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GAS CHROMATOGRAPHIC DETERMINATION FOR FORENSIC PURPOSES OF PETROLEUM FUEL INHALED JUST BEFORE FATAL BURNING

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SUMMARY

The determination of petroleum fuel in the blood of burned bodies was carried out by three different gas chromatographic procedures. Seven components of gasoline (isopentane, *n*-pentane, 2-methylpentane, benzene, 2-methylhexane, 3-methylhexane and toluene) and five of kerosene (xylene, C_9H_{20}, mesitylene, pseudocumene and $C_{11}H_{24}$) were chosen as indicators with a coefficient of variation of 5–24%. The methods were applied to four autopsy cases with a relatively low carboxyhaemoglobin (HbCO) content. When gasoline exposure had occurred, the blood concentrations determined were almost identical whatever the components selected. Great variations in the components determined were found after kerosene exposure, and hydrocarbons $\geqslant C_{14}$ were hardly inhaled by the victims. A higher content of fuel in the left than in the right ventricular blood observed in the autopsy cases suggests fuel inhalation just before death. The same phenomenon was also observed in the content of blood HbCO. Determinations of petroleum fuel and HbCO in both the right and left ventricular blood would be useful for the forensic diagnosis on burned bodies with a low HbCO content.

INTRODUCTION

The forensic diagnosis of burned bodies, whether or not the death occurred as a result of the fire, has been routinely assessed from "vital signs" such as erythaema of the skin, dilatation of subcutaneous vessels filled with blood, entry of

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soot into the airways and formation of carboxyhaemoglobin (HbCO) at levels above 20–30% in the blood. These vital signs, however, are often negative in cases where the victims are burned to death with petroleum fuel in a short time, misleading the diagnosis of the cause of death. The detection of fuel components in the blood of a victim should be useful for the diagnosis of death, but only a few reports [1,2] have offered analytical data on petroleum fuel in the blood of burned bodies. When making toxicological examinations for blood petrol, only an identification analysis is carried out in most laboratories. Trace fuel components, if identified in the blood of a burned victim, might be due to contamination with body surface petroleum fuel during autopsy.

We have therefore sought an easy and rapid quantitative method for the determination of petroleum fuel in the blood using gas chromatography (GC), and applied the method to four burned victims with a relatively low blood HbCO content. The diagnostic value of fuel concentrations in the blood from both sides of the heart ventricles is discussed.

EXPERIMENTAL

Materials

Gasoline and kerosene of the same brands that were used in the autopsy cases were obtained as standard materials. *n*-Butylbenzene was purchased from Aldrich (Milwaukee, WI, U.S.A.) and other chemicals were of analytical-reagent grade from Wako (Osaka, Japan). Stored blood for transfusion was obtained from Shimane University Hospital and fresh blood from healthy volunteers.

Analytical methods

The determination of petroleum fuel was carried out with three different procedures, as follows.

Procedure 1. A 2-ml volume of blood and 0.2 ml of 0.01% methyl acetate (internal standard) were placed in a 17-ml vial sealed with a rubber septum and an aluminium cap. The vial was placed in a water-bath at 60 °C and allowed to equilibrate for 20 min prior to analysis. Then, 1 ml of the headspace gas was withdrawn and injected into the GC column.

Procedure 2. An aliquot of 1 ml of blood and 0.2 ml of 0.01% isobutyl alcohol (internal standard) in a rubber-stoppered vial were warmed at 60 °C for 20 min, then 1 ml of the gas phase was analysed by GC.

Procedure 3. A 5-ml volume of blood and 0.2 ml of 0.001% *n*-butylbenzene (internal standard) were pipetted into a 15-ml glass tube fitted with a glass stopper. Fuel components were extracted with 7 ml of *n*-pentane, the organic layer was condensed to ca. 200 μl under a gentle stream of nitrogen in a water-bath at 10 °C and aliquots of 3–5 μl of the sample were injected into the GC column.

The peaks on the gas chromatogram were identified by gas chromatography–mass spectrometry (GC–MS) with electron-impact ionization.

TABLE I

GAS CHROMATOGRAPHIC CONDITIONS TESTED FOR THE DETERMINATION OF FUEL COMPONENTS

Procedure	Column	Temperature		Carrier gas (nitrogen) flow-rate (ml/min)
		Injection port	Column	
1	25% PEG 1000 on Shimalite (80–100 mesh), 3 m×2.6 mm I.D.	70°C	Initial 40°C for 4 min, to 60°C at 2°C/min	40
2	25%PEG 6000 on Shimalite (60–80 mesh), 1 m×2.6 mm I.D.	120°C	75°C	40
3	1.5% GE SE-30 on Chromsorb W (60–80 mesh), 2 m×2.6 mm I.D.	140°C	Initial 60°C for 2 min, to 120°C at 2°C/min	40

Instrumental conditions

GC. A Shimadzu GC-7AG instrument equipped with a flame ionization detector was used. The chromatographic conditions are given in Table I. Column packings were purchased from Shimadzu (Kyoto, Japan).

GC–MS. A Hewlett-Packard Model 5710A gas chromatograph interfaced to a computer-controlled JEOL Model D-300 mass spectrometer was used. GC separation was achieved under the same conditions as those for the above GC analysis. The temperature of both the separator and ion source was 150°C. The ionization current was set at 300 μA and the electron energy at 24 eV. The carrier gas was helium.

Autopsy cases

Blood was collected from four burned victims with a relatively low HbCO content and analysed using the above methods. Blood from right and left ventricles was stored at 4°C in 30-ml bottles until analysis. HbCO concentrations (Table II) were determined by the method previously reported [3,4]. Blood ethanol was also measured by GC with a negative result in all instances. Using the routine screening test, no toxicological drug was detected in any subject.

TABLE II

BLOOD CARBOXYHAEMOGLOBIN CONTENT IN AUTOPSY CASES

Mean values of duplicate analyses.

Ventricle	HbCO content (%)			
	Case 1	Case 2	Case 3	Case 4
Left	4.6	11.5	4.2	35.9
Right	3.8	9.6	3.8	36.7

Case 1. A 59-year-old woman was found dead in her house, which had been destroyed by fire. Two empty containers of gasoline were beside the body. No soot was found in the airways and carbonization had occurred over the whole body.

Case 2. A 61-year-old man had committed double suicide with his wife after setting fire to kerosene poured over themselves. No soot was found in the airways. The whole body was carbonized.

Case 3. A 87-year-old man was found dead in the front yeard of his burned house, seemingly after failure to escape from the fire. A very small amount of soot existed in the airways. Carbonization and combustio escharotica were evident over most part of the body.

Case 4. A 63-year-old woman was found dead in her house, which had been destroyed by fire. Firemen smelled burning kerosene at the scene. Soot was found in the airways and combustio had occurred over most of the body.

RESULTS

Detection of fuel components

Gas chromatograms obtained from the fuel analysis using procedures 1, 2 and 3 are shown in Figs. 1, 2 and 3, respectively. Procedure 1 detected paraffinic hydrocarbons (C_{5-8}) of gasoline and procedure 2 benzene and toluene. Components of kerosene could be detected with procedure 3. In the analysis of a blank sample using procedure 3, some aromatic hydrocarbons, such as *o*-, *m*- and *p*-xylenes, trimethylbenzenes and *sec.*- and isobutylbenzenes originating from the extraction solvent were observed on the gas chromatogram (Fig. 3) and the mass chromatogram. The GC patterns of gasoline and kerosene were very different from each other. As the gas chromatogram of fuel components was complicated, GC–MS was useful for identifying each fuel component (Fig. 4).

Heating for 20 min at 60°C was sufficient in the headspace analysis of gasoline components in procedures 1 and 2.

Accuracy and precision studies

The distribution of fuel components between the gas and liquid phases in headspace analysis was so complicated that a standard fuel solution in blood was required for the calibration graph in procedure 1, but not in procedure 2. The recoveries of kerosene from blood and water were very different, and a standard solution of kerosene in blood was also necessary in procedure 3. For the preparation of the standard kerosene solution, fresh untreated blood and not stored blood for transfusion was used in order to avoid peak interferences due to preservatives.

Stock solutions (10 mg/ml) were prepared by adding gasoline or kerosene to stored blood for transfusion (procedure 1), to water–acetone (50:50) (procedure 2) and to fresh blood (procedure 3). The solution was diluted with stored blood, water or fresh blood to give a standard solution for calibration. Quantification was achieved by plotting peak-area ratios of the selected fuel component to the internal standard against fuel concentrations. Components tested for the determination of blood petroleum fuels were isopentane, *n*-pentane, 2-methyl-

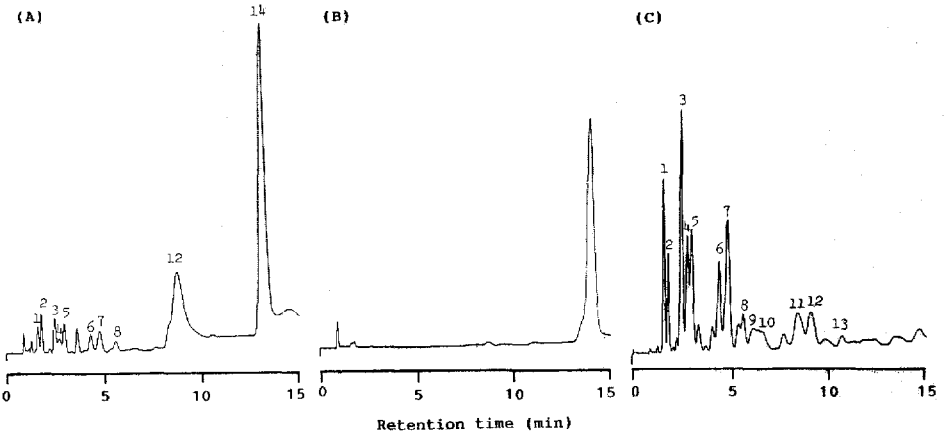

Fig. 1. Gas chromatograms obtained by procedure 1. (A) Case 1 (left ventricular blood); (B) case 4 (left ventricular blood); (C) standard gasoline in blood (200 μg/ml). Peaks: 1 = isopentane; 2 = *n*-pentane; 3 = 2-methylpentane; 4 = 3-methylpentane; 5 = *n*-hexane; 6 = 2-methylhexane; 7 = 3-methylhexane; 8 = *n*-heptane; 9–13 = C_8H_{18} isomers; 14 = methyl acetate (internal standard).

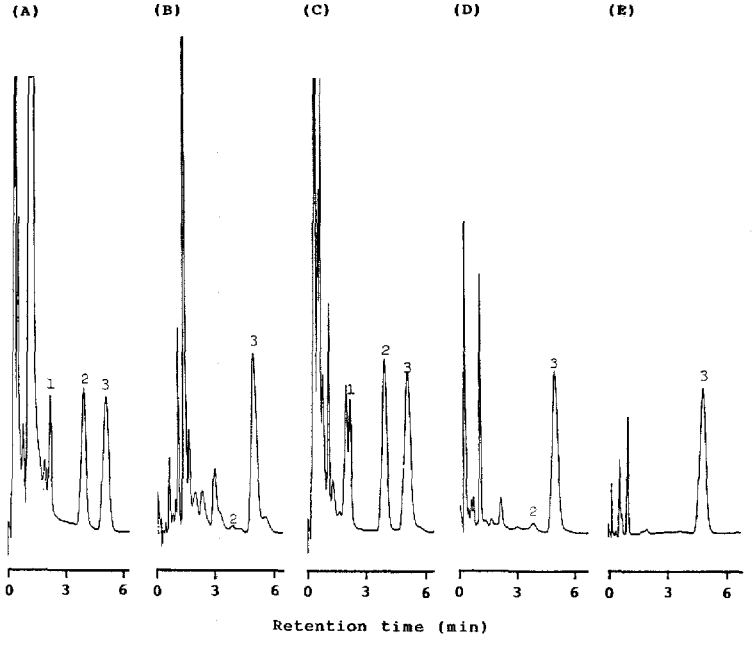

Fig. 2. Gas chromatograms obtained by procedure 2. (A) Standard gasoline in blood (25 μg/ml); (B) standard kerosene in blood (25 μg/ml); (C) case 1 (right ventricle); (D) case 2 (right ventricle); (E) case 3 (right ventricle). Peaks: 1 = benzene; 2 = toluene; 3 = isobutyl alcohol (internal standard).

pentane, benzene, 2-methylhexane, 3-methylhexane and toluene for gasoline and *m*- and *p*-xylenes (unseparated), C_9H_{20}, mesitylene, pseudocumene and $C_{11}H_{24}$ for kerosene. The calibration graphs showed good linearity for every component selected over the ranges 1.0–40 μg/ml for gasoline and 0.2–10 μg/ml for kerosene (correlation coefficient: $r = 0.97$–0.99). In Tables III and IV, the usefulness of

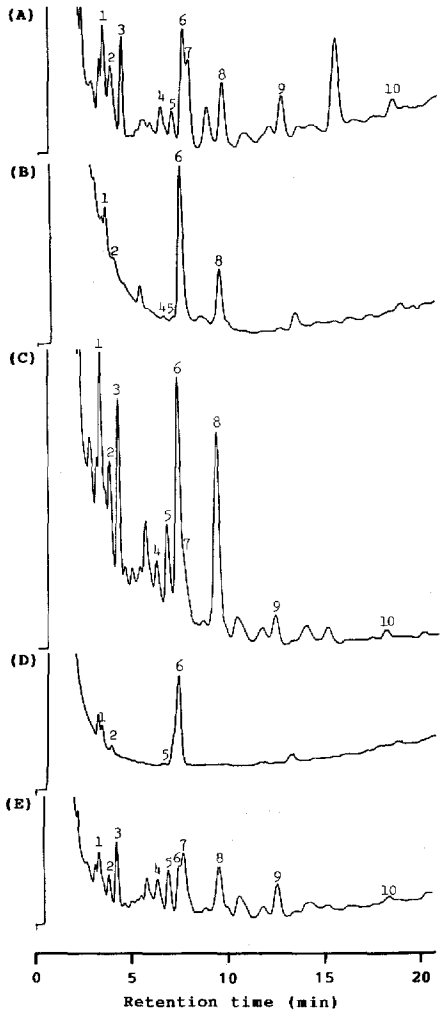

Fig. 3. Gas chromatograms obtained by procedure 3. (A) Case 2 (left ventricle); (B) case 3 (left ventricle); (C) case 4 (left ventricle); (D) blank blood; (E) standard kerosene in blood (4 μg/ml). Peaks: 1 = *m*- and *p*-xylenes; 2 = *o*-xylene; 3 = C_9H_{20}; 4 = mesitylene; 5 = pseudocumene; 6 = *sec.*- and isobutylbenzenes; 7 = $C_{10}H_{22}$; 8 = *n*-butylbenzene (internal standard); 9 = $C_{11}H_{24}$; 10 = $C_{12}H_{26}$.

this technique is demonstrated with respect to its accuracy and precision (between-run). The coefficients of variation of this assay technique ranged from 5 to 24%, which would be acceptable for practical applications. In procedure 3, the peak of $C_{10}H_{22}$ was not adequate for the determination of kerosene, as *sec.*- and isobutylbenzenes (unseparated) derived from the extraction solvent interfered with this peak (Fig. 3). The levels of xylenes and trimethylbenzenes in the extraction solvent were very low, so the use of *n*-pentane for blood kerosene determination was permissible in the GC assay (Fig. 3 and Table IV).

Autopsy cases

Many peaks were detected in the blood of case 1 when procedures 1 and 2 were employed (Figs. 2 and 3). These peaks corresponded to those of gasoline. The

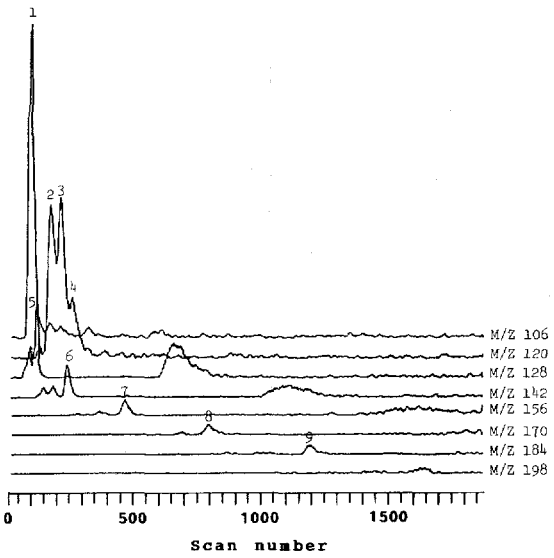

Fig. 4. GC–MS (electron-impact ionization) results for blood kerosene in case 4 (left ventricle). The sample was prepared using procedure 3. Mass numbers monitored were of molecular ions: m/z 106 = *o*-, *m*- and *p*-xylenes (peak 1); 120 = trimethylbenzenes (peaks 2, 3 and 4); 128 = C_9H_{20} (peak 5); 142 = $C_{10}H_{22}$ (peak 6); 156 = $C_{11}H_{24}$ (peak 7); 170 = $C_{12}H_{26}$ (peak 8); 184 = $C_{13}H_{28}$ (peak 9); 198 = $C_{14}H_{30}$ (peak undetected).

concentrations of blood gasoline determined were ca. 20 and 14 μg/ml in the right and left ventricles of the heart, respectively (Table V). In cases 2 and 4, where kerosene was used, no peak corresponding to gasoline components (except toluene) appeared on the gas chromatogram with procedures 1 and 2, and the chromatogram obtained by procedure 3 agreed approximately with that for standard kerosene obtained using both GC and GC–MS (Figs. 3 and 4). The blood kerosene concentration was calculated to be 5.8 and 1.7 μg/ml in the right and left ventricles of case 2 and 3.3 and 2.2 μg/ml in case 4, respectively, using C_9H_{20} as

TABLE III

ACCURACY AND PRECISION OF BLOOD GASOLINE DETERMINATION

Prepared concentration of gasoline in fresh blood: 30.0 μg/ml.

Procedure	Indicator component	Determined blood gasoline (mean ± S.D., $n=5$) (μg/ml)	Coefficient of variation (%)
1	Isopentane	30.1 ± 1.5	4.8
	n-Pentane	30.5 ± 2.8	9.3
	2-Methylpentane	29.5 ± 3.0	10.0
	2-Methylhexane	33.7 ± 6.4	19.1
	3-Methylhexane	37.5 ± 6.9	18.3
2	Benzene	24.4 ± 5.9	24.1
	Toluene	32.8 ± 3.4	10.2

TABLE IV

ACCURACY AND PRECISION OF BLOOD KEROSENE DETERMINATION BY PROCEDURE 3

Prepared concentration of kerosene in fresh blood: 4.0 μg/ml.

Indicator component	Determined blood kerosene (mean ± S.D., $n=5$) (μg/ml)	Coefficient of variation (%)
m,p-Xylene	4.22 ± 0.22	5.3
C_9H_{20}	4.52 ± 0.23	5.0
Mesitylene	4.46 ± 0.39	8.8
Pseudocumene	3.68 ± 0.29	7.8
$C_{11}H_{24}$	4.08 ± 0.75	18.5

TABLE V

DETERMINATION OF BLOOD GASOLINE CONCENTRATION IN CASE 1

Significant difference ($p<0.05$) for blood gasoline between left and right ventricles by Wilcoxon's signed ranks test.

Indicator component	Boiling point (°C)	Determined blood gasoline* (μg/ml)	
		Left ventricle	Right ventricle
Isopentane	27.9	18.8	16.8
n-Pentane	36.1	22.0	19.8
2-Methylpentane	60.3	15.6	14.2
Benzene	80.1	17.8	14.0
2-Methylhexane	90.1	23.4	10.4
3-Methylhexane	91.9	22.1	10.2
Toluene	110.6	19.2	13.3

*Mean values of triplicate analyses.

TABLE VI

BLOOD KEROSENE DETERMINATION IN CASES 2 AND 4

Significant difference ($p<0.01$) for blood kerosene between left and right ventricles by Wilcoxon's signed ranks test.

Case	Indicator component	Boiling point (°C)	Determined blood kerosene* (μg/ml)	
			Left ventricle	Right ventricle
2	*m,p*-Xylene	138.4, 139.1	10.3	3.5
	C_9H_{20}	150.8	5.8	1.7
	Mesitylene	164.7	1.4	1.0
	Pseudocumene	169.4	1.9	1.5
	$C_{11}H_{24}$	195.9	4.3	2.2
4	*m,p*-Xylene	138.4, 139.1	5.4	3.7
	C_9H_{20}	150.8	3.3	2.2
	Mesitylene	164.7	1.4	0.9
	Pseudocumene	169.4	1.4	0.5
	$C_{11}H_{24}$	195.9	0.6	0.3

*Mean values of triplicate analyses.

an indicator. A great variation was found, however, in the blood kerosene concentrations determined when five constituents of kerosene were chosen for quantitation (Table VI). In these cases of kerosene exposure, hydrocarbons having more than fourteen carbon atoms were hardly inhaled (Fig. 4). Fuel concentrations in the left and right ventricular blood were compared using Wilcoxon's signed ranks test and significant differences were observed for both blood gasoline ($p<0.05$) and blood kerosene ($p<0.01$). The fuel concentration in the left ventricle was higher than that in the right in all cases. No peaks of fuel components were detected in the blood of case 3. The blood HbCO content was lower in the right than in the left ventricle except for case 4 (Table II).

DISCUSSION

The methods examined proved to be rapid and simple for the determination of petroleum fuel concentrations in blood from burned bodies. Recently, petroleum fuel sniffing has caused increasing numbers of accidental deaths [5,6]. The present technique would be applicable to toxicological analysis in such cases.

Aliphatic and aromatic hydrocarbons (C_{5-7}) could be readily detected by the headspace method (procedures 1 and 2). For the detection of hydrocarbons with nine or more carbons, the solvent extraction technique was needed. Diethyl ether, which is used as an extraction reagent for the detection of kerosene from fire spots, was not suitable for the analysis of blood samples as the gas chromatograms obtained had large backgrounds and the solvent peak itself also interfered with some hydrocarbons (unpublished data). On the other hand, *n*-pentane used as an extraction solvent in this study contained some aromatic hydrocarbons, e.g., *o*-, *m*- and *p*-xylenes, trimethylbenzenes and *sec*.- and isobutylbenzenes, but not paraffinic hydrocarbons with nine or more carbon atoms. There was a clear distinction between the chromatograms obtained for the extraction solvent and those for samples from burned bodies with kerosene exposure. Hence the use of *n*-pentane as an extraction solvent can be recommended in such practical GC analyses.

The separation of gasoline and kerosene was very easily achieved on the gas chromatograms obtained using the three different procedures. As the gas chromatogram of petroleum fuel was complicated, analysis by GC–MS increased the reliability of the assay. Petroleum fuel is a complex mixture of hydrocarbons with several hundred components, for which the use of a capillary column is generally preferable to a packed column [7]. In trace analysis using the headspace method, however, packed columns gave better results (unpublished data).

The quantitative data from the victims showed a higher content of petroleum fuel in the left than in the right ventricular blood. This effect should have been due to circulation and diffusion in the living body, suggesting that fuel was inhaled just before death. The blood gasoline concentrations in case 1 were almost the same when any component was chosen. On the other hand, a great variation in the blood kerosene concentrations was found in cases 2 and 4. As the vaporization points of kerosene components are high and differ widely, the selection of a hydrocarbon having a higher boiling point resulted in the calculation of a lower

kerosene level in the blood. Hydrocarbons with 14 or more carbon atoms were hardly inhaled, whereas C_{8-13} hydrocarbons could be detected. It should be remembered, however, that the blood fuel levels determined in this study are not necessarily the real fuel contents but estimates based on selected fuel components inhaled.

There are only a few papers [1,2] that offer any quantitative data on blood petroleum fuels in burned bodies. The toxicological effects of fuel inhaled just before burning are also unclear. In a case of fatal poisoning by gasoline ingestion, the concentration of gasoline in the blood was 30–130 μg/ml, with the highest content in the pulmonary blood and the lowest in the blood from the brain [8]. In a case of fatal gasoline poisoning via inhalation, the gasoline level in the blood was calculated to be 1 mg/ml [9]. Schlunegger [10] carried out gasoline vapour experiments with rats and reported the lethal gasoline concentration in the blood to be 0.3–0.4 mg/ml. Recently, in a fatal case of gasoline inhalation combined with alcohol intoxication, the gasoline concentration in the heart blood was reported to be less than 1 μg/ml [11]. These data suggest that the blood gasoline detected in case 1 could have had some toxic effects on the victim before death. The period of exposure to the gasoline vapour, however, seemed to have been very short in the above case and the expected effects of inhaled fuel might be much lower. Quantitative data on burning with kerosene exposure have rarely been reported [2]. Hydrocarbons of kerosene have a higher boiling point than those of gasoline, so a lower inhalation of hydrocarbons is expected in kerosene exposure. For a more detailed discussion of the effects of petroleum fuel inhaled before burning, quantitative data similar to those for the present autopsy cases should be accumulated.

The cause of death in the four cases examined here was concluded to be burning in each instance. The inhalation of soot into the airways and the formation of HbCO are therefore often negative in subjects who have died from rapid burning with petroleum fuel. The same phenomenon is also observed in victims burned in an open space without exposure to petroleum fuel or in a flash fire [12]. In such cases, the determination of petrol and HbCO in both the right and left ventricular blood of victims would be helpful in the forensic diagnosis of death.

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