from a pretransfusion level of 1:32. Slight increases in osmotic and mechanical fragilities as well as clumps of spheroidal red cells persisted for more than eight hours.

These observations indicated that the agglutination of the red cells was indirectly responsible for their destruction. Accordingly, experiments were designed to test further this hypothesis in dogs. Anti-dog-red-cell-immune serum (ADRIS) was prepared in rabbits, and its effects on the red cells of 10 dogs were tested in vivo and in vitro. The findings of Moss were confirmed that homologous serum inhibits the isohemolysin of heterologous serum. However, the agglutinin titer was not inhibited by serum. Thus, in vitro, hemolytic effect could be avoided, while agglutinating effect could be preserved in vivo and in vitro. When ADRIS was injected intravenously, agglutination was observed in samples of venous blood for twenty-four hours, there was an initial fall in hematocrit, an initial increase in osmotic fragility of 10 per cent and after twenty-four hours of 75 per cent of the circulating red cells. Thus, the suggestion of Banti is confirmed that anti-red-cell-immune serum in vivo causes a specific hemolyzing activity of the tissues of the organism.

Next, gravity perfusion of a lobe of a normal dog liver via a portal vein radicle was established, and ADRIS added to the perfusing blood. Agglutinated red cells were retained in the liver and ischemic injury to liver cells was produced, and an increase in the osmotic fragility of the circulating red cells resulted.

Studies were made of the osmotic fragility of red cells removed by saline perfusion of the liver, lungs and aorta of dogs whose body temperature had been artificially maintained for
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three hours after death. Only the cells from the liver showed increased osmotic and mechanical fragility. It is thus inferred that these changes require close contact with actively autolyzing tissue cells.

It is concluded that red cell agglutination invokes the sequence of physical and chemical events: erythroagglutination, erythrostasis with local exclusion of serum, tissue ischemia and release of injurious substances from autolyzing tissues adjacent to stagnating red cells. The possible connection between red cell agglutination and the mechanism of acquired hemolytic anemias is also suggested.

This paper represents the results of a masterly application of established technics. T.R.T., Jr.

ERYTHROCYTE SURVIVAL STUDIES IN CHILDHOOD. I. METHODS AND GENERAL OBSERVATIONS.
II. STUDIES IN MEDITERRANEAN ANEMIA.

I. Methods and General Observations

A technic is described for the purpose of studying red cell survival in infants and children. The method is based upon that of Ashby and employs capillary blood samples for this purpose. The results obtained in normal subjects and with subjects with sickle cell anemia and congenital hemolytic icterus conform to those of other workers. The authors have, therefore, undertaken a series of studies which are summarized in the following two abstracts.

II. Studies in Mediterranean Anemia

Erythrocytes from 3 children with Mediterranean anemia were transferred into 3 normal patients. In each case there was a rapid initial elimination of the transfused cells as compared to the survival of normal cells. Between 25 and 50 per cent of the transfused cells were destroyed in the first twenty to thirty days. In the subsequent period the remaining cells were destroyed at a normal rate.

The survival time of normal red cells transfused into patients with Mediterranean anemia was normal.

Three women with the carrier state of Mediterranean anemia were studied and when their red cells were given to normal subjects, the survival times were within the normal range.

The existence of a hemolytic component in Mediterranean anemia is, therefore, confirmed. The possible mechanisms for this derangement are discussed.

III. Unusual Familial Hemolytic Anemias Associated with Intrinsic Erythrocyte Abnormality

Members of two families with atypical chronic familial hemolytic anemia were studied by means of observing the survival of transfused red cells. The pattern of elimination of the patients' erythrocytes in normal recipients was similar to that in sickle cell anemia and congenital hemolytic icterus.—T.R.T., Jr.


Evidence of hemolytic episode developed in an Indian aged 36, thirty-five days after starting treatment for leprosy with D.A.D.P.S. A total of 7.1 Gm. had been given by mouth. The patient recovered and after seven weeks, had normal blood findings and no evidence of liver dysfunction. An attempt to recommence treatment with small doses of the drug produced signs of recurrence of hemolysis.—S.T.C.
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Some anti-human globulin sera tested against weakly sensitized red cells were found to give a prozone phenomenon. Two possible explanations for this behavior are suggested: (1) that such anti-human globulin sera contain a blocking antibody and (2) that there is an inhibitor due to antibody being present in excess. Evidence is given in support of the second explanation. The importance of checking all anti-globulin sera for such prozone phenomenon before putting them to routine use is emphasized.—S.T.C.

A HERETOFORE UNREPORTED AGGLUTINABLE HUMAN BLOOD FACTOR AND ITS POSSIBLE RELATIONSHIP TO BLACKWATER FEVER. D. C. A. Butts. From the Department of Bacteriology, Division of Tropical Disease Research, University of Miami, Medical Research Unit, Coral Gables, Fla. Am. J. Trop. Med. 30: 663-667, 1950.

In an effort to explain the etiology of blackwater fever the author has undertaken studies to determine whether or not an unreported hemagglutinogen exists in human erythrocytes. Chimpanzee blood, group O, MN and type Rh negative was injected into rabbits. The serum produced by the rabbits was adsorbed with O, MN, Rh positive human red cells, and was then used in the testing of a number of group O human bloods. A total of 103 white and 77 Negro group O bloods have been so tested; 97 per cent of the former and 97.4 per cent of the latter showed positive agglutination. The reaction is not due to M or N, the P factor, or the Cellano factor. It is also reasoned that the Rh-Hr systems are probably not responsible.

It is concluded that a new factor, called the “Ch” factor has been found. Brief studies with blood highly parasitized with P. falciparium suggest that this malarial parasite possesses a relatively strong “Ch-like” agglutinogen. It is postulated that Ch negative individuals may become sensitized by malarial infection and that this may ultimately lead to agglutination of the host red cells.—T.R.T., Jr.


A statistical analysis of 308 liveborn Rh-positive infants of sensitized Rh-negative mothers has revealed the importance of jaundice in the pathogenesis of kernicterus and the effectiveness of exchange transfusion in reducing the incidence of this complication of erythroblastosis fetalis.

The important relationship of immaturity, high maternal antibody titer, male sex and family history of previous occurrence to the likelihood of kernicterus is by no means underestimated. In spite of these factors, however, a significant decrease in the incidence of kernicterus in infants given exchange transfusions occurred. A rather striking correlation was seen between the incidence of kernicterus and severity of jaundice. It was also demonstrated that jaundice was the common denominator in the above mentioned factors leading to a high incidence of kernicterus. It would appear, therefore, that exchange transfusions prevented kernicterus by lessening the jaundice.

A program for management of erythroblastosis fetalis is presented which includes avoidance of early induction of labor; early exchange transfusion with fresh Rh-negative female blood in any infant with clinical erythroblastosis or with any of the factors predisposing to severe icterus; and a second exchange transfusion if jaundice increases following the first one. Since the institution of this program only one case of kernicterus has occurred in a group of 106 babies with erythroblastosis and this infant was one who did not receive a second exchange transfusion.
While the fundamental cause of kernicterus still remains unknown, the results of this study demonstrate the progress that has been made in minimizing the incidence of this unfortunate complication of erythroblastosis fetalis.—H.W.B.


The unique opportunity of studying the comparative effectiveness of exchange transfusion and multiple small transfusions was afforded by the birth of a set of identical male twins with erythroblastosis fetalis.

Twin A, who was more icteric and had splenomegaly and hepatomegaly, appeared to have the poorer prognosis and was given an exchange transfusion followed by two small transfusions. Twin B, also severely jaundiced but less anemic and without clinical enlargement of liver and spleen, received conservative therapy including three small transfusions following a sudden drop in red cell count on the fifth day.

Of interest was the rapid recession of jaundice, splenomegaly and hepatomegaly, and a better weight gain in twin A. Twin B, on the other hand, gained weight more slowly and his jaundice persisted for four weeks. While both twins appeared to be normal at nine months, twin B was more advanced in motor development and it is stated in the Addendum that twin A has subsequently exhibited a slight weakness of the left upper and lower extremities.—H.W.B.


The author's experience with exchange transfusion in 24 cases of erythroblastosis fetalis (only 23 are actually tabulated) is described. The overall mortality rate of 16.7 per cent compares closely with that of other investigators. The cases were broken down into four classes: (1) those without abnormal obstetrical history and/or history of blood transfusion (10 cases all of whom recovered); (2) those with abnormal obstetrical history and/or history of blood transfusion (6 cases with 33.3 per cent mortality); (3) those with a history of erythroblastosis with recovery (5 cases with no mortality); and (4) those with a history of fatal erythroblastosis (2 cases both of whom died).

Exchange transfusions performed in 2 other infants with early severe jaundice due to duodenal obstruction and probable congenital hemolytic icterus, respectively, were thought to be helpful although one can hardly draw conclusions as to the superiority of this method of transfusion from this data alone.

The author's opinion regarding the greater effectiveness of exchange transfusion over multiple small transfusions in erythroblastosis fetalis is based in part on his observation of 2 cases that recovered in which the past history suggested a poorer prognosis.

One might take issue with his statement that the "timely" interruption of pregnancy in a sensitized mother is advisable if followed by immediate exchange transfusion.—H.W.B.


This is a most useful paper for those concerned with treatment of hemolytic disease of the newborn by exchange transfusion. The theoretical considerations and objectives of such exchange are discussed and the advantages of using a concentrated red cell suspension in-
stead of whole blood are emphasised. The formulas presented have been tested by the use of red cells labelled with $^{32}$P. An outline of recommended procedure is given together with nomograms from which the size of the transfusion necessary (1) to reduce the hematocrit of Rh positive cells to 5 per cent, (2) to add sufficient Rh negative cells to bring the total venous hematocrit to 50 per cent, can be rapidly deduced.—S.T.C.


Six cases of acute hemolytic anemia in infants were reported at the same meeting of the French Society of Pediatrics. In all cases the drug given was phenylsemicarbazide in a commercial form containing 0.375 Gm. for a suppository. After 4 or 5 Gm. of the drug, acute hemolytic anemia was observed. This dosage is much too high for the weight of infants, and the anemia may be considered as toxic.

Knowing the similarity between phenylcarbazide and phenylhydrazine it can be assumed that disintegration of phenylcarbazide is directly responsible for the hemolysis. Use of this antipyretic, known as cryogenin, ought to be prohibited in infants.—J.P.S.


This article is a scholarly review of the experimental data and hypotheses of hyper-splenism.

An excellent brief historical summary of the “incomplete” antibody concept is given, with a discussion of the Coombs test and the use of trypsin-treated red cells. It is pointed out that these tests are not infallible in differentiating acquired from congenital hemolytic anemia.

The question is raised whether or not there can be autosensitization to platelet and granuloctytic antigens independent of erythrocyte antigens.

The role of the reticulo-endothelial phagocyte is extensively discussed.

The physical and immuno-chemical mechanisms are conceived of as “varying from patient to patient and from time to time,” thus explaining “the variances reported in incomplete antibody titers in several cytologic syndromes, the lack of differentiating antibody findings” and the “occasional failure of splenectomy to arrest the cytologic process permanently.”—T.R.T., Jr.

NUTRITIONAL FACTORS

OBSERVATIONS ON THE ETIOLOGIC RELATIONSHIP OF ACYLLIA GASTRICA TO PERNICIOUS ANEMIA. XII. FAILURE OF THYMUS AMINOPOLYPEPTIDASE TO ACT AS INTRINSIC FACTOR. R. F. Schilling, J. S. Fruton, B. H. J. Hofstee, A. D. Welch, J. W. Harris, F. H. Gardner and W. B. Castle. From the Thorndike Memorial Laboratory, the Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.; the Department of Physiological Chemistry, Yale University, New Haven, Conn.; and the Department of Pharmacology, Western Reserve University School of Medicine, Cleveland, Ohio. J. Lab. & Clin. Med. 38: 942–949, 1950.

In 1944 and 1947 Ågren and Waldenstrom suggested that the intrinsic factor might be identical with the enzyme, aminopolypeptidase. The authors considered the possibility
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that the impure preparations which were used in these studies might contain significant amounts of intrinsic factor as a contaminant. They therefore obtained a relatively purified preparation of the enzyme and, in addition, determined the aminopolypeptidase activity of gastric juice of known intrinsic factor activity.

In 3 patients the hematopoietic activity of 5 μg. of vitamin B₁₂ (given orally) was not increased by the simultaneous administration of active preparations of aminopolypeptidase. In the same patients, neutralized normal human gastric juice, in amounts of either 10 ml. or 50 ml. daily, exhibited significant potentiating effect on 5 μg. of vitamin B₁₂. Neither fresh nor lyophilized normal human gastric juice demonstrated polypeptidase activity.

Inasmuch as only 10 ml. of normal human gastric juice possessed detectable intrinsic factor activity, it is suggested that "in persons without pernicious anemia, demonstration of histamine-fast achylia by the usual clinical methods is not necessarily evidence for physiologic absence of intrinsic factor."—T.R.T.,Jr.


The authors have produced a deficiency of vitamin B₁₂ in the presence of an excess of folic acid, using the baby pig as an experimental animal. This was accomplished by means of feeding a diet deficient in vitamin B₁₂ for three weeks and then adding "x-methyl" folic acid for two weeks. The bone marrow became depleted in erythroid elements and numerous basophilic normoblasts and erythroblasts appeared. These changes were corrected by either vitamin B₁₂ or folic acid therapy. The former therapy resulted in optimum growth whereas the latter produced temporary and suboptimal growth.

In a second experiment baby pigs were fed a lower protein diet deficient in both vitamin B₁₂ and folic acid, as well as containing sulfathalidine in order to produce a double deficiency without the use of a folic acid antagonist. These animals had a marked reticuloocyte response to vitamin B₁₂ and a second marked response to folic acid.

It is concluded "that the pig requires both vitamin B₁₂ and folic acid and that both are involved in hemopoiesis. In addition, vitamin B₁₂ is required for normal growth."—T.R.T.,Jr.

NEW DEVELOPMENTS IN THE STUDY OF FOLIC ACID. PRELIMINARY REPORT. A. D. Welch. From the Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland, Ohio. Tr. A. Am. Physicians. 63: 147-154, 1950.

An increase in the urinary excretion of the citrovorum factor ("a new factor or factors with growth-promoting activity for Leuconostoc citrovorum") which follows the administration of folic acid to human subjects, was found to be increased approximately threefold by the simultaneous administration of ascorbic acid.

It is postulated that this factor is a metabolite of folic acid which serves as the prosthetic group of an enzyme or as the precursor of such a group. In this connection reference is made to the work of Welch and Sakami who reported that rats, in vivo, and liver slices, in vitro, can synthesize the labile methyl group, and the rate of this synthesis appears to be influenced by catalysts derived from synthetic folic acid.

Aminopterin prevented the conversion of folic acid to the citrovorum factor, when added to liver slices. The toxic effects of aminopterin in rats were completely prevented by administration of citrovorum factor. It is thus suggested that "the activity of folic acid is dependent upon its conversion to citrovorum factor and that a major part of the action of aminopterin is due to its effective interference with this conversion."—T.R.T.,Jr.
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In this article, the third of a series of papers dealing with nutritional macrocytic anemia of swine, further studies are presented on the macrocytic anemia caused by deficiency of pteroylglutamic acid. This anemia is produced on a diet of casein supplemented by B vitamins, sulfasuxidine, and "crude" methylfolate acid antagonist. The response of this anemia to crystalline vitamin B₁₂ was suboptimal, as it was also to proteolyzed liver, marmite, crude deoxyribonucleic acid and crude ribonucleic acid. Thymine did not augment the activity of vitamin B₁₂.

The relationship between pteroylglutamic acid, ascorbic acid, and tyrosine metabolism is discussed, and data are presented on plasma iron, free erythrocyte protoporphyrin and plasma copper. The relationships of this nutritional anemia of swine to human pernicious anemia, refractory megaloblastic anemia and achrestic anemia are discussed.—T.R.T.,Jr.


The authors studied the response of 7 patients with pernicious anemia in relapse following the administration of vitamin B₁₂. The dose ranged from 75 to 300 μg. The response to this form of treatment was not consistent or dependable, and indicates considerable variation between patients in their deficiency of intrinsic factor, and in their absorption and utilization of vitamin B₁₂.—T.R.T.,Jr.

IRON METABOLISM AND HEMOCHROMATOSIS


This review of iron metabolism is well represented from the clinical point of view. The mechanisms of iron absorption, transport and excretion are discussed. The therapeutic uses of iron are reviewed, the importance of accurate diagnosis before using iron therapy is stressed, and the merits and limitations of oral and intravenous iron therapy are presented. It is emphasized that intravenous iron preparation should be used only in situations which are not amenable to, or cannot be treated with oral preparations. The danger of overloading the tissue stores by excessive doses of intravenous iron, thus producing hemosiderosis, is stressed.—T.R.T.,Jr.


Severe cerebral symptoms were observed in a woman of 54 treated for hypochromic anemia with a privately made preparation of saccharated oxide of iron. Convulsions and loss of consciousness occurred after a total dose of 350 mg. of iron, the maximal single injection being 75 mg. Minor symptoms such as nausea had been complained of earlier in the course. The patient recovered but was left with some residual symptoms.

The authors sound a warning against using unproved preparations for intravenous iron therapy.

(This report should also warn against (1) continuing treatment and increasing the dose when any signs of toxicity are shown and (2) giving intravenous iron unless there is the clear indication of oral treatment being unsatisfactory.)—S.T.C.
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