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Selection of Specimen in the Determination of Carboxyhemoglobin Saturation by Spectrophotometry*)

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ABSTRACT

Carboxyhemoglobin (HbCO) saturation in a liquid specimen collected at medicolegal autopsy was analyzed firstly by a spectrophotometric method, which was first reported by Fretwurst et al, modified by Fukui and then modified by the authors, and secondly by a carbon monoxide-total hemoglobin (CO-Total Hb) method developed by the authors. In 70 blood specimens collected from the heart or blood vessels of 59 cadavers, the values obtained by the spectrophotometric method were similar to those obtained by the CO-Total Hb method with the exception of three blood specimens, two of which were markedly putrefied and the other considerably denatured by heat, in which the values obtained by the spectrophotometric method were significantly higher than those by the CO-Total Hb method. In 62 specimens of reddish discolored body cavity fluids collected from 31 cadavers, nearly all values obtained by the spectrophotometric method were much higher than those obtained by the CO-Total Hb method. The results indicate that the spectrophotometric method should not be used for the quantitative determination of HbCO in body cavity fluids, blood mixed with body cavity fluid, or blood markedly denatured by putrefaction or heat.

INTRODUCTION

Carboxyhemoglobin (HbCO) saturation in a liquid specimen collected from a significantly putrefied or markedly burnt cadaver is not infrequently analyzed in medicolegal practice. Spectrophotometry has been widely used for the determination of HbCO in fresh blood and blood denatured by putrefaction or heat, because of its simplicity, rapidity and reliability. However, it has not been confirmed that spectrophotometry yields correct values in the determination of HbCO saturation in the case of significantly putrefied blood, blood markedly denatured by heat, or body cavity fluids. So, the HbCO saturation in a liquid specimen collected at medicolegal autopsy was analyzed by a spectrophotometric method [developed by Fretwurst et al.[1], modified by Fukui[2] and then modified by authors[7]] and compared with that analyzed by a carbon monoxide–total hemoglobin (CO-Total Hb) method developed by authors[4,5]. The results indicate that spectrophotometry should not be used for the quantitative determination of HbCO in body cavity fluids, blood mixed with body cavity fluid, markedly putrefied blood, or blood considerably denatured by heat.

MATERIALS

The liquid specimens used were blood collected at medicolegal autopsies from the heart or blood vessels, and body cavity fluids from thoracic, pericardial and abdominal cavities of cadavers deceased by fire, drowning or other causes.

Seventy blood specimens were collected from 59 cases. They were classified according to the time elapsed between death and collection of the specimen, and degree of burn on the body

*) 小嶋 亨, 屋敷幹雄, 宇根伊津子：分光光度法による一酸化炭素ヘモグロビン飽和度測定のための試料の選択

Table 1. Classification of blood specimens according to time elapsed after death, and degree of burn on the body

Time after death (days)	Degree of burn	Number of cases	Heart blood*	Vascular blood
≦2	Without burn	18	18	0
	With burn	13	14	0
	With heavy burn**	12	13	8
	Total	43	45	8
2< <7	Without burn	7	7	1
	With burn	0	0	0
	With heavy burn	0	0	0
	Total	7	7	1
≧7	Without burn	9	9	0
	With burn	0	0	0
	With heavy burn	0	0	0
	Total	9	9	0

* Left and right heart blood were collected separately or together.
** Chest wall partially burnt down.

Table 2. Classification of body cavity fluid specimens according to time elapsed after death, and existence of burn on the body

Time after death (days)	Existence of burn	Number of cases	TCF	PCF	ACF
≦2	Without burn	6	3*	1	4
	With burn	4	3	3	0
	Total	10	6	4	4
2< <7	Without burn	8	9	8	2
	With burn	0	0	0	0
	Total	8	9	8	2
≧7	Without burn	13	20	6	5
	With burn	0	0	0	0
	Total	13	20	6	5

* One specimen included blood from thoracic cavity. TCF: Thoracic cavity fluid. PCF: Pericardial cavity fluid. ACF: Abdominal cavity fluid.

(Table 1).

Sixty-four specimens of body cavity fluids were collected from 31 cadavers. They were classified according to the time elapsed between death and collection of the specimen, and the existence of burn on the body (Table 2).

METHODS

1. The spectrophotometric method[7]

Each specimen was diluted with 0.1% sodium carbonate solution containing 0.3% sodium hydrosulfite. After the Hb was denatured by sodium hydroxide, maximum absorbancies at around 530 nm (A) and at around 560 nm (B) were determined on an absorption spectrum recorded from 500 to 600 nm. The HbCO saturation (%) was obtained by plotting the ratio of B/A against the calibration curve, which had been obtained by analyzing standard HbCO blood.

2. The CO-Total Hb method[4,5]

CO released from the specimen was deter-

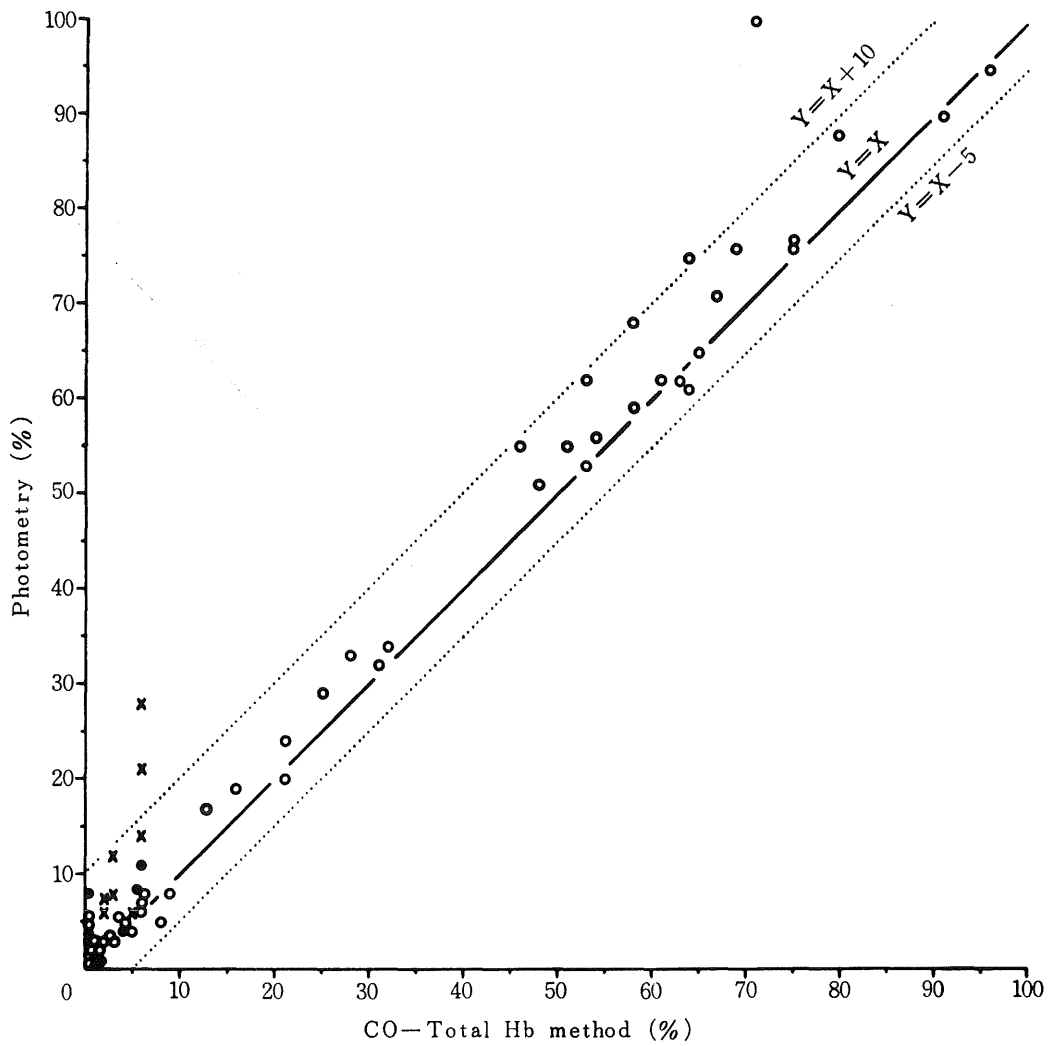

Fig. 1. Comparison of HbCO saturations in blood obtained by the spectrophotometric method with those obtained by the CO-Total Hb method. ○ : Specimen collected 2 days or less after death. ● : Specimen collected between not less than 2 days after death and not more than 7 days after death. × : Specimen collected 7 days or more after death.

mined by gas chromatography using methane as an internal standard, and the total concentration of Hb was measured as cyanmethemoglobin. The HbCO saturation (%) was calculated by the ratio of CO content/CO-binding capacity.

RESULTS

A comparison of the HbCO saturations in blood obtained by the spectrophotometric method, with those obtained by the CO-Total Hb method, is shown in Fig. 1. The differences in the values obtained by these two methods were within 10%, with the exception of three specimens, namely two heart blood specimens collected from drowned cadavers 9 and 31 days respectively after death, and one heart blood collected from a burnt cadaver, the chest wall having been partially burnt.

A comparison of the HbCO saturations in body cavity fluids obtained by the spectrophotometric method, with those obtained by the CO-Total Hb method, is shown in Fig. 2. Most values obtained by the spectrophotometric method were much higher than those obtained by the CO-Total Hb method.

DISCUSSION

According to Maehly[6], hemoglobin will be

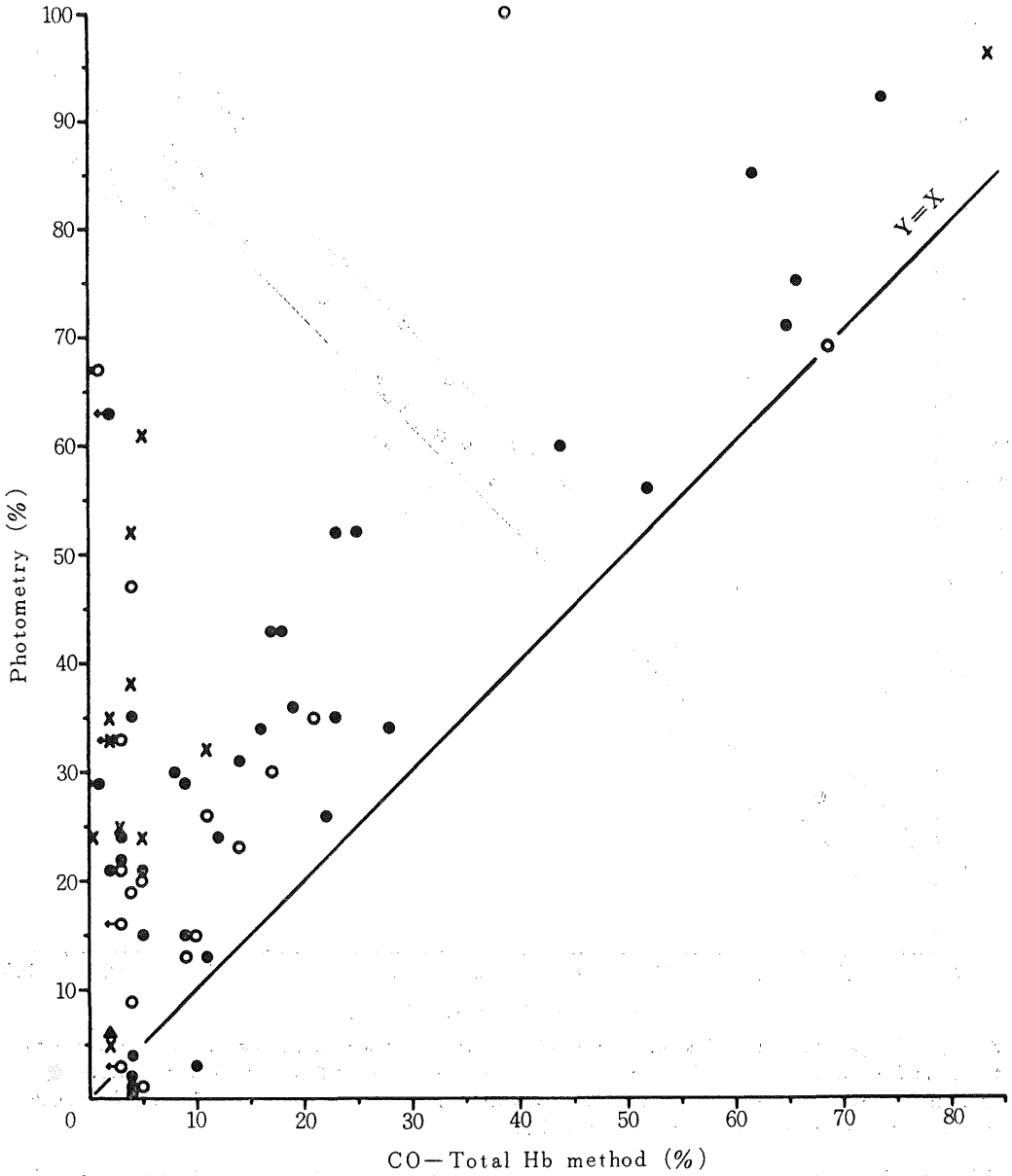

Fig. 2. Comparison of HbCO saturations in body cavity fluids obtained by the spectrophotometric method with those obtained by the CO-Total Hb method. ● : Thoracic cavity fluid. ○ : Pericardial cavity fluid. × : Abdominal cavity fluid. ▲ : Blood in thoracic cavity. ← : HbCO saturation (%) obtained by the CO-Total Hb method was not more than the value shown in the figure.

almost exclusively in the form of HbO_2 and HbCO in a fresh blood sample from a living person. However, other Hb compounds may be present in blood obtained at autopsies due to bacterial decomposition or heat. Schwerd et al.[8] reported that when CO-free blood was allowed to stand at 45°C, analysis by spectrophotometry revealed a 40% rise after 2 days and a 75% rise after 3 days in what appeared to be HbCO saturation, but that this rise was in fact due to a disturbance in the spectrophotometry caused by denatured Hb.

The spectrophotometric method used was one of the methods recommended for CO analysis of aged blood[6]. However, large differences between HbCO saturations obtained by the spectrophotometric method, and those obtained by the CO-Total Hb method, were observed in

body cavity fluids, significantly putrefied blood, and blood markedly denatured by heat. Therefore, the results indicate that the spectrophotometric method should not be used for the quantitative determination of HbCO in body cavity fluids, blood mixed with body cavity fluids, and blood significantly denatured by putrefaction or heat.

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