HEMOGLOBIN, METHEMOGLOBIN AND BILIRUBIN METABOLISM


Knowledge that fetal goat's hemoglobin has a higher affinity for oxygen than maternal hemoglobin has led to further investigations along developmental and comparative lines. In the present study, various elasmobranchs and turtles were obtained in the adult, fetal and embryonic forms. In general, sharks had a higher loading capacity and a lower unloading capacity for oxygen than the rays. Unlike other animals, the fetal ray had an affinity for oxygen like that of the adult. Lack of material did not permit a study of the more embryonic stages. In the turtles, the terrestrial forms had a higher affinity than the aquatic forms. In the development of turtles, no changes in oxygen affinity occur until after the embryos hatch. By the end of the second year the adult type of hemoglobin is established. These and similar results may be correlated with ontogenetic, phylogenetic, ecologic and physiologic factors.

O. P. J.


One certain and one probable case of methemoglobinemia are reported in infants whose formulae included well water containing from 110 to 250 parts of nitrate per million. The upper limit of safe nitrate content is given as 10 parts per million. In both cases cyanosis disappeared without treatment within one or two days after the use of well water was discontinued.

Four similar reports, including one fatal case, in the literature are cited, all cases occurring in infants during the first two months of life. It is suggested that the determining factors are body weight, nitrate content of well water and amount of water ingested. The incidence of well-water methemoglobinemia would, therefore, be highest in areas where farm sanitation is poor, wells poorly constructed and where dried milk mixtures are extensively used.

The efficacy of methylene blue and ascorbic acid in treating methemoglobinemia is briefly discussed. Prompt recognition of the condition is most important, since spontaneous recovery follows when use of contaminated well water is abandoned, provided the methemoglobin content of the blood has not risen too high.

L. E. Y.


The early studies of Warburg on the mechanism of erythrocyte methemoglobin reconversion are extended by these authors. Methylene blue was used as a part of the system because of its known ability to accelerate the reaction. While glucose functions as a substrate in the intact erythrocyte, hexose diphos-
ABSTRACTS

Phosphate or lactate are necessary in hemolysates. Methemoglobin reversion occurred in the hemolysate when nicotinamide was employed to suppress pyridine nucleotide hydrolysis. The essential substrates for the reduction of methemoglobin in these experiments appeared to be reduced DPN and phosphorylated glucose or lactate. These observations, unfortunately, do not throw any light on the one clinical state in which the cell reconversion mechanism does not function—congenital methemoglobinemia. This would suggest that the reconversion system is more complex than herein described.

C.A.F.


In 1890, Heinz was the first to notice the appearance of basophilic corpuscles in the red cells during phenylhydrazin intoxication. These corpuscles are stained by supravital staining, but in contrast to reticulocyte granulations, they disappear when later stained by a panoptic method (May-Grunwald-Giemsa). Several hemolytic poisons, and some sulfonamide drugs bring on the corpuscles, in vivo as well as in vitro. Their chemical nature and their biologic significance have been the subjects of numerous researches. Some think they are due to methemoglobin; others that they are related to verdoglobins (intermediary substances between hemoglobin and bile pigments).

The experiments of A. Gajdos and G. Tiprez bring new arguments for the close relations between Heinz corpuscles and verdoglobins. They show that all substances able to transform hemoglobin into verdoglobin induce Heinz corpuscles in vivo and in vitro, in particular ascorbic acid. Moreover, the same factors are acting (pH, temperature, speed of formation) for both verdoglobin and Heinz corpuscles. Thus, these corpuscles seem to represent an alteration of the hemoglobin in the red cells by hemolytic poisons.

J.P.S.


The authors studied the incidence of physiologic icterus in the newborn and attempted to decide whether it is due chiefly to hemolysis or to some other factor, such as hypofunction of the liver. They found first that up to 81 per cent of newborn infants had a plasma bilirubin in excess of 1 mg. per cent (i.e., laboratory icterus), although much fewer (e.g., 18 per cent of one group) had clinical jaundice. At two weeks of age, all infants studied had levels of mg. per cent or less. The level of neonatal plasma bilirubin roughly paralleled that of the cord blood, but there was not a strict correlation; nor was there a correlation between the plasma bilirubin and the maturity of the fetus. Jaundice, however, was more likely to occur the more premature the baby:

<table>
<thead>
<tr>
<th>pregnancy</th>
<th>jaundice</th>
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<tr>
<td>31-35 weeks</td>
<td>100% of subjects</td>
</tr>
<tr>
<td>36-37</td>
<td>65%</td>
</tr>
<tr>
<td>38-39</td>
<td>47%</td>
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<td>40 (term)</td>
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It is generally taught that 'physiologic icterus' is the result of hemolysis, which is said to cause the fall in hemoglobin and red cell count from the prenatal to neonatal levels. Against this concept the authors adduce the following data:

1. The most rapid fall in hemoglobin and red count occurs in the second week of life, whereas the hyperbilirubinemia occurs largely in the first week. The fall in blood values continues for months, long after the jaundice is gone. (L. Findlay. Arch. Dis. Childhood 22: 195, 1946.)

2. The rate of fall of hemoglobin was found to be the same in jaundiced and in nonjaundiced infants.

3. None of the usual concomitants of hemolysis (reticulocytosis, normoblastosis) occurred postnataally, whereas fetal umbilical blood was found regularly to contain increased reticulocytes and normoblasts.

4. The erythrocytes of fetal blood showed increased fragility to hypotonic solutions; those of neonatal blood showed decreased fragility (Arch. Dis. Childhood 22: 64, 1945).
The authors believe that neonatal physiologic icterus is the result of immaturity of function of the liver which, like many other organs, is relatively immature at birth. In utero, they postulate, the fetus's bilirubin is excreted largely by way of the maternal placenta, so that not till birth does the liver begin to excrete bilirubin. Attempts to study neonatal liver function gave inconclusive results, but more bilirubin was found in the feces of nonjaundiced than of jaundiced infants.

SE.

HEMOGLOBINEMIA AND HEMOGLOBINURIA


A preliminary report is made of studies carried out on the blood of a syphilitic patient with paroxysmal cold hemoglobinuria. It was shown that saturation of the patient's blood with carbon dioxide produced hemolysis of the erythrocytes, an effect which was not dependent on a reduction of the pH of the serum (as is the case in paroxysmal nocturnal hemoglobinuria). This hemolysis was dependent on the presence of a hemolysin in the patient's serum.

Inhibition of the carbonic anhydrase of the erythrocytes with cyanide or with sulfanilamide blocked the hemolytic action of chilling. This blockage was due to inhibition and not to destruction of the carbonic anhydrase.

Microscopic observation of erythrocytes being hemolyzed by the patient's serum showed that these cells underwent considerable swelling and morphologic changes just prior to lysis.

J.F.R.


The chief point of interest in this patient with a five year story of anemia and a two year story of episodes of blueness, tingling, and numbness of the hands on exposure to the cold, is the extraordinarily high titer of the autoagglutinin circulating in the blood serum. The patient had no physical abnormalities except after chilling, when bluish red blotches appeared over the fingers, hands, and tips of nose and ears. The blood studies showed a persistent macrocytic anemia with reticulocytosis and thrombocytosis, and normoblastic hyperplasia of the bone marrow. The titer of autoagglutinin was 1:12,000,000 at ice-box temperature, and the red cells were very fragile to mechanical trauma when kept at low temperatures. Actual rupture of the red cells was visible in vivo under the capillary microscope.

S.E.


The case reported is that of an apparently normal 18 year old soldier who for three months exhibited hemoglobinemia, hemoglobinuria, albuminuria and abdominal distress after walking for periods as short as thirty minutes. For at least part of the three months the appearance of symptoms seemed to depend upon the presence of food in the upper digestive tract. Further study of this phenomenon was prevented by spontaneous remission. A brief review of march and other types of paroxysmal hemoglobinuria is given and the association of albuminuria with hemoglobinuria is discussed.

L.E.Y.


A method is described for preparation of sterile, nonpyrogenic solutions of oxyhemoglobin which have the approximate protein content and electrolyte composition of plasma. The procedure involves precipitation of stroma by adding 0.1 NHCl to laked red cells and removal of excess potassium by treatment with sodium zeolite.

The authors report that large volumes of solution can be rapidly prepared with 95 to 98 per cent of the hemoglobin in active form capable of combining with oxygen. Solutions stored at 4 C. showed no
conversion of hemoglobin to methemoglobin over a period of two and a half months, but a small and variable conversion was detected over a six month period.

L.E.Y.


The methods used for freezing, drying and preserving plasma in vacuo were successfully applied to hemoglobin solutions, but only after hemoglobin was deoxygenated to prevent formation of methemoglobin during the drying process. It was found that deoxygenated hemoglobin dried and preserved in vacuo retained all its oxygen-binding capacity for 180 days when stored at temperatures from 4 to 30°C, for 92 days at 38°C, and for 7 days at 56°C. Dried deoxygenated hemoglobin was partly converted to methemoglobin by even momentary contact with oxygen, but it was stable when dissolved in water before being exposed to air and could be stored for months at 4°C in contact with air, without significant loss of activity. The dried hemoglobin had a foam structure which caused it to dissolve immediately upon contact with water.

Deoxygenation was accomplished by repeated shaking of hemoglobin solutions under diminished pressure. All but traces of oxygen were removed by alternately degassing the solutions and saturating them with oxygen-free nitrogen. It is pointed out, however, that after completion of this work, Pennell, Smith and Werkheiser reported a procedure for deoxygenation by action of enzymes in laked cells after addition of nicotinic acid and glucose. This method is considered better adapted to large scale preparations.

L.E.Y.


Dogs were bled, 50 cc. per kilo body weight, and the blood withdrawn was quickly replaced by equal volumes of 0.9 per cent NaCl solution, heparinized dog plasma, or 7 per cent oxyhemoglobin or methemoglobin solution. The effects of more prolonged hemorrhagic shock were not studied. When the blood was not replaced, oliguria or anuria developed and the urea clearance was depressed for several hours after bleeding, but renal function returned to normal within twenty-four hours. Infusion of 0.9 per cent NaCl solution or plasma promptly relieved the oliguria and elevated the urea clearance. Injection of oxyhemoglobin solution also relieved oliguria promptly, but in some cases there followed a period of three to five days in which urea clearance was depressed to about 25 per cent of normal and plasma urea nitrogen was moderately elevated. The clearance then returned to normal during the next five or six days. No histologic observations are reported.

When acidosis was produced before bleeding by giving NH₄Cl, the results after infusion of plasma or hemoglobin were unchanged, but since hemorrhage itself caused nearly as much acidosis as the NH₄Cl, acidosis was not excluded as a factor in these experiments.

It was shown that methemoglobin was rapidly converted into active hemoglobin after injection, and that there was no significant difference between infused oxyhemoglobin and methemoglobin either in effect on renal function or in rates of disappearance from circulation and excretion in the urine.

The authors conclude that, although the immediate effects of hemoglobin solutions are favorable in treating posthemorrhagic shock in dogs, the subsequent transitory depression of urea clearance indicates sufficient possibility of renal damage to prevent recommending the use of such solutions as blood substitutes.

L.E.Y.

From the Department of Medicine and Pathology, Peter Bent Brigham Hospital and Department of Pathology, Harvard Medical School, Boston, Massachusetts. New England J. Med. 235: 657-665, 1947.

A case of massive intravascular hemolysis following a therapeutic abortion is presented and the management of the subsequent anuria is discussed in detail. The etiology of the hemolysis was attributed to
either ingress of a hemolytic substance by way of the uterus or by sensitivity reaction to an orally ingested drug. Dr. Hetselene has called the attention of the authors to the article by Hill on Post-abortal and Puerperal Gas Gangrene (J. Obst. & Gynec. 43: 201, 1937) and the similarity of this case to the reported cases of hemolysis due to B. welchi is striking.

The recovery of the renal lesion was followed by renal function tests, and upon the death of the patient three months later with acute hepatitis, the residual anatomic lesion was found to consist of only minimal scarring. The literature pertaining to pathogenesis of hemoglobinuric nephrosis and its treatment are reviewed.

C.A.F.

THE PATHOGENESIS OF THE RENAL INJURY PRODUCED IN THE DOG BY HEMOGLOBIN OR METHEMOGLOBIN.

H. E. Harrison, H. Bunting, N. K. Ordway, and W. S. Albrink. From the Department of Pathology, Yale University School of Medicine, New Haven, Conn. J. Exper. Med. 86: 339-356, 1947.

Severe and persistent impairment of renal function was produced in dogs by extreme intravascular hemolysis due to arsine and by intravenous injection of large amounts of dog hemoglobin and methemoglobin. Methemoglobin caused more severe renal injury than equal amounts of oxyhemoglobin. In oliguric dogs, azotemia and reduction of creatinine clearance followed injections of methemoglobin in amounts resulting in plasma concentrations of 1 Gm. per 100 cc., whereas much higher concentrations were tolerated by dogs with greater urine output. Acidosis, produced by administration of 0.1 N hydrochloric acid, appeared to have less effect than oliguria in the production of renal damage after injections of methemoglobin. In acidotic dogs, the pH of the urine rose promptly with the onset of hemoglobinuria, sometimes from approximately 5.2 to 7.0, but no explanation of this change is given.

Kidneys of these animals were examined by the ferrocyanide histochemical technic to determine whether the renal tubules were functionally obstructed. In addition, casts were teased out of frozen sections, dissolved in buffer solutions and the absorption spectra and solubilities of their pigments were determined.

Material filling the lumina of the tubules was found to be chiefly methemoglobin in concentrated solution of gel-like consistency. No evidence of formation of pigments such as hemochromogen or hemosiderin, insoluble at the pH of the urine, was found. Obstruction to the flow of urine through the tubules appeared to be an important factor in early reduction of renal function and was attributed to increased viscosity of tubular contents. It is worthy of note, however, that the concentrations of hemoglobin and methemoglobin produced in the plasma in these experiments were for the most part considerably in excess of those encountered clinically. The mechanism of cast formation under these conditions is not yet clear.

Necrosis of proximal convoluted tubule cells was found as a late lesion and was deemed a contributing factor in the persistent depression of renal function. Following disappearance of most of the intratubular pigment, many collapsed tubules lined with hemosiderin-filled cells were found and were considered to represent nonfunctioning nephrons.

Direct measurements with a T-tube cannula inserted into the renal vein in two animals failed to reveal any reduction of renal blood flow following injection of methemoglobin in amounts sufficient to produce renal injury. It should be pointed out, however, that the method here employed might fail to measure a reduction in effective renal blood flow, if such occurred, due to diversion of flow from cortex to medulla by recently discovered shunting mechanisms.

L.E.Y.

IRON METABOLISM

THE PATHOGENESIS OF CYTOSIDEROSIS (HEMOCHROMATOSIS) AS EVIDENCED IN MALNOURISHED AFRICANS. J. Gillman and T. Gillman. From the Department of Anatomy, Medical School, University of the Witwatersrand, Johannesburg, South Africa. Gastroenterology 3: 19-23, 1947.

Based on numerous histochemical studies of liver biopsy material and tissues obtained postmortem from nutritionally deficient African natives, many of whom presented clinical and pathologic evidences of hemochromatosis, a theory is proposed by the authors regarding the sequence of events operative in the pathogenesis of that disease. Initially, as a complication of malnutrition, commonly pellagra, there occurs a degradation of intracellular iron compounds, possibly cytochrome or catalase. During this stage of the process a striking porphyrin-like fluorescence is demonstrable on examination of liver tissue; it is
presumed that other tissues are likewise involved in this process of deterioration. Iron liberated within the liver is excrated into the bile, then promptly and completely reabsorbed into the intestinal mucosa. The cells lining the intestinal tract, similarly affected by the underlying metabolic disorder, become laden with iron. Iron accumulates in the lymphocytes of the tunica propria and subsequently enters the lymphatic circulation, chains of mesenteric lymph nodes becoming progressively pigmented. Eventually sufficient iron becomes absorbed into and distributed by the systemic circulation to effect a generalized siderosis of the entire reticulo-endothelial system, including the Kupffer cells, which now serve as an additional source of iron delivered to the upper abdominal lymph glands, already pigmented from iron-containing lymph derived from the liver itself. In severe cases these glands draining the small intestine, stomach, pancreas and liver become extensively involved. Successful arrest of the process necessarily involves an attack on the fundamental disorder of intracellular metabolism, which is believed to be secondary to a nutritional deficiency.

C. P. E.


A case of this unusual syndrome was diagnosed clinically from the appearance of the pulmonary roentgen picture and the hematologic status. This was characterized by the mixture of sideropenic and hemolytic symptoms that is typical for the malady. The hemoglobin responded well to iron therapy. There was noted a constant reticulocytosis, urobilinuria and periodically increased serum bilirubin. Of special interest is the demonstration of large amounts of hemosiderin in microscopic sections of the patient’s sputum. Another finding that may possibly be of importance for the understanding of the disease in the future was a sarcoid-like structure found in an excised cervical lymph gland. No autopsy was performed but the clinical diagnosis was quite convincing. It is probable that this syndrome is sometimes overlooked and should be diagnosed more frequently.

J. W.


This is an extension of previous studies by these authors on the anemia produced by pyridoxine deficiency in swine. Normal values of erythrocyte protoporphyrin in swine were found to be 118 ± 43.4 μg/100 cc., serum copper 106 ± 16.3 μg per cent, and serum iron 169 ± 38.8 μg per cent. In pyridoxine deficiency, protoporphyrin was reduced to an average of 47 ± 13.6 μg. Copper was reduced to 160 ± 38.8 μg per cent while serum iron showed an increase to 408 ± 166.6 μg per cent. Urinary coproporphyrin was not altered. Following intravenous administration of pyridoxal and pyridoxamine, serum iron dropped to normal limits within twenty-four hours, and erythrocyte protoporphyrin rose over several days to above normal limits.

The authors suggest that in view of the reduced free erythrocyte protoporphyrin, the fundamental disturbance in erythropoiesis may be a failure to synthesize protoporphyrin. The elevation of serum iron and tissue hemosiderosis is a natural sequence of this block in hemesynthesis.

These investigations are the first to suggest such a defect in protoporphyrin metabolism as a cause of anemia. Investigations along this line are warranted in Cooley’s anemia which has certain similarities to the anemia of pyridoxine deficient swine.

C. A. F.


A method is described of measuring ferrous and ferric iron in the presence of each other. Under conditions simulating gastric digestion, the degree of reduction and binding by various foods and biologic materials was tested. The reduction of iron varied with different foods from 0 to 98 per cent. Ascorbic acid and proteins were felt to be in part responsible for this reduction.
ABSTRACTS 709

Recent studies have indicated that dietary factors are important in determining iron absorption. Since there is some evidence that iron must be in the ferrous form for absorption to occur, this reduction capacity of the diet may be a determining factor.

C.A.F.


The authors describe 4 cases of human anemia treated with 2 Gm. methionine daily, in which the amount of serum iron and copper were estimated. (Iron estimated by the method of Heilmeyer and Plotner, modified by Lederer and De Mouschalt; copper estimated by the method of Callan and Henderson, modified by Briskas.) In all 4 cases, the amounts of serum iron and copper increased considerably at the beginning of the treatment, despite a diet poor in these elements. The increase of serum iron is more important than that of serum copper. While the anemia regresses, the copper, and above all the serum iron, decreases. It seems, therefore, that methionine aids the mobilization of iron and copper. It would be interesting to give iron with methionine therapeutically.

J.P.S.

LEUCOPENIA AND AGRANULOCYTOSIS

THE EFFECT OF TRIMETHYLAZOLOZIDINE DIONE (TRIDIONE) ON THE BLOOD. J. P. Davis and W. G. Lennox. From the Department of Neurology, Harvard Medical School and The Children's and Infants' Hospital, Boston, Mass. J. Pediat. 31: 24-33, 1947.

Periodic hematologic examinations of 127 patients receiving tridione demonstrated, as the only alteration of possible serious significance, the gradual development of a definite neutropenia in 6.3 per cent, the neutrophile counts in this group falling to values between 600 and 1600 per cubic millimeter. An additional 7 per cent exhibited a mild neutrophilic depression, counts descending below 2,000 per cubic millimeter. The total leukocyte count was relatively unaffected, due to a concomitant absolute lymphocytosis. The occurrence of granulopenia was unrelated to dosage of the drug, or to the therapeutic schedule employed; no premonitory or accompanying clinical manifestations or hematologic abnormalities were observed; in no instance was its onset abrupt, or its progression rapid. Restoration of a normal granulocyte count promptly and invariably followed discontinuance of the drug. Therapy was resumed in 3 cases without recurrence of neutropenia. It is emphasized that, whereas drugs with this type of molecular structure may be potential bone marrow depressants, the administration of tridione may be safely controlled and the development of a marked or irreversible neutropenia forestalled by conducting monthly hematologic examinations which should always include a leukocyte differential, as well as total white cell count.

C.P.E.


The 68 year old hyperthyroid patient who forms the subject of this report developed sudden acute neutropenia ("agranulocytosis") after the previously uneventful administration of some 13,400 mg. of propylthiouracil in a period of ten weeks. The blood showed 1,000 white cells with no granulocytes at all, and granulocytes were virtually absent from the marrow puncture. About the same time, however, a non-hemolytic jaundice supervened, attributed to hepatocellular damage caused by the propylthiouracil. Treatment consisted of penicillin, streptomycin, amino acids, liver extract, vitamin injections, and blood transfusion; and improvement gradually occurred. The thyrotoxicosis remained unchanged and required subsequent thyroidectomy.

It is impossible to evaluate this curious case from the published article, in which no tables were included because of lack of space. The neutropenia is amenable to explanation on the basis of known damage to granulocytopoiesis in the marrow, which has been well demonstrated in the case of various drugs, including thiouracil, and has been reported for half a dozen cases with propylthiouracil. The occurrence of hepatic involvement must be very rare or even unique; and the question of its etiology remains unexplained from the data presented.

S.E.

The authors report a patient of 6 with hyperthyroidism, who developed a leukopenia and neutropenia (white cells 3,400; neutrophils, 40 per cent) after administration of some 36 grams of thiouracil in a period of five months. Cessation of drug therapy was followed by prompt return of the leukocyte and neutrophil values to normal, but relapse in the hyperthyroidism occurred within two months. The patient was therefore put on propylthiouracil, 50 mg. three times a day, and later 75 mg. daily. There was no effect on the blood counts, but the thyrotoxicosis remained uncontrolled, and the dose of propylthiouracil was raised to 100 mg. daily. This increase in dosage was followed by a rapid fall of white count to 3,100 per cu. mm., with 22 per cent neutrophils. This drug, too, was therefore stopped, and the count returned to normal values. Thyroidectomy was subsequently used to control the hyperthyroidism.

According to the authors, of some 471 cases treated with the drug this is the seventh case in the literature of leukopenia due to propylthiouracil.

S.E.

THE Rh FACTOR AND ERYTHROBLASTOSIS FOETALIS


This is the substance of a lecture delivered by Diamond in England in May 1947. Several points covered in this review are of interest.

1. About 13 per cent of all marriages involve an Rh negative woman and an Rh positive man, but only one in 150 of all deliveries results in an infant with erythroblastosis foetalis. In other words, less than one susceptible woman in 20 is sensitized by pregnancy alone; whereas a single transfusion of Rh positive blood, followed by subsequent pregnancy, in an Rh negative woman, increases the chances that the child will be erythroblastotic from 0 per cent to over 2 per cent.

2. The results of treatment of affected infants over a period of some twenty years are reviewed. During fifteen years in which the only treatment was transfusion of compatible blood, usually from the baby's father (i.e., Rh positive), the mortality, including stillbirths, was 40 per cent. During three years in which treatment consisted of multiple small transfusions with Rh negative blood, the mortality was 30 per cent. In the period of 1944 to 1946, when early delivery in affected cases was combined with Rh negative transfusions after delivery, the mortality was 20 per cent. The newest technic, combining early delivery with careful studies at birth and, when indicated, exsanguination-transfusion of affected infants, resulted in a mortality of about 10 per cent in the first 50 cases. Of those that recovered, most responded to the single procedure of exsanguination-transfusion, and only a few needed a subsequent small transfusion in the third week of life.

3. Diamond gives flexible indications for exsanguination-transfusion. The procedure is indicated (a) when the mother has Rh antibodies in the serum, and the infant has clinical erythroblastosis foetalis; (b) when the mother has Rh antibodies in the serum, the infant has no symptoms at birth, but cord blood shows the baby to be Rh positive and his serum to have Rh antibodies. If the infant looks good and, although Rh positive, has no free antibody by suitable tests, no therapy is attempted at birth; in most of this group, no treatment became necessary, although a few such infants subsequently required a single transfusion of blood.

The experiences of Diamond are in keeping with generally held ideas concerning the mechanisms of erythroblastosis foetalis.

S.E.


The 3 cases of erythroblastosis foetalis cited in this report are presumably the first to be described among South African natives. It is emphasized that only 5 per cent of the natives are Rh negative and that only 18 per cent of Bantu bloods react with anti-C (anti-Rh3) serum as compared with 70 per cent among Euro-
peans. It is extremely rare, moreover, to encounter native bloods which react with anti-E (anti-Rh") serum (30 per cent among Europeans).

All of the 3 cases reported occurred in infants born of Rh positive mothers, and in each case incomplete antibodies were present while abnormal agglutinins were absent. In the first case the baby's type was CDE (Rh;Rh;e) and the mother's type was CDe (Rh;); incomplete antibodies against CDE (Rh") were found in the maternal serum—a finding of rarity even among Europeans. In the second case the baby's type was CDe (Rh;e), the mother's Cde (Rh;); anti-D (Rh;0) incomplete antibodies were present. In the third case the baby's type was CDe (Rh;e), the mother's cDe (Rh;0), and incomplete anti-C (Rh") antibodies were detected.

L.E.Y.


Forty-three unselected cases of erythroblasto sis fetalis who survived were studied for intellectual development by means of Gesell's development quotient (D.Q.) and standard intelligent quotients (I.Q.). It was found that 5 children who had gross neurologic involvement consistent with kernicterus, all had low D.Q. and low I.Q. In all, jaundice and anemia had been present after birth. Only two other children had slightly low D.Q. and I.Q. On the other hand, 7 other children with slight physical abnormalities not suggestive of kernicterus, had normal D.Q. and I.Q., and in 2 of these, jaundice had been extremely severe.

These results suggested that severe mental defects in such patients is closely associated with the physical characteristics of neurologic damage. In the absence of such damage due to kernicterus, the mentality is perfectly normal. Erythroblastosis fetalis in itself, in other words, has no particular relationship to impaired intellectual development, unless the latter is due directly to brain damage attributable to kernicterus.

S.E.

DETERMINATION OF ANTI-RH ANTIBODY IN INFANTS WITH ERYTHROBLASTOSIS FETALIS. W. E. Wheeler and M. L. L. Scholl. From the Department of Pediatrics, Ohio State University College of Medicine, and the Children's Hospital, Columbus, Ohio. Am. J. Dis. Child. 274-282, 1947.

A superior method for liberation and demonstration of both complete and incomplete anti-Rh antibodies attached to erythrocytes of babies with erythroblastosis is described. Washed packed red cells from the infant are divided into two equal portions, one of which is resuspended in 3 volumes of saline, the other in a similar amount of a 2.0 per cent solution of bovine albumin. Both portions are heated at 45 to 50 C. for 30 to 40 minutes, then centrifuged rapidly at the same temperature and the supernatant fluids tested with Rh; Rh;e and Rh negative cells of the same OAB group. In 10 of 11 cases, the albumin supernate gave a positive conglutination test, but all of the saline supernates failed to produce agglutination. In 6 cases, however, Rh positive cells incubated in the saline supernates became sensitized as demonstrated by their agglutination when anti-human-serum rabbit serum was added.

It is emphasized that these procedures establish the specificity of the antibody attached to the baby's cells, whereas the Coombs, Mourant and Race (CMR) test with rabbit serum, when carried out by itself, merely demonstrates the presence or absence of cell-bound globulin. The ease with which Rh antibodies are separated from cells by heat suggests to the authors that the antibodies have low avidity. They offer this as an explanation for the puzzling situation present at birth in which there may be found free antigen in agglutinable red cells, free antibody in the baby's serum and antibody combined with antigen in the red cells. It is further suggested that after an Rh positive cell is damaged, Rh antibody may be released in vivo to attack fresh Rh positive cells introduced into the circulation, and that antibody may not be bound to fresh cells permanently. The importance of these considerations in exchange transfusions and in the transfusion of Rh positive cells to erythroblastotic infants is stressed.

L.E.Y.

The nature of conglutinin was explored by adding plasma and plasma constituents singly and in combination to Rh and Rh2 cells sensitized in respective tubes with serial dilutions of Rh antisera containing incomplete antibodies. Supernatant fluid was removed as completely as possible from the cells before conglutinin was added.

It was found that dilution of oxalated human plasma with more than an equal volume of saline destroys its ability to produce conglutination of cells sensitized by univalent antibody. Plasma showed greater conglutinating activity than serum, presumably due to the presence of fibrinogen, which the authors consider an important component of the colloidal complex of proteins making up conglutinin. Heating at 56° C. for 30 minutes weakened slightly the activity of plasma by causing precipitation of fibrinogen, but similar treatment of serum improved its activity slightly.

Although there was little variation in conglutinin activity of sera from different normal adults, fetal plasma and serum yielded much lower titers. The rapid increase in conglutinin content of the blood after birth is correlated with the abrupt onset of icterus gravis. The use of whole citrated blood in exchange transfusion of an erythroblastotic baby caused a rise in total plasma proteins and conglutinating activity, whereas replacement of transfused plasma with saline eliminated this unfavorable change in the infant's plasma.

Although 15 per cent human albumin solution yielded titers only half as high as plasma, a mixture of 1 part albumin with 3 parts plasma produced a fourfold increase in titer of the plasma. Addition of more or less than this optimal amount of albumin resulted in lower titers. Albumin solutions of less than 12.5 per cent and immune globulin solutions of less than 4.6 per cent concentration had little conglutinin activity, but mixtures of these dilute solutions in optimal proportions showed activity greater than that of plasma. On the basis of these observations it is emphasized that intensity of conglutination does not depend merely on the total protein content of the medium of suspension. It is further suggested that there may be substances in normal plasma which tend to maintain albumin and globulin in molecular dispersion, and that fractionation may render albumin and globulin less hydrophilic, thus increasing their tendency to form colloidal aggregates.

MALIGNANT LYMPHOMA

LA DIAGNOSI CITOTOLOGICA DEL GRANULOMA MALIGNO PER PUNTURA MIDOLLARE, SPLENUCA E GHIAIADARE. (Cytologic diagnosis of Hodgkin's disease by bone marrow, splenic and lymph node aspiration.)


The authors studied 7 cases of Hodgkin's disease by means of organ punctures and biopsies. They found in bone marrow punctures, an increase in plasma cells which were frequently atypical, but only occasionally noted Sternberg cells. In the splenic punctures there was an inversion of the normal ratio: lymphocytes/granulocytes as a result of increase in the granulocytes; and Sternberg cells were often noted. In lymph node punctures, Sternberg cells were found in all cases and there was a striking cellular polymorphism, with granulocytes (neutrophils and eosinophils) lymphocytes and reticular cells.

In other words, of these three procedures, lymph node puncture was the easiest and the safest way to insure a diagnosis of Hodgkin's disease.


This article is part of a series on early diagnosis and treatment of various forms of cancer. The author summarizes present principles of treatment of the lymphomatous disorders. A few highlights are:

1. Any "inflammatory" lymphadenopathy which persists for three weeks should be considered malignant until disproven by thorough investigation, including biopsy.

2. The treatment of choice for Hodgkin's disease or lymphosarcoma is intensive x-ray therapy to the involved areas. It is emphasized that the minimum doses just sufficient to cause regression of masses are not adequate therapy, but much larger doses—probably to the limit of skin tolerance—are indicated. (Specific schedules are given.)
ABSTRACTS

3. The treatment of choice for chronic leukemias is also x-ray.

4. The nitrogen mustards are of palliative value in certain disorders, notably generalized Hodgkin’s disease. Radioactive isotopes are of palliative value, notably radioactive phosphorus or sodium in chronic leukemia. Urethane may be effective in some chronic leukemias, but inconstantly (25 to 33 per cent of cases). None of these agents is considered the treatment of choice.

5. Certain early cases of Hodgkin’s disease, which seem to be localized, may be cured by complete local excision followed by intensive postoperative irradiation, or, perhaps, by intensive local irradiation alone.

S.E.


The author summarizes his own experience at the Memorial Hospital and that of other recent observers in a well organized review. Particular emphasis is placed on (1) improvement in palliative results in Hodgkin’s disease, lymphosarcoma and chronic lymphatic leukemia by early detection of local lesions and treatment with roentgen irradiation; (2) use of nitrogen mustards in patients with generalized Hodgkin’s disease; (3) use of P32 in polycythemia and in chronic myeloid and lymphatic leukemia with minimal enlargement of spleen, liver and nodes. The limited application of urethane in some cases of leukemia and of stilbamidine in multiple myeloma is briefly discussed.

In looking to the future, the cure of some cases of Hodgkin’s disease and lymphosarcoma is considered a possibility. If these diseases have a unicentric origin, sufficiently early obliterator roentgen therapy or even radical surgery might prove curative, in the author’s opinion. Considerable hope is also held for more specific attack on these disorders by means of further modifications in radioactive isotope therapy and by discovery of more effective chemical agents.

L.E.Y.


Several points of interest are included in this informal discussion of the treatment of the lymphomatous diseases. The generally accepted doctrine that, on the whole, treatment does not influence duration of life, although it may affect comfort and economic usefulness during life, is supported by comparative data. Comparison of patients with Hodgkin’s disease treated with x-ray (1939) and without x-ray (1933) showed no statistical difference in survival times, although improvement of symptoms following x-ray is well known. A similar result is noted in the leukemias and “reticuloses.” Scott points out that surgical excision of a localized group of nodes involved in Hodgkin’s disease may allow permanent cure, and mentions 3 such personal cases. Isolated instances of such result are well known, although the procedure is accepted with hesitation by most.

It is of interest that splenectomy is recommended (by Scott) in the occasional selected case of Hodgkin’s disease or leukemia in whom symptomatic hemolytic anemia or symptomatic thrombocytopenia supervenes. The principle that splenectomy is not necessarily contraindicated once the diagnosis of lymphomatous splenomegaly has been made, but that the operation, by affording relief, especially from a hemolytic process superimposed on the underlying disorder, is becoming increasingly recognized as good medical practice in properly selected cases.

S.E.


The effects of various nitrogen and sulfur mustards were investigated in albino mice, albino rats and New Zealand rabbits. Whenever possible, the effect of these compounds in other species was presented. In general, leukopenia was the most distinctive feature of the intoxication. Bone marrow reacted by showing initial degenerative changes followed by a rapid depletion of all hematopoietic cells. Weights of the spleens, thymuses and lymph nodes decreased markedly. These changes were reflected histologi-
cally by a loss of follicles in lymph nodes, an atrophy of malpighian corpuscles in the spleens and a shrinkage of the thymic cortex followed by involution. The effects of different routes of administration were discussed as well as the effects on other systems and tissues.

O.P.J.

THE EFFECTS OF \(\beta,\beta'-\)DICHLORODIETHYL-METHYLMAMINE HYDROCHLORIDE ON THE BLOOD-FORMING TISSUES.

A nitrogen mustard in the form of its hydrochloride was administered to rabbits and dogs for the purpose of studying its effect on the blood and hematopoietic organs. Doses given either intravenously or subcutaneously produced similar results. There was a generalized damage to the blood-forming tissues but they did not all respond in the same manner nor to the same degree. Germinal centers of the lymph nodes and the spleen showed necrosis as early as three to six hours after administration of the drug. This was reflected in the blood by a lymphopenia and in the thoracic duct lymph by a decreased output. Damage to the bone marrow was reflected by a granulocytopenia following an initial increase. This granulocytopenia could be maintained by repeated doses of the drug. Since red corpuscles have a longer life than granulocytes in the circulating blood, anemia was produced only after repeated doses of the nitrogen mustard. Experimental anemias were produced in treated and untreated animals to determine the extent of bone marrow damage. The reticulocyte response was delayed in treated animals but it rose quite rapidly after the drug was discontinued. The action of this nitrogen mustard on the hematopoietic organs is very rapid and the recovery from it equally so.

O.P.J.

NITROGEN MUSTARD AS A THERAPEUTIC AGENT FOR HODGKIN'S DISEASE, LYMPHOSARCOMA, AND LEUKEMIA.
M. M. Wintrobe, C. M. Huguley, Jr., M. T. McLennan, and L. P. de C. Lima. From the Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah. Ann. Int. Med. 27: 519-540, 1947.

Seventy-seven patients were treated with di(B-chlorethyl) methyl amine hydrochloride, employing in most cases a dose of 0.1 mg./kilo repeated four to six times. In Hodgkin's disease along with general improvement and decrease in size of the involved lymphoid tissue, there was a striking alleviation of fever. Pruritis was variably affected, dependent on the general response to treatment. Response was considered to be good in 61 per cent of 28 cases, and remission occurred in several patients considered to be x-ray resistant. In lymphosarcoma, response was good in 4 of 11 patients and in chronic leukemia one-third of the cases responded well. Results in acute leukemia were generally unsatisfactory although mention is made that small doses of mustard were very effective in bone pain. The authors observed a consistent reduction in both granulocytes and lymphocytes following therapy, but in only one case did they encounter the clinical picture of agranulocytosis. The red count and platelet levels were variably affected.

C.A.F.

NITROGEN MUSTARD THERAPY IN CUTANEOUS BLASTOMATOUS DISEASE.

This report concerns the effect of the nitrogen mustards in 4 patients with lymphomatous disorders of the skin, and 1 patient with diffuse lupus erythematosus. Two patients with mycosis fungoides showed prompt and dramatic improvement in both signs and symptoms, but rapid relapse with progression (and death in 1 case) occurred. One patient with lymphosarcoma showed dissolution of skin lesions, lymph nodes, and abdominal masses, but died within two weeks with progressive severe leukopenia. It is of interest that no gross or microscopic evidences for lymphosarcoma were present at autopsy, although biopsy before treatment showed definite lymphosarcoma. In a patient with combined Kaposi's sarcoma and Hodgkin's disease, the nitrogen mustard therapy had no effect. In a patient with chronic diffuse lupus erythematosus, who had had no response to x-ray therapy, the use of nitrogen mustard was followed within four days by virtual disappearance of the eruption. The patient continued to improve,
ABSTRACTS

although a few areas of chronic discoid lupus erythematosus appeared on the face and neck within three months.

Most dramatic in the patients' responses was relief of the severe, stubborn itching present in mycosis fungoides and in the patient with lupus. The results here presented duplicate, in general, other observations of the response of lymphomatous disorders to the nitrogen mustards. The authors emphasize that they do not advocate the general use of these agents in nonmalignant conditions such as disseminated lupus.

S.E.


The results of the treatment of 25 patients with leukemia, and 8 patients with malignant neoplasm, with urethane are summarized. The drug was most effective in producing remission in patients with chronic myelogenous leukemia. Its effect was inconstant in chronic lymphatic leukemia. It was of no value in the acute leukemias, or in the patients with carcinoma or sarcoma. These results are in line with previous investigations of the effects of urethane in these disorders. The drug is recommended as an adjunct form of therapy in selected cases of leukemia, and not as a general substitute for x-ray therapy in these diseases.

S.E.


In this report the author attempts to classify various lymphomata according to their response to x-ray therapy; and proposes a therapeutic test with x-rays. His list places giant follicular lymphoblastoma at the top as most radiosensitive, and follows it with lymphatic leukemia, lymphosarcoma, "polymorphous-cell sarcoma," and Hodgkin's disease.

A therapeutic test is recommended for deep-seated tumors in which biopsy is not feasible, such as mediastinal masses. In such tumors, the author believes, lymphatic leukemia is easily excluded by examination of blood and bone-marrow preparations. If this diagnosis has been ruled out, he then measures the size of the mass on an x-ray film, and then gives 600 roentgens to the lesion in two days, and re-x-rays and measures the mass three days later. If there has been a decrease in the size of the mass of 25 per cent or more, a diagnosis of giant follicular lymphoblastoma or of lymphosarcoma is in order, and a tumor-killing dose of x-ray is given. Recurrence after this suggests lymphosarcoma.

If the second x-ray examination shows no diminution in the size of the tumor, the author gives further irradiation to the tumor. If regression occurs when 1,000 to 2,000 roentgens have been given, the mass is considered a "polymorphous-cell sarcoma." If regression occurs only after 2,000 to 3,000 roentgens, the mass is probably Hodgkin's disease. If no response has occurred after 3,000 roentgens have been given; the mass is radioresistant and probably belongs to a heterogeneous category including neurogenic sarcoma, carcinoma, various cysts, etc.

There is no discussion in the article of other forms of therapy of lymphomata, such as the nitrogen mustards.

S.E.


Anemia is generally considered as a sign of slight importance in the course of Hodgkin's disease. Marchal, on the contrary, considers it as an important factor and one of the essential signs of malignity. He has made a very comprehensive and well documented study of it.

Clinically, the author distinguishes three forms: anemias of an acute type, anemias connected with bony involvement, and anemia with splenomegaly. Clinical, anatomic and experimental studies show
ABSTRACTS

that the mechanism of these anemias is essentially hemolytic (shown by the reticulocytosis and the indirect bilirubinemia) and also erythrophagic (shown by the macrophage hyperplasia found particularly in the spleen).

J.P.S.

IMMUNOHEMATOLOGY

PREPARATION FROM HUMAN RED CELLS OF A SUBSTANCE INHIBITING VIRUS HEMAGGLUTINATION. P. M. de Burgh, Pen-Chung Yu, C. Howe, and M. Bovarnick. From the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass. J. Exper. Med. 81: 1-9, 1948.

Methods are described for extraction and purification of a substance inhibiting the agglutination of red cells by influenza (PR8) and mumps viruses. Human red cells of all types served as the chief source of inhibitor, activity being associated with the elinin fraction rather than with the stromatin fraction described by Calvin and coworkers. Material having similar properties was also found in human lung, but not in human liver, kidney or serum.

Active extracts were purified to the extent that 0.1 gamma of material inhibited one hemagglutinating dose of virus. The most highly purified fractions contained 1.6 per cent nitrogen, at least 50 per cent of polysaccharide, and no phosphorus. In the ultracentrifuge the purified preparation behaved as a polydisperse macromolecular substance. The active material was obtained from red cell stroma in an ether- and chloroform-soluble form which, on further treatment, was converted into a chloroform-insoluble form. It is considered possible that the former represents more closely the virus receptor as it exists in the intact cell.

The purified inhibitor was inactivated on incubation with virus at 37 C.

This report, and many others in the recent literature, serve to emphasize the importance of virus-red cell relationships. It seems likely that further attempts to purify the various substances present in erythrocyte stroma may produce results that will aid in clarifying the nature of viral parasitism. The possible therapeutic implications of these studies are also apparent.

L.E.Y.

IRON AND PORPHYRIN METABOLISM


The work begins with a discussion of previous results regarding the transportation of iron. A short survey of the importance of the serum iron fraction is also given, together with a discussion of the iron-binding capacity of serum. In this connection, the author mentions some of his own experiments aiming at an isolation of the specific iron-binding protein in the serum.

Holmberg's and Laurell's earlier work on the saturation limit for iron in the serum shows that there is a mean value of 312.7 per cent for this limit, which agrees quite well with the mean maximal serum iron concentration of 291.7 per cent found by Waldenström after the intravenous injection of iron into normal subjects. The iron-binding capacity of the serum is usually not fully utilized. The difference between the actual amount of serum iron and the saturation limit is called latent capacity. Above this saturation limit, the iron is loosely bound and reacts with phenanthroline directly. After a discussion of the different methods invented and used by the author, he finds that an indirect method is the easiest and best for the determination of the saturation limit. In the next chapter, the mode of binding of iron that has been added to the serum in vitro is discussed. With the aid of a number of ingenious adsorption experiments, the author shows that up to a certain concentration, iron is firmly bound and cannot be adsorbed. This holds true for both ferric and ferrous iron. The natural occurrence of ferric or ferrous iron linked with the serum protein cannot be determined. A large number of dialyzing experiments against serum seem to show that iron above the saturation limit is easily dialyzable and thus loosely bound. This would explain why the toxic effects of intravenously injected iron are seen only when the 'ceiling' observed by Waldenström was reached. This ceiling thus corresponds to the saturation limit.

In some later experiments it was shown that the iron may be liberated from the natural serum iron complex without changing the capacity for iron-binding of this protein. The process is thus reversible.
The mean value for the saturation limit in a number of normal persons was found to be $315 \pm 3.3\%$
per cent. Ingestion of iron does not increase the iron-binding capacity of the serum and the author
concludes that the iron must leave the mucosa in ionized form and not as an iron-protein complex.

During the latter part of pregnancy, the values for the serum iron show a steady rise. There is a drop
immediately before parturition. A study of maternal and fetal blood gives some interesting pieces of
information. It is obvious that the iron-binding component of the maternal serum cannot pass freely through
the placenta.

In posthemorrhagic anemia, the saturation limit is high but the serum iron values low. In untreated
pernicious anemia, the mean saturation values were lowered (256.7 per cent). The saturation limit was
not affected by the rapid drop in serum iron under treatment. The limit was low in acute infections and
paralleled the drop in the serum iron value. Cirrhosis of the liver and uremia showed a very low limit.

The work ends with a discussion of the theoretic implications of these results.

In a later paper published with Ingelman in Acta chem. scand., the author has given a more detailed
account of his preparative work. Swine serum was used for the experiments as it shows very little color
from bilirubin. It is therefore easier to use the color intensity of the iron binding protein as an indicator
for further fractionations. After addition of ferrous salts the color of the serum becomes reddish. Am-
monium sulphate was added up to 60 per cent saturation. The precipitate was separated and about 90 per
cent of the iron-binding component was still present in the solution. When the salt concentration was
increased to 75 per cent, albumin and the iron containing protein came down as a precipitate; it was re-
dissolved and dialyzed free from salt at pH 5.2 at $0^\circ$C. Ethyl alcohol was added to give a concentration of
20 volumes per cent. The solution was brilliantly red after the removal of the precipitate. After further
precipitation of a new fraction, the solution of the iron containing protein was left at $-15^\circ$C, when this
protein was precipitated as a red sediment. Judging from its solubility in concentrated electrolyte solu-
tions this protein cannot be regarded as a globulin. On electrophoresis, the isoelectric point was found to
be about 4.4. At ultracentrifugation, the sedimentation constant was found to be 5.8. The diffusion con-
stant was $5.8 \times 10^{-7}$. The parcel specific volume was not determined and the value given by Cohn and
colleagues for their iron binding $\beta$-globulin was used for the calculation of the molecular weight. It was
found to be $88,000$. This agrees very well with the value given by Cohn for the metal-combining protein,
but the isoelectric point was found by Cohn to be 5.6.

J.W.

**SERUM IRON LEVELS IN ADOLESCENT GIRLS: A STUDY OF THREE CASES.** F. A. Johnston. From the Depart-

The concentration of iron in the serum was determined eleven times over a period of five months in 3
adolescent girls whose well controlled diet contained 4 to 5 mg., then 9 mg. and 11 mg. iron per day for
the three successive periods of the study. This investigation was carried out with the expectation that
changes in serum iron on a controlled diet might provide an estimate of the iron requirement for growth
and menstruation.

Hemoglobin levels of the subjects were near average values for girls, while the mean serum iron con-
centrations of 72, 62, and 46 micrograms per 100 cc. respectively were low, thus suggesting that serum iron
might be more sensitive than hemoglobin as an index of iron stores. Since serum iron levels did not fall
significantly, however, over a nine week period on low iron intake or rise over a period of twelve weeks
on a fair iron intake, it was concluded that serum iron concentration does not respond to dietary intake
quickly enough for use as a criterion of adequacy in short-term studies.

L.E.Y.

**A MICROMETHOD FOR THE QUANTITATIVE DETERMINATION OF THE URINARY COPROPORPHYRIN ISOMERS**
(1 AND III). S. Schwartz, V. Hawkins, S. Cohen, and C. J. Watson, From the Department of Medicine,
University of Minnesota Hospital, Minneapolis, Minn., and the Metallurgical Laboratory, University

A method is described for the extraction of coproporphyrin quantitatively from 100 cc. aliquots of
urine and for its measurement fluorophotometrically. There are two alternate procedures for further
purification and for isomer analyses, both dependent on the difference in fluorescence stability of the two
porphyrin methyl esters in 30 to 35 per cent aqueous acetone in the cold.

C.A.F.

A case of congenital porphyria in a 17 month old girl is presented and its significance as related to an understanding of normal and pathologic pigment metabolism is emphasized. The clinical features of the case included red urine, erythrodontia, hydroa-aestivale and hirsutism. Analysis of the urine revealed the presence of uroporphyrin I and coproporphyrin I in addition to an unidentified porphyrin, the methyl ester of which melted at 209° to 212°C. The feces contained coproporphyrin I and another porphyrin with a somewhat higher melting point, but no uroporphyrin I was detected in the feces. A concise review of the literature on porphyrin metabolism is included in the report.

L.E.Y.

Hemoglobinuria


Available fluid was measured by the thiocyanate method before and after a three day period of water deprivation in 20 rabbits, 15 of which were then given nine or ten intravenous injections of hemoglobin, totalling 1.8 Gm. per Kg. over a three day period. Wide variation in available fluid was found in different animals, especially in the females, some of which had appreciably lower volumes than any of the males. Wide variation was also noted in response to water deprivation. Inverse relationship is claimed between quantity of available fluid and severity of hemoglobinuric nephrosis, but the number of animals in each category is too small to permit conclusions in this respect. It should be noted, moreover, that during the period of hemoglobin injection all of the rabbits that urinated had an acid urine. The deleterious effect of dehydration might have been more clearly established if alkalinity of the urine could have been maintained.

Intravenously administered hemoglobin solutions were found to exert a diuretic effect in 3 of 7 rabbits tested, but when available fluid was depleted, elimination of hemoglobin appeared to be delayed or inhibited. It is postulated that under such circumstances, hemoglobin enters the renal tubules, pigment casts are formed, degeneration of tubular epithelium appears and uremia follows.

Additional findings of interest were necrosis of the liver and pulmonary edema in some of the rabbits that died of uremia. The combined weight of the kidneys of rabbits dying of hemoglobinuric nephrosis was significantly in excess of that of the control rabbits and of the test animals with transient nephrosis.

L.E.Y.

Renal Athrocytosis and Intracellular Digestion of Intraperitoneally Injected Hemoglobin in Rats. L. J. Rather. From the Department of Pathology, Stanford University School of Medicine, San Francisco, Calif. J. Exper. Med. 87: 163-174, 1948.

Rats were injected intraperitoneally with solutions of human hemoglobin in total doses averaging 5 Gm. per Kg. Serum hemoglobin concentration, rate of excretion of hemoglobin in the urine and urine output were measured, and the rats were then killed at varying intervals for the purpose of studying the tissues.

In rats killed two hours after the first injection of hemoglobin, discrete bodies with the staining characteristics (Dunn’s stain) of hemoglobin were found in small numbers within the epithelium of the proximal convoluted tubules. In rats killed at seventeen hours, the renal epithelium was packed with these particles, and it was found that formation of casts did not occur to any extent until the amount of athrocyted material reached a maximum. Although it was inferred that break-up of the hemoglobin began prior to release of inorganic iron, the appearance of hemosiderin was the first visible evidence of intracellular splitting of hemoglobin. Deposits of hemosiderin were most marked at about sixty-five hours after which time they slowly disappeared. It is of interest that very little hemosiderin and no hemoglobin were demonstrable in the parenchymal or Kupffer cells of the liver, nor in phagocytic cells of spleen or femoral marrow.
There was no evidence that intracellular accumulation of hemoglobin damaged renal epithelium, and it was not until large amounts of hemoglobin were present within lumina of the nephrons that injury could be detected. Oliguria was attributed to interference with flow through lumina due to increased viscosity of protein-rich fluid.

These observations are in general agreement with those reported by other investigators and they support the hypothesis that hemoglobin filtered by glomeruli undergoes athrocytosis by tubular cells and is then returned to the blood in a simpler form.

L.E.Y.