Z Rechtsmed 85, 275–281 (1980)

Zeitschrift für
Rechtsmedizin

Effect of Heating on CO Content in the Blood

An in Vitro Study

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Summary. Rabbit blood (2 ml) containing varying concentrations of COHb in an unsealed glass vessel (35 ml) was heated in a water bath and a change in CO content was determined gas chromatographically. Four temperatures (55°C, 75°C, 90°C, 100°C) and three exposure times (15 min, 30 min, 45 min) were used. To the 55°C-group alone, experiments with longer exposure times (65 min, 85 min) were added extra. When blood was heated at 55°C, it remained fluid even after the longest exposure time, while heating at temperatures above 75°C coagulated blood at all times during exposure. Coagulated blood was stirred with a needle or cut into pieces with a scissor before being mixed with potassium ferricyanide solution. Thermal coagulation was divided into three degrees by the appearance after CO releasing procedures had been finished. Blood that had formed smooth solution with degassing agent was classified into the first degree. On the other hand, in higher degrees, coarse pieces of coagulated blood were observed in the solution. In the third degree, blood that had been cut into pieces did not change its initial shape in spite of vigorous stirring. With fluid blood, the loss of CO was at most 20%, whereas blood of the third degree had lost 65–95% of CO initially present.

The applicability of a spectrophotometric method devised by van Kampen et al. to the heated sample was discussed.

Key words: COHb determination, gas chromatography, spectrophotometry – Thermal coagulation, COHb determination

Zusammenfassung. Kohlenoxidhaltiges Kaninchenblut in einem offenen Glasgefäß wurde in einem Wasserbad erhitzt und der Abfall des Kohlenoxidgehaltes wurde mit Hilfe einer von uns entwickelten gaschromatographischen Methode gemessen. Folgende Versuchsbedingungen wurden angewandt; Temperaturen: 55°C, 75°C, 90°C, 100°C; Zeitdauer: 15, 30, 45, 65, 85 min. Die letzten zwei Zeiten wurden nur bei der 55°C-Gruppe angewandt. Blutproben blieben flüssig, wenn sie bei 55°C erhitzt wurden, und das Ausmaß des

0044-3433/80/0085/0275/$ 1.40

Kohlenoxidverlustes betrug höchstens 20%. Bei Erhitzungstemperaturen über 75° C waren Blutproben während der Versuche hitzekoaguliert. Koagulierte Massen wurden mit einer Nadel oder einer Schere zerkleinert, bevor sie mit Kaliumferrizyanid-Lösung gemischt wurden, die zur Dissoziation des Gases von Hämoglobin verwendet wird. Hitzekoagulierte Proben wurden in drei Klassen unterteilt. Blut des ersten Grades vermischte sich homogen mit Kaliumferrizyanid-Lösung. In der zweiten und dritten Klasse blieben grobe hitzekoagulierte Blutmassen trotz kräftigen Umrührens noch in der Lösung. Blut, das mit einer Schere zerkleinert wurde, wurde im dritten Grade klassifiziert. Blut dieses Grades gab während Erhitzung 65–95% des ursprünglichen Kohlenoxidgehaltes ab.

Die Verwendbarkeit einer von van Kampen u. a. entwickelten spektrophotometrischen Methode zu erhitzten Blutproben wurde diskutiert.

Schlüsselwörter: COHb-Bestimmung – Gaschromatographie, Spektrophotometrie – thermische Koagulation, COHb-Bestimmung

To solve the question as to whether a badly charred victim was still alive or already dead at the start of fire, it is necessary to examine blood for CO. However, cases in which blood has been thermocoagulated present difficult problems. In such cases, interpretation of the obtained COHb values as well as CO determination procedure itself becomes much complicated in comparison with cases, in which effect of heat can be excluded. So far there has been only a limited number of studies [1, 2, 3, 4a, 4b] on this subject with the exception of Miyauchi et al.'s systematic paper published recently [5]. Miyauchi et al. have carried out gas chromatographically extensive experiments on the release of CO from heated blood.

In the present study we have investigated the change of CO content in the blood subjected to heat by using a gas chromatographic method newly devised in our laboratory [6]. A spectrophotometric method was also used in the present study to test the applicability of this method to the heated sample in comparison to the gas chromatographic method.

Material and Methods

Blood was taken from the carotid artery of heparinized rabbits. CO-saturated blood was prepared by introduction of pure CO into a blood-containing separating funnel, the inside of which had been made subatmospheric by aspiration. Blood samples containing varying concentrations of COHb were prepared by mixing CO-saturated blood with various amounts of CO-free blood. As a vessel, in which a sample is heated, a glass reaction vessel of about 35 ml capacity was used throughout the present study. The reaction vessel, which has been originally devised for gas chromatographic determination of CO in the blood [6], consists of two (main and side) chambers and two side tubes. A blood sample (2 ml) was placed into the main chamber and heated in a thermostated water bath. Four temperatures (55° C, 75° C, 90° C, 100° C) and three exposure times (15 min, 30 min, 45 min) were used. Experiments with longer exposure times (65 min, 85 min) were added extra to the group heated at 55° C (hereafter, termed as 55° C-group). During heating, the vessel was kept unclosed.

The gas chromatographic procedure for CO content determination, the details of which have been described elsewhere [6], is outlined briefly as follows. Blood and a magnetic bar are

placed into the main chamber and potassium ferricyanide solution is placed into the side chamber. A helium-containing saran bag is connected to one of the side tubes. After sealing the vessel, the degassing solution is transferred into the main chamber by tilting the vessel, and the mixture is stirred by a magnetic stirrer. CO released is drawn manually with helium into a gas sampler connected to the other side tube. This procedure is repeated at about 3 min intervals until only a negligible amount of CO appears on the chromatogram. A sample is injected into a gas chromatograph via a heated gas sampler, and the CO content is calculated by using a reference gas containing a known concentration of CO. In the present study, a gas sampler of 100 ml was used.

The CO content of a sample, which remained fluid during exposure to heat, was determined directly according to the above-mentioned procedure. When a sample coagulated during exposure, it was stirred by a needle to facilitate subsequent mixing of the blood with potassium ferricyanide solution. With a sample being thermocoagulated into a hard membranous sheet, it was cut into pieces by a scissor.

For spectrophotometry, van Kampen et al.'s method [7] was chosen. This method using a sample diluted with 0.1% ammonia water to 1:200 is one of the typical two-wavelength methods, which makes use of the isosbestic point (I.P.) of carboxy Hb (COHb), and deoxy Hb at about 580 nm. The position of the I.P. and absorbancy ratio were determined on both heated (at 55°C for 85 min) and unheated blood. In the present experiment, blood samples were saturated with CO prior to addition of sodium dithionite. After blood samples with varying concentrations of COHb were divided into two parts, one part was allowed to stand at room temperature (18°C) while the other was heated at 55°C for 85 min in the water bath. The spectrophotometric determination of COHb was carried out on the both groups by using a calibration line made from unheated blood (as to the reason for this, see Results and Table 2).

Results

When blood was heated at 55°C, it remained fluid after the longest exposure time although it became dark and thick. On the other hand, when blood was heated at temperatures above 75°C, it always coagulated during exposure. After CO releasing procedure had been finished, thermal coagulation was divided into three classes by the appearance of the sample as follows. First degree: Blood has formed smooth solution with degassing agent and no coarse pieces of coagulated blood were observed in the solution. Second degree: In contrast to the first degree, several coarse pieces were observed. Third degree: Pieces, into which coagulated blood has been cut, have maintained their original shape in spite of vigorous stirring, although the color of potassium ferricyanide solution has changed from yellow to brown. In the third degree, more time was required for releasing of CO from samples than in the other degrees.

The CO preservation rate of each group was calculated as a ratio to the CO content of the unheated sample and is given in Table 1, together with distribution of samples into each class. The CO preservation rate decreased with exposure time in all experimental groups, however, except the 100°C-group, the decline of CO content was not linear. There was not clear correlation between decreasing rate and initial CO content.

On an assumption that the magnitude of the effect of heat can be expressed in terms of a product of heating temperature with exposure time, a relation of the product with CO preservation rate was examined. As Fig. 1 shows, CO content decreased as product became larger, however, the decreasing rates varied among experimental groups. The 55°C-group showed a higher CO preservation rate

Table 1. The effect of heating on CO content. Blood in an unclosed glass vessel was heated in a water bath and changes in CO content were determined gas chromatographically. The CO preservation rate was calculated as a ratio to the CO content of the unheated blood. Thermal coagulation was divided into three classes by the appearances after CO releasing procedure had been finished (for details refer to text)

Temperature (°C)	Exposure time (min)	CO preservation rate (%, mean ± s.d. number of samples)	Distribution of samples			
			fluid	thermo-coagulated		
				grade 1	grade 2	grade 3
55	15	98 ± 6 8	8	0	0	0
	30	91 ± 5 8	8	0	0	0
	45	90 ± 7 8	8	0	0	0
	65	89 ± 2 3	3	0	0	0
	85	80 ± 3 3	3	0	0	0
75	15	61 ± 6 8	0	8	0	0
	30	52 ± 8 8	0	8	0	0
	45	47 ± 11 8	0	6	2	0
90	15	62 ± 6 8	0	5	3	0
	30	58 ± 5 8	0	0	8	0
	45	46 ± 8 8	0	0	4	4
100	15	67 ± 4 8	0	5	3	0
	30	35 ± 4 8	0	0	0	8
	45	15 ± 6 7	0	0	0	7

Table 2. The effect of heating on the isosbestic points (I.P.) and absorbancy ratios. Blood was heated at 55°C for 85 min in a water bath. No shift was observed in the position of the I.P.

Control blood				Heated blood		
	I.P. (nm)	Absorbancy ratio		I.P. (nm)	Absorbancy ratio	
		CO-free	CO-saturated		CO-free	CO-saturated
Rabbit No. 1	580.2	1.058	1.537	580.4	1.053	1.474
	580.4	1.045	1.544	580.4	1.063	1.471
	580.6	1.049	1.558	580.6	1.058	1.490
Rabbit No. 2	579.6	1.068	1.571	579.6	1.062	1.449
	579.8	1.053	1.566	579.8	1.065	1.463
	579.8	1.042	1.555	580.0	1.068	1.466
Rabbit No. 3	580.0	1.066	1.562	580.0	1.102	1.444
	579.8	1.054	1.539	580.0	1.096	1.428
	580.0	1.057	1.533	579.8	1.088	1.420

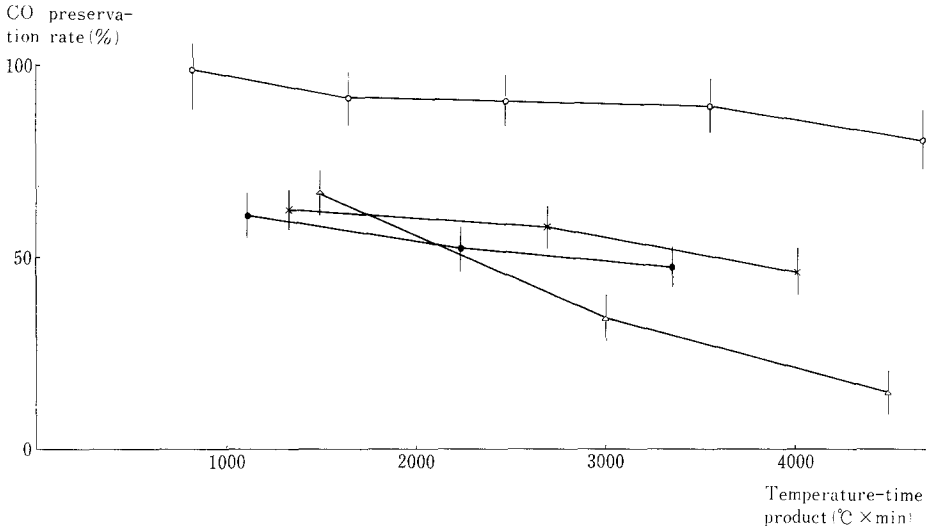

Fig. 1. Relations between the CO preservation rates and effects of heating. The effect of heating is expressed in terms of a product of temperature and exposure time (°C × min). Open circles, closed circles, crosses, and triangles indicate 55°C-, 75°C-, 90°C-, and 100°C-groups, respectively. Bars represent standard deviations

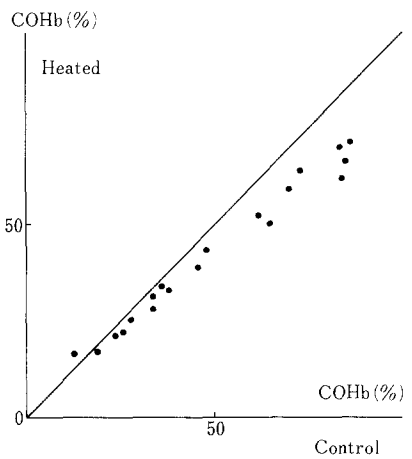

Fig. 2. Spectrophotometric determinations of COHb on the heated (at 55°C for 85 min) blood. The spectrophotometric method devised by van Kampen et al. was used. After blood samples with varying concentrations of COHb were divided into two parts, one part was left at room temperature, while the other was heated. The determinations of COHb on the both groups were carried out by using a calibration line made from the unheated blood (for details refer to text). COHb concentrations were lower in the heated blood in 18 of 19 samples. The line shows identity line drawn at 45°C

(80 ± 3% after 85 min heating) than any other groups. On the other hand, the 100°C-group, except from its 15-min exposure sample, showed the lowest CO preservation rate (15 ± 6% after 45 min heating). As to the effect of dilution on CO preservation rate, diluted blood seemed to be more susceptible to the effect of heating. For example, when blood was heated at 55°C for 85 min, the mean peak height of six undiluted blood samples was about 2.5 times larger than that of six samples diluted 2-fold with water (96.7 ± 2.0 vs. 38.8 ± 1.9, expressed in terms of division).

As Table 2 shows, the position of the I.P. of the heated blood was nearly identical with that of the unheated blood. As to the absorbancy ratio of CO-saturated blood, it was lower in the heated blood than in the unheated blood. As the results of the spectrophotometric determinations show in Fig. 2, COHb concentration decreased in 18 of 19 samples during exposure. The decreasing rate, ranging from 6 to 26%, was consistent with the results from gas chromatography (Table 1).

Discussion

Miyauchi et al. [5] heated 0.5 ml of blood sample in a glass reaction vessel of about 5 ml capacity and determined CO released gas chromatographically. During heating blood was being stirred with a magnetic bar. They have reported that in the presence of air 65% of CO initially present in the sample was released for 15 min heating at 85° C. This amount of CO released corresponds to a CO preservation rate of about 35% and this is much lower in comparison with our data.

As to the relation between CO preservation rate and degree of thermal coagulation, the preservation rate decreased as a degree became higher, although there was considerable overlap particularly between the first and the second degrees. Needless to say that great care is needed in applying the present results to the practical cases. However, as long as blood from a charred victim remains fluid and retains still its original red color, it can be safely said that loss of CO will have been at most 20–30% of CO initially present. Even in those cases in which blood has been charred badly and a considerable amount of CO is expected to have been lost, detection of CO should not be given up since the present study shows that not all of the CO initially present has always been lost even from such blood.

Spectrophotometrically, detection of CO may also be possible even on badly charred blood. After a hard membranous sheet of blood, obtained by heating blood at 100° C for 45 min, was ground in a mortar and dissolved in distilled water, this solution was examined for peak of COHb at the visible region with a Shimadzu multipurpose recording spectrophotometer (MPS 5000). With CO-saturated blood, shoulders were observed at about 540 nm and 570 nm, while no shoulder was observed with CO-free blood treated similarly. The diluted solution from CO-saturated blood was more rosy than that from CO-free blood. COHb concentration determination of heated blood, as long as it remains fluid, was considered to be possible by this spectrophotometric method also. Because the results that the heating did not change the position of the I.P. was considered to justify for COHb concentration of heated sample to be determined by using a calibration line made from the unheated blood. The lower absorbancy ratios of CO-saturated samples from heated blood are probably due to formation of methemoglobin (met-Hb) [8]. In fire victims also, significant amounts of met-Hb have been found [9].

The spectrophotometric method devised by van Kampen et al. [6] is indeed speedy, simple, and accurate, but its accuracy depends greatly on the extent to which the position of the I.P. of a sample to be measured coincides with that of a

reference sample used for calibration line. Since variations of the I.P. are considered to be great among samples encountered in medico-legal practice, this method seems to be more suitable for laboratory use rather than for medico-legal practice.

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Received February 11, 1980