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Simultaneous Determination of Carboxyhemoglobin and Methemoglobin in Victims of Carbon Monoxide Poisoning

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ABSTRACT: Concentrations of methemoglobin (Met-Hb) and carboxyhemoglobin (HbCO) were simultaneously determined in blood samples from the victims of city gas poisoning, fires, and exhaust fumes poisoning. All the samples contained high concentrations of HbCO, although the concentrations for victims of city gas poisoning were significantly higher than those for victims of fires or exhaust fumes poisoning. Only negligible amounts of Met-Hb were detected in the samples from cases of city gas poisoning, while 4.9 to 31.6% of Met-Hb were found in all but one (1.8%) of the samples of the other two causes. Heat denaturation was considered to be the main cause of Met-Hb production in cases of fires, and inhalation of oxides of nitrogen in cases of exhaust fumes poisoning.

KEY WORDS: pathology and biology, carbon monoxide, poisons

Postmortem blood, the material for the determination of carboxyhemoglobin (HbCO) in medicolegal practice, often contains methemoglobin (Met-Hb). There have been some reports concerning the simultaneous determination of HbCO and Met-Hb [*1,2*]. However, scanty information is available to date as to the mechanism of Met-Hb production in postmortem blood [*3*].

In the present study, HbCO and Met-Hb in blood were measured simultaneously in the victims of carbon monoxide poisoning to see if there were any differences in the production of HbCO and Met-Hb among the different sources of carbon monoxide.

Materials and Methods

Blood samples from victims of city gas poisoning, fires, and exhaust fumes poisoning were obtained by cardiac puncture. Determinations of reduced hemoglobin (HHb), oxyhemoglobin (HbO_2), and HbCO were made as described by Katsumata et al [*2*] using a polarographic oxygen analyzer (Model 1008, Beckman Instruments Inc.). Determination

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of total Hb was made according to the cyanmethemoglobin method [4], and Met-Hb was estimated both by subtracting the sum of HHb, HbO_2, and HbCO from total Hb [2] and by the method of Leahy and Smith [5]. For spectrophotometry, a Hitachi 139 spectrophotometer was used. All the determinations were made within 48 h after the death of the victims.

All the reagents used were of the highest purity commercially available.

Results

Blood concentrations of HbCO and Met-Hb in victims of city gas poisoning are summarized in Table 1. Each sample contained a large amount of HbCO and a negligible amount of Met-Hb. Inert hemoglobin concentrations determined as described by Katsumata et al [2] ("calculated" Met-Hb in Table 1) were closely comparable to those determined by the method of Leahy and Smith [5] ("observed" Met-Hb in Table 1).

Blood concentrations of the two Hb derivatives in victims of fires and exhaust fumes poisoning are shown in Tables 2 and 3, respectively. Most blood samples showed high contents of Met-Hb as well as HbCO. Again "calculated" Met-Hb concentrations were almost the same as those "observed." The HbCO concentrations for city gas poisoning were significantly higher than those for fires ($P < 0.001$, student's t test) or for exhaust fumes poisoning ($P < 0.005$).

Discussion

Calculated Met-Hb in the tables represents an inert Hb that loses its reactivity towards either carbon monoxide or oxygen [2]. Although sulfhemoglobin (HbS) as well as Met-Hb could be counted as inert Hb, HbS seemed not to be produced in the blood samples because the calculated values of Met-Hb were closely comparable to those observed (Tables 1 to 3).

It is reasonable to assume that production of Met-Hb in blood is due to heat denaturation of specimens in cases of fires. However, it is not likely that heat denaturation

TABLE 1—*Blood levels of HbCO and Met-Hb in victims of city gas poisoning.*

Case	Age, years	Sex	HbCO,[a] %	Met-Hb, %	
				Calculated	Observed
1	22	f	71.2	0	0.8
2	44	m	68.2	0	0.7
3	42	f	79.6	2.8	0.7
4	50	f	67.4	1.1	1.1
5	25	m	83.1	0	0
6	4	m	79.7	0.9	0.8
7	10	m	76.8	0.7	1.0
8	14	m	71.3	0	0.7
9	15	m	75.7	4.4	1.2
10	26	f	64.3	1.7	0.9
11	29	f	70.9	3.9	1.5
12	34	m	80.2	1.6	0.8
13	35	f	74.7	0.8	1.4
14	41	f	77.7	1.3	1.5
15	49	m	80.5	0.5	0.7

[a]Mean ± standard deviation is 74.8 ± 5.6.

TABLE 2—*Blood concentrations of HbCO and Met-Hb in victims of fire.*

Case	Age, years	Sex	HbCO,[a] %	Met-Hb, %	
				Calculated	Observed
1	78	m	46.8	13.5	12.7
2	41	f	54.4	9.5	5.9
3	25	m	58.5	30.2	31.6
4	3	f	63.0	19.5	20.1
5	1	m	50.5	12.7	12.5
6	3	f	52.7	23.3	20.8
7	14	m	67.2	8.4	8.5
8	41	m	51.7	20.7	19.9
9	45	m	60.7	22.6	20.8
10	58	m	68.5	5.3	5.7
11	67	f	40.1	11.6	11.4
12	87	f	45.2	10.3	9.8

[a]Mean ± standard deviation is 54.9 ± 8.9.

TABLE 3—*Blood concentrations of HbCO and Met-Hb in victims of exhaust fumes poisoning.*

Case	Age, years	Sex	HbCO,[a] %	Met-Hb, %	
				Calculated	Observed
1	39	m	53.1	28.8	26.9
2	37	m	72.6	13.7	12.3
3	19	m	50.3	25.4	22.3
4	42	m	81.6	0.9	1.8
5	46	m	55.7	23.9	20.5
6	44	m	54.2	22.3	20.2
7	32	m	68.7	14.6	13.7
8	30	m	72.3	5.5	4.9
9	23	m	68.4	14.3	14.6
10	34	m	69.5	15.8	15.2

[a]Mean ± standard deviation is 64.6 ± 10.5.

is the cause of methemoglobinemia in cases of exhaust fumes poisoning, since victims in the car (mostly suicides in Japan) are not exposed to high temperature. Schwerd and Schulz [3] observed methemoglobinemia resulting from the inhalation of oxides of nitrogen that were produced by burning plastics. Furthermore, massive conversion of HbO_2 to Met-Hb has been found in a series of anesthetized dogs exposed to oxides of nitrogen [6]. Therefore, oxides of nitrogen in the fumes seem to be the main cause of the methemoglobinemia in the victims of exhaust fumes poisoning. In some fire cases, Met-Hb might be produced by the inhalation of oxides of nitrogen.

In the present study, all the determinations were made within 48 h after death. In addition, only negligible amounts of Met-Hb were detected in cases of city gas poisoning (Table 1). Accordingly, aging of specimens seems not to be a cause of the Met-Hb production.

It should be noted that victims containing high concentrations of Met-Hb in blood (cases of fires and exhaust fumes poisoning) showed significantly lower concentrations of HbCO than those containing negligible amounts of Met-Hb (cases of city gas poisoning).

In some fire cases, intoxication from a combination of carbon monoxide and cyanide was reported along with low HbCO concentrations in the blood of victims and experimental animals [*7-9*]. Other fire victims showing low concentrations or even absence of HbCO in blood were also reported [*3,6,10*]; the postulated causes were the inhalation of oxides of nitrogen, impregnable laryngospasm, or an extremely well-ventilated fire.

The present study indicates that factors other than carbon monoxide should be taken into account as the cause of death in cases of fires or poisoning by exhaust fumes. The inhalation of oxides of nitrogen may be one of the factors, especially in cases of poisoning by exhaust fumes.

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