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Changes of Contents of CO and Water in Blood Exposed to Heat—As to a Possibility of Estimating Pre-exposure CO Content of Thermally Coagulated Blood on the Basis of Its Water Content

K. Yamamoto and C. Kuwahara

Dept. of Legal Medicine, Faculty of Medicine, Kyoto University, Kyoto, Japan

Summary. A blood sample containing CO in a glass vessel was heated in a thermostated water bath at various temperatures for varying lengths of time to compare changes in CO and water contents.

With higher temperatures and longer exposure periods the degrees of thermocoagulation advanced and the contents of CO and water decreased. At the individual temperatures examined, changes of CO and water contents showed significant correlations. However, with different temperatures regressions differed significantly. The above was considered to show that the temperature, to which a blood sample had been exposed, must be known for successful application of the water content method to estimate pre-exposure CO content of heat-exposed blood.

However, this requirement is considered difficult to be met.

Key word: Blood CO determination

Zusammenfassung. In einem offenen Glasgefäß wurde kohlenoxidhaltiges Kaninchenblut während verschiedener Zeiteinheiten und bei verschiedenen Temperaturen erhitzt. Der Verlust an Wasser und der Abfall der Kohlenoxidkonzentration wurden gemessen. Je nach der Größe der thermischen Koagulation wird der Wassergehalt und die Kohlenoxidkonzentration bei steigender Temperatur und längeren Einwirkungszeiten vermindert. Eine Korrelation zwischen den Wasser- und Kohlenoxidgehalten und den Temperaturen konnte hergestellt werden. Um den Verlust an Kohlenoxid bei Hitzeeinwirkung abzuschätzen, wäre es nötig, die Temperatur, der das Blut ausgesetzt war, zu kennen. Diese Bedingung ist jedoch nur schwer zu erfüllen.

Schlüsselwort: CO-Bestimmung

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Introduction

Blood COHb determinations are necessary procedures on bodies recovered from fires for elucidating contribution of CO to death as well as for determining victim's lives at start of fire. With heat-exposed blood, interpretation of obtained values poses some problem, the situation becoming more complicated in cases with thermally coagulated blood. Since CO is released from blood during exposure to heat, the degree of release seeming to depending on heat applied [1], values obtained at autopsy are considered to be different from those which would have been obtained before exposure to heat. It has been pointed out that much emphasis should not be placed on a COHb concentration itself from severely burnt corpse [2]. However, accurate estimation of pre-exposure COHb values, if possible, undoubtedly contributes to clarification of the circumstances surrounding deaths. The pre-exposure COHb value can be estimated by use of an another parameter, the change of which by heat exposure correlates with that of COHb. The accuracy of such an estimation depends on how closely the two are related. There is only one report dealing with the estimation of pre-exposure COHb value of thermally coagulated blood. Berg et al. [3] estimated the pre-exposure COHb concentration on the basis of the water content of a sample.

In the present study, the applicability of water content was examined by comparing the change of CO content with that of water content of samples exposed to heat. If the water content is found to satisfy the above-mentioned criterion, the pre-exposure COHb concentration can be estimated from contents of CO and water of the sample assuming that water content and Hb concentration of the sample would have lain in the normal concentration ranges before exposure to heat.

Materials and Method

Heparinized rabbit blood was used. CO-containing blood was prepared by introduction of pure CO into a blood-containing separating funnel, the inside of which had been made sub-atmospheric by aspiration. A glass flask of about 35 ml capacity, consisting of two (main and side) chambers and two side tubes, was used for CO content determination [4]. For the water content experiment, a vessel without a side chamber was used. Except for the side chamber, these two vessels were alike. After 2 ml of blood sample was placed into each vessel, the vessels were heated in a thermostated water bath, during heating the vessels being kept unclosed. The temperatures examined were 55°, 65°, 75°, 82°, 89°, and 95° C. The duration of heating ranged 30 min to 210 min at 30 min intervals.

CO content of coagulated blood was determined by the method described in the previous paper [1]. It is outlined as follows. After the vessel was taken out of the water bath, 1.5 ml of potassium ferricyanide solution was placed into the side chamber and 2 ml of water, one drop of octyl alcohol, and a magnetic bar were added into the main chamber. After blood stirring with a needle, the vessel was sealed and potassium ferricyanide transferred into the main chamber. A magnetic stirrer was used to facilitate release of CO. Afterward, a He-containing Saran bag was connected to one of the side tubes, 50 ml of sample is drawn manually into a glass syringe connected to the other side tube and it is injected into the gas chromatograph. The procedure is repeated at about 3 min intervals until only a negligible amount of CO appears on chromatogram.

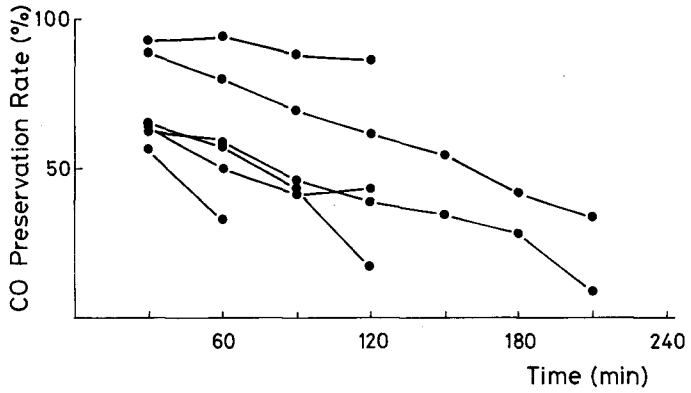

Fig. 1. Time-course of preservation rates (%) of CO contents. Temperatures: from top (at 30 min) 55°, 65°, 89°, 82°, 75°, and 95° C

CO initially present in the blood is calculated by using a standard gas with a known concentration of CO. When a sample had thermally coagulated so extensively as to change into a dark-red sheet-like material, it was cut into pieces by a scissor before being subjected to CO determination procedure.

Water content was determined by Shimadzu electronic moisture balance (ED-200M0). With this instrument water content (%) of a sample is converted automatically from decrease of weight caused by irradiation of infrared rays and is displayed continuously. After the sample had been blotted with a sheet of tissue paper, it was taken out from the vessel, cut into pieces, and heated on the scale of the instrument to constant weight. The temperature immediately above the scale during irradiation was about 60° C. About 50 min was required for determination.

For CO content as well as for water content, preservation rates were calculated as a ratio to each not-heated control sample.

Results

Gross Appearances of the Sample

Heated at 55° C blood remained fluid even after 120 min; on the other hand, at temperatures over 65° C it coagulated. According to the appearances of samples after CO determination had been finished, thermocoagulation was divided into the following three degrees [1]: (1) Blood having formed smooth solution with potassium ferricyanide solution. No coarse pieces of coagulated blood are present. (2) Several coarse pieces are present. (3) Blood having coagulated so extensively as to change into a sheet-like material. Pieces, into which coagulated blood was cut, have maintained their original shape in spite of stirring. Heating at 65° C for 210 min did not induce thermocoagulation of the third degree, while heating at 75° C had caused such a change after 120 min. With higher temperature, the time required to induce thermocoagulation of the third degree became shorter.

CO Content

With higher temperature and longer heating period, the CO content decreased. Figure 1 shows the time-course of preservation rates of CO content. At 30 min, the

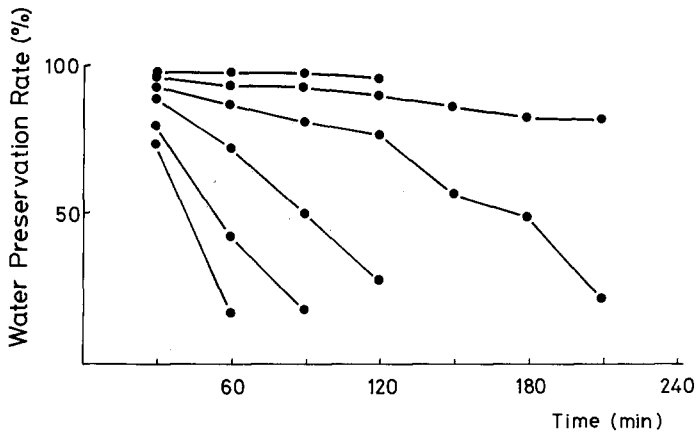

Fig. 2. Time-course of preservation rates (%) of water contents. Temperatures: from top, 55°, 65°, 75°, 82°, 89°, and 95°C

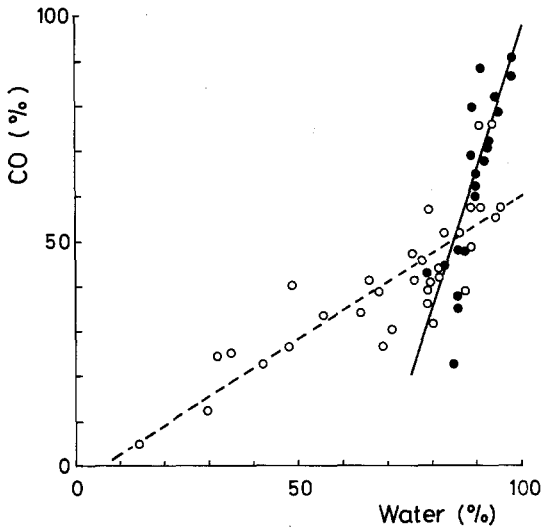

Fig. 3. Scatter diagram of preservation rates (%) of CO and water contents. *Open circle:* 75°C group; *closed circle:* 65°C group. A solid and a dotted line show regression of CO content preservation rate on water content preservation rate for the 65° and 75°C groups, respectively

Table 1. Correlations between preservation rates of CO and water contents

Temperature (°C)	Sample size	Correlation coefficient	Regression
65	21	0.83	$Y=3.15X-218$
75	35	0.83	$Y=0.63X-\ 3.1$
82	26	0.62	$Y=0.29X+\ 32.2$
89	22	0.70	$Y=0.48X+\ 29$

Correlation was significant in all the temperature groups. Regression means that of CO on water

Table 2. Relationship between preservation rates and degrees of thermocoagulation

Degree	Sample size	Temperature groups (°C)	Preservation rates (%)		Correlation
			Water content	CO content	
1st	9	65, 75	78- 96	55–92	NS
2nd	64	65, 75, 82, 89, 95	33–100	22–83	S ($r=0.85$)
3rd	44	75, 82, 89, 95	10- 82	5–72	NS

NS: not significant; S: significant

There were considerable overlaps among the degrees of thermocoagulation for the both preservation rates. The lack of significant correlation in the first group is considered due to small sample size and narrow range of water contents

loss of CO content in lower temperature groups (55° and 65° C) was about 10%, while that in higher temperature groups (over 75° C) was about 40%, there being not significant difference among the 75°, 82°, and 89° C groups. From 30 min onward, the CO content of the 65° and 75° C groups decreased parallel with each other.

Water Content

Figure 2 shows the time-course of preservation rates of water content. The pattern of decrease was not the same as that of the CO content. At 30 min there was clear temperature-dependent decrease. The rate of decrease became faster as temperatures increased.

Figure 3 shows a relationship between the preservation rates of CO and water contents in the 65° and 75° C groups. Table 1 shows the summarized data for respective temperature groups. At individual temperatures, significant relationship was present between preservation rates of CO and water contents, the correlation being stronger in the 65° and 75° C groups. However, the slopes of the regression lines were markedly different between these two groups. CO seemed to be lost easily relative to water at 65° C as compared to 75° C. The regression equations for 82° C and 89° C were similar to that for 75° C. When four temperature groups were combined, the distribution of points became wider, though the correlation was still significant ($r=0.58$).

As to the relation with the degrees of thermocoagulation (Table 2), the correlation was significant in the second-degree thermocoagulation alone.

Discussion

For water content to become a reliable index for estimation of pre-exposure CO content of thermally coagulated blood, it is necessary that there is a close correlation between the changes of CO and water contents by heat. At the individual temperatures examined, there was significant correlation. However,

even with the 75° C group which showed the closest correlation, the correlation was far from being perfect. To make matters worse, the manner of correlation varied as temperatures were different. The slope of the regression line of 65° C group was significantly different from those of the higher temperature groups. Therefore, to apply the estimation method based on water content successfully, it is absolutely necessary that the temperature to which blood has been exposed is known. However, it is extremely difficult to estimate the temperature from gross appearance of the sample alone. When temperature is unknown, loss of CO content from thermally coagulated blood must be estimated on the basis of its gross appearance, and even in the case of thermocoagulation of the second degree, this estimation will give values with too wide a range. Berg et al. [3] estimated a pre-exposure COHb concentration of a thermally coagulated blood sample on the basis of a relation between the preservation rates of COHb concentration and water content. Unfortunately, it is not known whether the temperature to which the reference sample was exposed was equal to that to which the test sample had been exposed.

It can be said conclusively that water content has only a limited applicability to the estimation of pre-exposure CO content of thermally coagulated blood.

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