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Postmortem Methemoglobin Concentrations and Their Significance

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ABSTRACT: Small concentrations of methemoglobin are present in the blood of normal individuals. Increased concentrations of methemoglobin can be formed by the action of certain chemicals or drugs, or in individuals with specific genetic defects. There is little information available concerning the validity of postmortem methemoglobin concentration as an indicator of antemortem methemoglobinemia. We measured blood concentrations of methemoglobin in 49 autopsy specimens. We conclude that postmortem methemoglobin concentrations are not valid indicators of antemortem methemoglobinemia.

KEYWORDS: toxicology, methemoglobin, blood

Methemoglobin is a product of hemoglobin oxidation in which the ferrous iron in the hemoglobin molecule is converted to its ferric form. In this state, it is unable to combine with oxygen and function in the transport of oxygen to tissues. Under normal circumstances a small amount of methemoglobin is formed and that which is present is reduced by the methemoglobin reductase pathway which utilizes nicotinamide adenine dinucleotide (NADH) and nicotinamide adeninedinucleotide phosphate (NADPH). Rare congenital deficiencies in the reductase pathway can produce chronically elevated levels of methemoglobin. Various chemicals and drugs have been identified as causing increased concentrations of methemoglobin; these include nitrogen oxides in products of combustion (either from fires or internal combustion engines), as well as drugs and chemicals in the nitrite, nitrate, chlorate, and quinone classes.

Katsumata et al [*1*] reported elevated methemoglobin concentrations in blood from fatalities of fire or exhaust fumes and suggest that methemoglobin may contribute to death in such individuals. They further suggested that methemoglobin levels may be useful in assessing hemoglobin oxidation by the oxides of nitrogen which are produced in combustion.

Butyl nitrite and its congener amyl nitrite are currently promoted as aphrodisiacs and the

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use of nitrites by inhalation is occurring within increased frequency. Production of methemoglobin by inhalation of butyl nitrite has been demonstrated experimentally by Horne et al [2]. In their experiments methyl and butyl nitrite concentrations were determined in blood after subjects sniffed proprietary butyl nitrite. The concentrations of methemoglobin produced were directly related to the concentrations of butyl nitrite in the blood.

Shesser et al [3] reported three cases of oral ingestion of isobutyl nitrite, one of which was fatal. In the fatality, the methemoglobin concentration in the blood was reported as 38% of total hemoglobin: it was not stated whether antemortem or postmortem blood was analyzed. In the two nonfatal cases, methemoglobinemia was diagnosed on clinical information and methemoglobin analysis was not reported.

This study reports methemoglobin levels in the series of 49 postmortem blood samples. These samples were obtained from autopsies on individuals who died under a variety of circumstances and from various causes but without evidence of exposure to products of combustion or use of nitrites or other agents. We evaluated postmortem methemoglobin concentrations to determine their potential usefulness in reflecting antemortem values.

Methods

Blood samples were taken from the heart in 49 autopsies performed at the King County Medical Examiner's Office in Seattle, WA. Aside from the exclusion of victims exposed to fire and combustion engine exhaust, no particular medical criteria were used in the selection; the victims were autopsied because their deaths were violent or sudden and unexpected. Of the deaths 78% were violent while 22% were a result of natural causes. The ages of the victims and the estimated postmortem interval were the only information used in this study. The samples were collected in tubes containing sodium fluoride, immediately frozen, and maintained at 0 to −4°C until analysis. The samples were batched analyzed, a method commonly used in toxicological studies.

The analysis was performed using an IL 282 Co-oximeter®. The method uses hemolyzed whole blood and measures absorbance at 626.6μm. The absorbance is directly related to the concentration of methemoglobin [4]. The results obtained are reported as a percentage of total hemoglobin. Normally methemoglobin accounts for less than 1.5% of total hemoglobin. Sulfhemoglobin was not determined in the samples studied.

Results

The subjects ranged in age from 1 to 82 years with an average of 41 years. The postmortem interval, as estimated by scene investigation and corroborated by autopsy findings, showed a range of 2 to 72 h. The mean postmortem interval was 24.67 h with a standard deviation of ±15.61 h. The amount of methemoglobin expressed as percentage of total hemoglobin ranged from 0.8 to 57%. A mean value of 17.27% was calculated and a standard deviation of ±13.28% (Table 1) was determined. Postmortem interval and methemoglobin levels were plotted using regression analysis (Fig. 1). The correlation coefficient r^2 value showed no significant relationship between the postmortem interval and methemoglobin values.

Discussion

We evaluated postmortem methemoglobin concentrations to determine their potential usefulness in reflecting antemortem methemoglobinemia. Our results show a wide range of methemoglobin concentrations in postmortem blood from individuals who should have had normal antemortem concentrations. Furthermore, there was no correlation with the antemortem circumstance of death, autopsy findings, or postmortem interval from death to autopsy examination.

TABLE 1—*Mean and standard deviation of postmortem methemoglobin determination.*

	Range	Mean	Standard Deviation ($n - 1$)
Age of victim	1-82 yrs.	41 yrs.	...
Postmortem interval	2-72	24.67 h	±15.61 h
Methemoglobin, %	0.8-57	17.27	±13.28

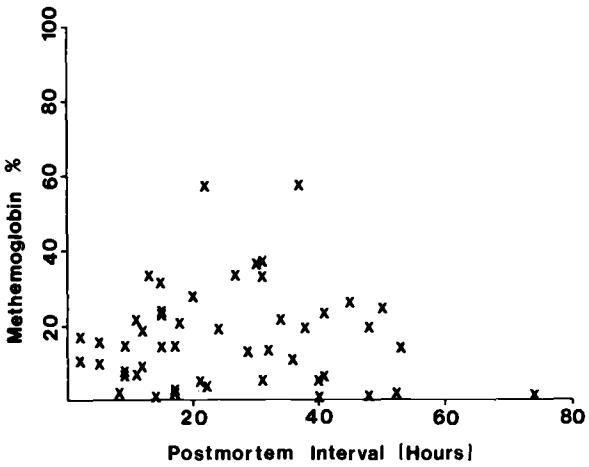

FIG. 1—*Scatter diagram demonstrating the relation between postmortem methemoglobin blood levels and postmortem interval.*

Postmortem blood chemistry determinations frequently fail to reflect the antemortem state of the victim and are subject to a wide range of interpretative analysis [5]. The variability of postmortem methemoglobin concentrations which we determined is not surprising. The methemoglobin reductase system requires the presence of reduced diphosphopyridine nucleotide (DPNH) or triphosphopyridine nucleotide (TPNH) and in the absence of oxidative metabolism these would be decreased or absent. Postmortem bacterial growth and bacteremia is a common occurrence, particularly in forensic science investigations where bodies are recovered in a variety of ambient conditions and at different times after death. During postmortem autolysis, spontaneous hemolysis occurs, intracellular hemolysins release, and hydrogen peroxide may be formed by bacterial metabolism: any of these factors would promote a conversion of free hemoglobin to methemoglobin [6, 7]. Finally other unidentified oxidizing agents may be released by the body after death and contribute to this transformation.

Conclusion

Our observations indicate that methemoglobin concentrations determined by standard analytical methods are unsuitable as a chemical parameter of antemortem methemoglobin concentrations and methemoglobin production is a random function of postmortem change. Although methemoglobin determinations may be of some value in fire related deaths when blood samples are obtained shortly after death, postmortem methemoglobin determinations are of limited value in assessing antemortem methemoglobinemia in routine autopsies.

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