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# ABSTRACTS

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## ABNORMAL BLOOD PIGMENTS

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THE BLOOD PIGMENTS; THE PROPERTIES AND QUANTITATIVE DETERMINATION WITH SPECIAL REFERENCE TO THE SPECTROPHOTOMETRIC METHODS. *H. O. Michel and J. S. Harris. J. Lab. & Clin. Med., 25: 445-463, 1940.*

The authors present an orientational discussion of the nature and chemical properties of the various blood pigments derived from hemoglobin. The absorption spectra of these substances and their reactions to certain chemicals are described. Particularly useful is their outline of the qualitative identification by simple spectroscopy of methemoglobin, sulfhemoglobin, hemein, and pseudomethemoglobin (methemalbumin). Quantitative methods of analysis, i.e., gasometric, combined gasometric and colorimetric, and spectrophotometric are reviewed. The authors present a spectrophotometric method with experimental data based on extinction coefficients for the determination of mixtures of sulfhemoglobin, methemoglobin, and oxyhemoglobin.

C. A. F.

A RAPID SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF METHEMOGLOBIN AND CARBONYLHEMOGLOBIN IN BLOOD. *B. L. Herecker and F. S. Brackett. J. Biol. Chem., 152: 669-677, 1944.*

This method utilizes the same principle as the method employed by Evelyn and Malloy (*J. Biol. Chem. 126: 655, 1938*). The instruments used were the Coleman spectrophotometer and especially constructed photometers. Measurements were made at 8000 Å and at 4965 Å. Results by the method were compared to the Van Slyke gasometric determinations. The procedure is simple, and subject to an error of about 1%. This method appears to be the most satisfactory for routine use.

C. A. F.

THE EFFECT OF METHEMOGLOBIN ON THE EQUILIBRIUM BETWEEN OXYGEN AND HEMOGLOBIN. *R. C. Darling and F. J. W. Roughton. Am. J. Physiol., 137: 56-68, 1942.*

Experiments were performed on ox and on human blood. The methemoglobin was made by aerobic oxidation, by ferricyanide, or by nitrite. The presence of methemoglobin caused a shift in the oxygen dissociation curve to the left, qualitatively the same but not as great as that produced by carbon monoxide. The shift was reversible once the methemoglobin had reverted to the ferrous form.

The authors conclude that "this newly discovered effect means that in methemoglobinemia the tissues are liable to anoxemia, not only from loss of oxygen capacity of the blood, but also from increasing difficulty in the unloading from the blood of such oxygen as is available."

The mechanism is believed to be the formation of compounds intermediate between reduced hemoglobin (entirely ferrous) and methemoglobin (entirely ferric), the conversion of one or more of the four ferrous atoms in the hemoglobin molecule to the ferric valence leading to an increased affinity of the remaining ferrous atoms for oxygen.

C. A. F.

THE COMPARATIVE ANOXEMIC EFFECTS FROM CARBON MONOXIDE HEMOGLOBIN AND METHEMOGLOBIN. *D. Lester and L. Granberg. J. Pharm. & Exper. Therap., 81: 182-188, 1944.*

Carbon monoxide hemoglobin and methemoglobin, both functionally inert, would seem to differ physiologically only in the degree to which they shift the oxygen dissociation curve and interfere with normal tissue oxygen exchange. In this study the levels of these pigments at which death occurred are re-

ported. Cats became unconscious and died when 66 to 71% of hemoglobin had been converted to carbon monoxide hemoglobin. Cats and dogs could tolerate more than 80% conversion of hemoglobin to methemoglobin without becoming unconscious. The asphyxial effects of methemoglobin thus were significantly less than those of carbon monoxide.

C. A. F.

CONGENITAL IDIOPATHIC METHEMOGLOBINEMIA. FAVORABLE RESPONSE TO ASCORBIC ACID THERAPY. R. F. Sievers and J. B. Ryan. Arch. Int. Med., 76: 299-307, 1945.

The article includes the most complete tabulation of clinical reports of this rare disease, summarizing 18 cases and adding one. (In the opinion of the reviewer the case reports of Miller, and of Schwartz and Rector should be excluded; their discrepancies are mentioned by the authors.)

In the majority of cases the cyanosis had been first attributed to a congenital cardiac defect. Symptoms were mild, including dyspnea on exertion, tachycardia, and headache. Methemoglobin levels varied from 10 to 57% of the total hemoglobin. The mean was 30 to 40%.

The authors reported one case and demonstrated the efficiency of ascorbic acid and methylene blue in reducing the degree of methemoglobinemia. Before ascorbic acid therapy the blood of their patient had no capacity to revert methemoglobin on standing. Normal plasma was able to effect some slight reduction of the methemoglobin of the patient's cells. After four days of treatment there was 1.6 Gm. reduction of methemoglobin in the patient's blood on standing 24 hours. Equal disappearance of methemoglobin at this time was found with the red cells suspended in either plasma or sodium chloride, which pointed to the operation of an intracellular rather than a plasma reducing substance. They felt that adding ascorbic acid partially replaced the defective cell reducing system.

C. A. F.

FAMILIAL IDIOPATHIC METHEMOGLOBINEMIA AND ITS TREATMENT WITH ASCORBIC ACID. H. Barcroft, Q. H. Gibson, D. C. Harrison, and J. McMurray. Clin. Sci., 5: 145-157, 1945.

The effect of ascorbic acid therapy, 100-200 mgms. per day, in one case of familial idiopathic methemoglobinemia was described in detail. The initial level of 7.3 grams methemoglobin fell to 0.8 grams over a period of 30 days while the total hemoglobin level remained fairly constant. The decrease in ferric pigment paralleled the rise of blood ascorbic acid which was initially low. The same reducing action of ascorbic acid was demonstrated *in vitro* with hemolyzed or non-hemolyzed blood aerobically and anaerobically.

The authors felt that the increase in ascorbic acid concentration coincidental with a decrease in methemoglobin concentration was consistent with Gibson's report that the reaction may be expressed by a bimolecular formula (that the rate of reduction of methemoglobin is proportional to the product of the concentration of the two reactants).

The normal erythrocyte is able to reduce methemoglobin to hemoglobin in the presence of either lactate or glucose. Methylene blue greatly catalyzes this reaction. The patient's blood shows a greatly decreased ability to effect this reversion with either substance. Methylene blue in the presence of glucose produced a normal catalytic reduction in the patient's red cell, but did not catalyze with lactate as a substrate. This was interpreted as a defect in the enzyme system of the red cell which functions to convert methemoglobin to hemoglobin.

C. A. F.

CYANOSIS IN INFANTS CAUSED BY NITRATES IN WELL WATER. H. H. Comly. J. A. M. A., 129: 112-116, 1945.

Two infants are reported who on a formula containing boiled water developed severe cyanosis which was determined spectrophotometrically to be due to methemoglobinemia. The administration of in one case 1.1 mgm./kilo. and in the second of 1.5 mgm./kilo. of methylene blue within a few minutes reverted the abnormal pigment to bivalent hemoglobin. The well water used in both cases contained large amounts of nitrates and was heavily polluted with bacteria, presumably nitrate formers. The ingested nitrate was apparently converted by bacteria of the intestine to nitrite which, following absorption into the blood stream, produced methemoglobinemia.

C. A. F.

DYE POISONING IN THE NURSERY. A REVIEW OF 17 CASES. *J. Granbarth, C. J. Bloom, F. C. Coleman and H. N. Solomon.* *J. A. M. A.*, 128: 1155-1157, 1945.

Another episode of aniline poisoning through skin absorption is described. Seventeen babies wearing new aniline-stamped diapers developed cyanosis; fifteen other babies with previously washed diapers did not. Many of the affected infants apparently developed anemia. Three died of infection and one of intracranial hemorrhage. Methemoglobinemia was assumed to be present and treatment consisted of methylene blue, oxygen and 5% carbon dioxide, and transfusions. The authors felt that methylene blue was without apparent effect and expressed skepticism as to its value in the treatment of methemoglobinemia. This opinion seems unwarranted in the absence of spectroscopic data identifying the pigment as methemoglobin.

C. A. F.

METHÄMOGLOBIN, INNENKÖRPER DER ERYTHROCYTEN UND ANÄMIE. *W. Heubner.* *Klin. Wchnschr.*, 20: 137-141, 1941.

In a review article Heubner discusses the concept that the formation of methemoglobin is a reversible oxidative process without associated hemoglobin breakdown. An entirely different type referred to as deep oxidation is represented by the splitting open of the porphyrin ring and the production of verdohemochromogen. Heubner believes sulfhemoglobin is a product of deep oxidation and that it does not contain sulfur. (His ideas on sulfhemoglobin seem poorly substantiated and are at variance with most present opinions.)

Considerable attention is given to the early descriptions by Heinz, Ehlich and others of the "inner bodies" in erythrocytes, and of their production by hemolytic aromatic compounds. "Inner bodies" and anemia may be produced with little or no methemoglobinemia; methemoglobin is not a stage in the development of the "inner body" and anemia.

On the basis of rather inadequate *in vitro* evidence, the author feels that hemolytic agents may inactivate erythrocyte catalase and thus leave the cell vulnerable to oxidation by hydrogen peroxide.

While many assertions of the author cannot be accepted, his general thesis of separating methemoglobinemia from hemolytic anemia is undoubtedly valid and well documented.

C. A. F.

THE CONTROL OF METHEMOGLOBINEMIA WITH METHYLENE BLUE. *W. B. Wendell.* *J. Clin. Investigation*, 18: 179-185, 1939.

The normal rate of reconversion of methemoglobin (produced by injection of sodium nitrite) to hemoglobin by the red cell was 0.028 vol. %/min. Methylene blue administered intravenously at the peak of methemoglobin formation increased the reversion rate about five fold.

Methemoglobin was demonstrated in the blood of patients on sulfonamide. Intravenous injection of 0.1 cc. to 0.2 cc. per kilo. of body weight of a 1% aqueous solution of methylene blue converted in 45 minutes all of the methemoglobin into functionally active hemoglobin. The duration of this action was 12 to 24 hours and no toxic effects were observed.

C. A. F.

STUDIES ON SULFHAEMOGLOBIN. *R. Lemberg, H. F. Holden, J. W. Legge, and W. H. Lockwood.* *Aust. J. Exper. Biol. & Med. Sci.*, 20: 161-167, 1942.

This study was directed at the confused subject of the structural nature of sulfhemoglobin. The authors uphold the idea that the compound is composed of a closed porphyrin ring. They differentiate spectroscopically the open ring reduced choleglobin from sulfhemoglobin by its behavior to alkali or carbon monoxide. Sulfhemoglobin was reconvertible into protohemochromogen. Artificially produced sulfhemoglobin generally contained as a by-product 20 to 50% of choleglobin. The properties of this decomposition product have led to confusion. In clinical sulfhemoglobinemia little or no choleglobin was found, although it was present in the blood of rats fed sulfur and acetanilide.

C. A. F.

METHEMOGLOBIN: A NORMAL CONSTITUENT OF BLOOD. *W. D. Paul and C. R. Kemp.* *Proc. Soc. Exp. Biol. and Med.*, 56: 55-56, 1944.

By a spectrophotometric method methemoglobin was found in 99 of 100 hospital patients in amounts

ranging from 0.01 to 0.5 Gm./100 cc. blood. In 20 blood donors the range was from 0.03 to 0.13 Gm.%. This confirms the previous observation of Ammundsen, E. (J. Biol. Chem., 138: 563-570, 1941) and has been corroborated by F. J. W. Roughton, R. C. Darling and W. S. Root (Am. J. Med. Sc., 208: 132, 1944) and by D. L. Drabkin and C. F. Schmidt (J. Biol. Chem., 157: 69-83, 1945) using gasometric methods.

The demonstration of methemoglobin in the blood of normal individuals helps to establish the concept of an equilibrium between the reduced and oxidized forms of hemoglobin. The extreme degree that the hemoglobin-methemoglobin equilibrium has been displaced in favor of the reduced form is an expression of the efficiency of the intracellular reducing system of the mammalian red cell.

C. A. F.

METHEMALBUMIN. PART I. CLINICAL ASPECTS. N. H. Fairley, M.D. Quart. J. Med., 10: 95-114, 1941.

The blood of patients with severe hemolytic anemia (e.g., blackwater fever, and paroxysmal nocturnal hemoglobinuria) is of peculiar chocolate color and the plasma is brown. This discoloration is caused by the presence of an abnormal pigment, methemalbumin, in the plasma. Fairley and Bromfield identified this pigment and differentiated it from methemoglobin and sulfhemoglobin, which in contrast to methemalbumin, are practically always intra-erythrocytic pigments. Methemalbumin is found in the plasma when intravascular hemolysis has been excessive. It is apparently formed in the plasma by the combination of the heme complex (derived from the destruction of hemoglobin) with plasma albumin. Cases described as "hematinaemia" are actually cases of methemalbuminemia, since hematin in plasma immediately combines with albumin to form methemalbumin. It can readily be differentiated from other heme pigments by its characteristic alpha absorption maximum at 623-624 $\mu\mu$  (methemoglobin 630 $\mu\mu$ , sulfhemoglobin 618  $\mu\mu$ ), and by the effect of certain chemical agents on its alpha absorption bands.

Fairley believes that all cases of hemolytic anemia may be classified into three groups accordingly as they show a.) hyperbilirubinemia alone, b.) hyperbilirubinemia and methemalbuminemia, c.) hyperbilirubinemia, methemalbuminemia, and haemoglobinemia. He suggests that hyperbilirubinemia in the absence of methemalbuminemia suggests intracellular (reticulo-endothelial) blood destruction whereas methemalbuminemia implies lysis of corpuscles in the circulating blood.

J. F. R.

METHEMALBUMIN. PART II. ITS SYNTHESIS, CHEMICAL BEHAVIOUR, AND EXPERIMENTAL PRODUCTION IN MAN AND MONKEYS. N. H. Fairley, M.D. Quart. J. Med., 10: 115-138, 1941.

Methemalbumin was invariably formed *in vitro* when purified alkaline hematin (ferric) was added to plasma or to the albumin fraction of plasma or to crystalalbumin. It was not formed when hematin was added to euglobin, pseudoglobulin seroglycoid or globuglycoid. The methemalbumin so formed was identical spectroscopically and chemically with that found in the plasma of patients with hemolytic anemia. Numerous chemical and physical studies indicated that methemalbumin is a definite chemical compound consisting of a prosthetic group, oxidized hematin (ferric), and a protein component, native serum albumin. On reduction, hemalbumin, a ferrous compound, was formed, but this compound differed from hemoglobin in being unable to combine loosely with oxygen.

Injection of alkaline hematin (ferric) or of reduced alkaline hematin (ferrous) into human subjects and into monkeys resulted in the immediate formation of methemalbumin. Interestingly enough Fairley reports that there was no rise in plasma bilirubin following the injection of hematin, observations in conflict with those of Pass, Schwartz and Watson.

Fairley suggests that the mode of extracorporeal hemoglobin disintegration following intravascular hemolysis in man may occur as follows: hemoglobin is split in the circulation into globin and reduced hematin (ferrous); the latter is immediately oxidized to haematin (ferric) which combines with serum albumin to form methemalbumin. The subsequent fate of the methemalbumin is not discussed.

J. F. R.

METHEMALBUMINEMIA DURING COMBINED THERAPY WITH PAMAQUINE AND QUININE. W. D. Blake, C. G. Zubrod, and M. Rosenfeld. Fed. Proc., 5: 167, 1946.

Methemalbumin appeared consistently in the serum of patients receiving pamaquine and quinine in combination for the treatment of malaria, although this pigment was not produced in patients receiving either of the two drugs alone in the same dosage. The concentration of methemalbumin progressively increased during fourteen days of combined therapy.

There were concurrent disturbances in pigment metabolism consisting of methemoglobin production in the erythrocytes, increased fecal urobilinogen and increased fecal and urinary coproporphyrin.

J. F. R.

THE CONVERSION OF HEMATIN TO BILIRUBIN FOLLOWING INTRAVENOUS ADMINISTRATION IN HUMAN SUBJECTS. *I. J. Pass, S. Schwartz and C. J. Watson.* J. Clin. Investigation, 24: 283-291, 1945.

The normal conversion of hemoglobin to bilirubin is supposed to occur with an opening of one of the methene linkages of the porphyrin ring and the formation of an intermediary "pseudohemoglobin," a biliverdin-iron-globin compound. Hematin, a compound in which the porphyrin ring is still intact but which has been split free from the globin fraction of hemoglobin, is not formed in the usual *in vivo* mechanism of hemoglobin degradation. Fairley showed, however, that hematin is formed when excessive intravascular hemolysis occurs, and that it immediately combines with plasma albumin to form methemalbumin.

The way in which the body rids itself of hematin and methemalbumin is obscure, but of some fundamental interest because of the frequency of its formation in severe hemolytic states, and because malarial pigment is identical with hematin. Duesberg, Bingold, Fairley and other investigators have reported that once hematin and methemalbumin were formed intravascularly they were not converted to bilirubin. Pass, Schwartz and Watson present definite evidence that hematin is converted into bilirubin. Following injections of hematin into human subjects an increase of fecal urobilinogen occurred which was proportional to the amount of hematin given. The serum bilirubin was not uniformly elevated, however, and methemalbumin persisted in the serum for prolonged periods of time, suggesting that the conversion of hematin to bilirubin occurs relatively slowly in contrast to the rapid conversion of hemoglobin to bilirubin.

J. F. R.