

Methemoglobin: A Normal Constituent of Blood.*

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The conversion of hemoglobin to methemoglobin by various chemical compounds and drugs has been reported repeatedly. Methemoglobin, so produced, has been held responsible for the toxic symptoms associated with its presence in the blood stream. However, there is little conclusive evidence that methemoglobin is the single causative factor in such toxemias. Methemoglobin may be a secondary factor in the chain of events associated with its presence in the blood stream.

The present work was undertaken to ascertain whether or not methemoglobin could be detected in the blood of normal persons, and to establish its normal range of concentration if it could be shown to be present. It was felt that either an affirmative or a negative answer to this question would have some bearing upon the problem of correctly interpreting the role of methemoglobin in pathologic conditions. If methemoglobin is a constituent of blood in normal persons, even in small concentrations, the view that it, in itself, is not toxic, and that its presence in higher concentrations in pathologic conditions is secondary to other factors which are responsible for the toxic symptoms, would gain support.

Experimental. Determinations were made upon two groups: (A) One hundred patients were selected at random from the wards of the Department of Medicine of the University of Iowa Hospital. No degree of selection was exercised other than to exclude those patients who were receiving sulfa drugs or other recognized methemoglobin-formers. The distribution in terms of sex was about 3 males to one female. The ages of the patients ranged from 7 to 78 years; no one age group predominated. A wide range of diseases was represented in order to rule out the possibility that methemoglobinemia was part of any given pathologic condition.

Blood samples were obtained by venipuncture from the forearm, using potassium oxalate as the anticoagulant. All blood samples were taken immediately to the laboratory, and, in most cases, complete analysis was made in 15 to 20 minutes. In no case did the period between withdrawal of the blood and completion of the analysis exceed one hour.

The method of analysis was that of Michel and Harris,¹ using a Coleman spectrophotometer, model 10S, and a Coleman electrometer, model 3E. This method utilizes the change in the extinction coefficient on conversion of methemoglobin to cyanmethemoglobin. One ml of whole blood is pipetted into a 25 ml volumetric flask. The blood is hemolyzed by the addition of a few milligrams of saponin. It is then diluted to approximately 23 ml with distilled water. One and one-fourth ml of 1M phosphate buffer, pH 7.4, is then added, and the solution is diluted to 25 ml. Without filtering, the extinction coefficient is ascertained on a portion of the sample of 634 mu. Two to 4 mg of dry KCN are then added to the solution in the spectrophotometer cuvette, and the contents mixed by inversion. One minute later the extinction coefficient is again ascertained, and the methemoglobin calculated as follows:

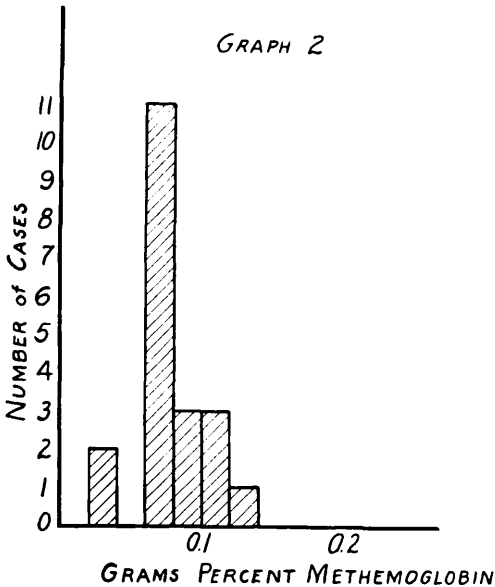
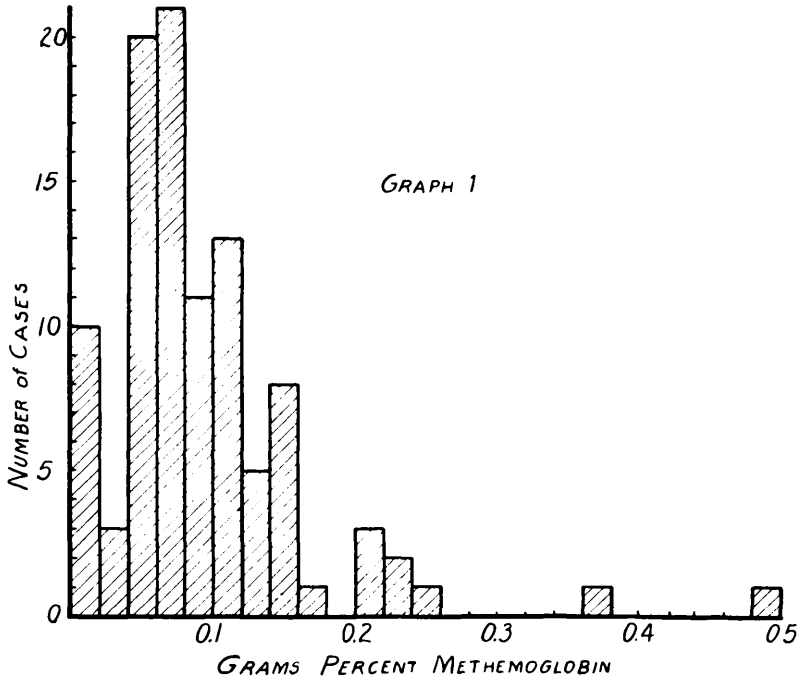
$$\frac{\text{Grams \% methemoglobin} = \frac{E_{634} - E_{634 \text{ with cyanide}}}{F}}$$

where $F = 0.0728$ at pH 7.4

(B) Twenty blood samples were obtained directly from donors to the blood bank of the University Hospitals. This group, therefore, was highly selected, in that no known pathologic condition existed, and no known medication preceded the estimation. All other factors in this series of estimations were

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¹ Michel, H. O., and Harris, J. S., *J. Lab. and Clin. Med.*, 1940, **25**, 445.



identical with those of group (A).

Results. Methemoglobin was found in the plasma of all patients except one. The values in the remaining cases ranged from 0.01 to 0.5 g %. Thirteen percent gave values ranging up to 0.04 g %, and 9 % of the group ranged from 0.16 to 0.38 g % of methemoglobin. The mean methemoglobin for one hundred cases was 0.09 g %. Graph I shows the distribution of methemoglobin levels for this group. No correlation was observed between methemoglobin and hemoglobin levels.

In group (B) methemoglobin was detected in the blood of all donors studied. The minimum amount was 0.03 g %; the maximum, 0.13 g %. Graph II shows the distribution of methemoglobin values in this group.