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Intratracheal Gas Analysis for Volatile Substances by Gas Chromatography/Mass Spectrometry—Application to Forensic Autopsies

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ABSTRACT: Intratracheal gas analysis was carried out by gas chromatography/mass spectrometry (GC/MS) in 20 burned body cases (13 males and 7 females). Volatile aromatic and aliphatic hydrocarbons were detected by GC/MS using a GS-Q column with the intratracheal gas as well as the blood in 19 cases. The characteristic patterns of mass chromatograms for gasoline, kerosene (gas oil), and liquid petroleum gas could be differentiated from each other using the intratracheal gas. The burned body in one case showed no presence of volatile substances in the intratracheal gas, nor intratracheal soot, although high concentrations (1 µg/g and more) of volatile substances were detected on the clothes. The victim also had normal CO-Hb concentrations (0.1 to 0.2%) in the heart blood. The results of intratracheal gas analysis were consistent with signs of the vital reaction. In conclusion, intratracheal gas analysis provides a supportive method for diagnosing the cause of death in burned bodies, and yields for at least 48 hours valuable information on volatile hydrocarbons (being detected in deliberate or accidental fire cases) to which the body had been exposed just before death.

KEYWORDS: forensic science, forensic toxicology, burned body, vital reaction, intratracheal gas, volatile hydrocarbons, GC/MS

Japan in 1997 recorded 61 889 cases of fires that claimed a total of 2095 lives. A large number (1305) of the victims died in structure (e.g., building, house) fires, and 283 died in car fires. It is difficult, however, to obtain information on the volatile hydrocarbons (accelerants) present. There are some familiar fuels in Japan, for example, liquid petroleum (LP) gas (for cooking), gasoline (for cars), kerosene (for heating), and gas oil (for cars). Also, thinners and mixed oil (gasoline + gas oil) are often utilized for painting and machine fuel, respectively.

It is very important for forensic and police investigations to determine whether or not accelerants were present. If accelerants were present, we must be clear as to which kinds. Using gas chromatography (GC) or GC-mass spectrometry (GC/MS), or both, volatile substances can be identified from the debris of a fire scene (1–8). Furthermore, in parallel with the police investigation of the

fire scene, it must also be apparent whether accelerants are present in the blood of a burned body (9–13).

In burned body cases, confirmation of the vital reaction is carried out using both autopsy findings and results of toxicological analyses—for example, the presence of first- and second-degree burns on the skin, the presence of intratracheal soot, rubor of the intratracheal mucosa, carboxy-hemoglobin (CO-Hb) (10% ≤) in the blood, and the presence of volatile hydrocarbons (e.g., petroleum) in the blood and tissues (9–14).

In previous reports, accelerant analysis was performed only on the blood and tissues (9–13). We had hypothesized that the presence of accelerants could be proved by GC/MS analysis of the intratracheal gas. To the best of our knowledge, intratracheal gas analysis has received little attention. Also, it presents technical difficulties, since the gas collected can not be stored for a long time.

We performed intratracheal gas analysis by GC/MS in 20 interesting cases of burned bodies.

Materials and Methods

Materials

We selected burned body cases where the tracheal wall was intact. From the situation in which the burned body was found, we categorized 20 cases into three groups; A (12 cases), burned body cadavers found indoors; B (5 cases), burned body cadavers discovered outdoors; C (3 cases), burned body cadavers found in cars. The total of 20 cases included 13 males and 7 females (ages, 1 to 89 years old; mean age 47.4).

Sample Collection and Preparation

After external examination, the trachea was exposed by a frontal neck incision. The intratracheal gas (1.5 to 2 mL) was collected through a steel needle with a 2 mL glass syringe. The gas collected was analyzed as soon as possible by GC/MS.

Standard substances (e.g., *n*-pentane, *n*-hexane, *n*-heptane, *n*-octane, *n*-nonane, benzene, toluene, xylene) were dissolved in 10% (v/v) Tween[®] 20 (polyoxyethylene sorbitan monolaurate, Nacalai tesque, Kyoto, Japan) aqueous solution at a concentration of 10 µg/mL (stock solution). Stock solutions were diluted (2 to 100 times) with water to make the working solution.

GC/MS Analysis for the Intratracheal Gas

A model of JMS-DX303 and DA5000 systems (JEOL, Tokyo, Japan) with a GS-Q column (0.53 mm × 30 m; J&W Scientific,

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U.S.) was used for the analysis. The column temperature was programmed to increase from 50 to 230°C at a rate of 16°C/min and then remain at 230°C for 15 min. The injection temperature was set at 200°C and the flow rate of the carrier gas (He) was 15 mL/min. The ionization voltage and current for electron impact (EI) and positive ion detection modes were set at 70 eV and 300 μ A, respectively. Scan (multiple ion monitoring) mode was used over the range of 10 to 400 amu (15).

For semi-quantitative radar-chart pattern analysis, intensities (logarithm scale) of hydrocarbons among C3 and C9 were used for C3 (propane/propylene) with m/z 41; C4 (butane/butene) with m/z 41; C5 (pentane/pentene) with m/z 42; C6 (hexane/hexene) with m/z 43; C7 (heptane/heptene) with m/z 43; C8 (octane/octene) with m/z 57 and C9 (nonane/nonene) with m/z 57.

Analytical Methods for Volatiles in the Blood and Organ Tissues

The heart blood and organ tissues were analyzed by headspace GC/MS with the same apparatus and conditions as described above. An aliquot (2 mL (g)) of sample was put into a 10 mL vial,

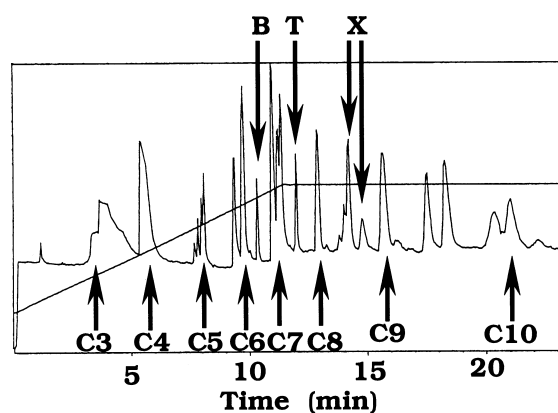


FIG. 1—A typical total ion chromatogram of some aliphatic and aromatic hydrocarbons using a GS-Q column. B, T and X represent benzene, toluene and m(p)-o-xylene, respectively. C3 to C10 indicate each retention time (position) of aliphatic hydrocarbons from C3 (propane) to C10 (n-decane). Analytical conditions are described in the text.

and then warmed for 15 min at 55°C. After incubation, 2 mL of head space gas was injected into the GC/MS apparatus described above (15).

CO-Hemoglobin Analysis in the Blood

The CO-Hb concentration in the blood was measured by a CO-oximeter (model 270, Ciba-Corning, Medfield, MA; current providing company in Japan, Bayer Medical Co., Tokyo) (16).

Results

The standard volatile substances (C3 to C9 aliphatic hydrocarbons, benzene, toluene, and xylene) were well separated by GC/MS using the GS-Q column (Fig. 1). Their detection limits with EI and positive ion detection modes ranged from 0.1 to 5 ng per single injection (Table 1).

Analytical results for volatile substances by GC/MS with the intratracheal gas in 20 cases are listed in Table 2. Figures 2–4 show typical mass chromatograms of some volatile substances detected from the intratracheal gas, in the heart blood and of the clothes in Case 4 (Group A), Case 15 (Group B), and Case 18 (Group C), respectively. Aromatic (e.g., benzene, toluene, xylene) and aliphatic (e.g., n-hexane, n-heptane, n-octane) hydrocarbons were detected in the intratracheal gas as well as in the blood in 19 of the 20 cases (Table 2).

In Group A, 11 cases out of 12 were positive for intratracheal soot and had high concentrations of CO-Hb in the heart blood, but one case (Case 12) showed no intratracheal soot and had normal level of CO-Hb concentrations (0.1 to 0.2%) in the heart blood. In this case, no volatile substances were detected in the intratracheal gas or in the blood, although high concentrations (1 μ g/g and more) of volatile substances were detected in the clothes (Fig. 5). The cause of death in this case (Case 12) was asphyxia due to compression of the neck.

In Group B, aliphatic and/or aromatic hydrocarbons were detected in the intratracheal gas and in the heart blood in all of the cases (Fig. 3). These results were identical to the components of the accelerant(s) used.

In Group C, one case (Case 20) had a very low CO-Hb concentration (3.9%) in the blood, although aromatic hydrocarbons were detected both in the blood and in the intratracheal gas (Table 2).

TABLE 1—Detection of volatile substances by GC/MS using GS-Q column and their detection limits.

Volatile Substance	Molecular Weight	Main Fragment Ions* (m/z)	Retention Time† (min)	Detection Limits (ng)
Aliphatic hydrocarbons				
pentane	72	43, 42, 41	8.1	5.0
n-hexane	86	57, 43, 41	9.9	1.0
l-hexene	84	56, 41, 55	9.8	0.5
n-heptane	100	71, 43, 57	11.3	0.5
l-heptene	98	56, 41, 55	11.2	0.2
n-octane	114	85, 57, 43	12.9	0.2
l-octene	112	55, 70, 43	12.8	0.2
n-nonane	128	57, 85, 71	15.7	0.2
l-nonene	126	56, 55, 43	15.6	0.2
Aromatic hydrocarbons				
benzene	78	78, 77, 45	10.4	0.1
toluene	92	91, 92, 77	12.0	0.1
p(m)-xylene	106	91, 105, 106	14.2	0.1
o-xylene	106	91, 105, 106	14.8	0.1
styrene	104	104, 78, 103	15.0	0.1

* Electron impact (EI) and positive ion detection modes.

† Conditions of separation with GS-Q column are described in the text.

TABLE 2—Summary of the autopsy findings and toxicological analyses in 20 burned body cases.

Group* Case	Sex/Age (Year)	PI [†] (h)	Degree of the burn	Intra-tracheal soot	CO-Hb in the heart blood (%) Left Right	Intratracheal gas analysis	Volatile analysis with the blood [‡]	Volatile analysis with clothes [‡]	Volatile hydrocarbons (Accelerant) [§]
A 1	F/42	17	1-2	+	84.8 95.4	Bez, Tol, Xyl, Sty, C3-C6**	Bez, Tol, Xyl, Sty**	Bez, Tol, Xyl, Sty	Gasoline
A 2	F/56	14	1-3	+	93.1 77.6	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C3-C9+	LP gas + Kerosene
A 3	M/77	18	2-4	+	76.2 76.4	Bez, Tol, Xyl, Sty, EtOH, C3-C9+	Bez, Tol, Xyl, Sty, EtOH	NT [¶]	Kerosene
A 4	M/17	21	1-3	+	69.9 70.0	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C4-C9+	Kerosene
A 5	F/86	16	1-3	+	96.1 68.8	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C3-C9+	LP gas + Kerosene
A 6	M/21	10	2-4	+	61.4 59.5	Tol, EtOH ^{¶¶}	Tol, EtOH ^{¶¶}	NT	Thinner
A 7	M/23	9	1-3	+	64.0 57.7	Bez, Tol, Xyl	Bez, Tol, Xyl, Sty	Bez, Tol, Xyl	Not used
A 8	M/52	18	1-3	+	63.6 50.1	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C3-C5	NT	LP gas + Kerosene
A 9	M/69	19	1-3	+	21.7 26.9	Bez, Tol, Xyl, Sty, C3-C6	Bez, Tol, Xyl, Sty, C4-C6	NT	Kerosene
A 10	F/49	12	1-3	+	15.5 15.5	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C4-C9+	LP gas + Kerosene
A 11	M/89	11	2-4	+	13.2 15.0	Bez, Tol, Xyl, C3-C6	Bez, Tol, Xyl, Sty, C3-C6	NT	Kerosene
A 12	F/60	13	(3) ^{¶¶}	-	0.2 0.1	ND [§]	ND	Bez, Tol, Xyl, Sty, C4-C9+	Kerosene
B 13	M/16	20	1-4	+	30.7 25.3	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C3-C9+	Gasoline + gas oil
B 14	M/44	22	1-4	+	17.9 19.8	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C4-C9+	Bez, Tol, Xyl, Sty, C3-C9+	Gasoline + gas oil
B 15	F/31	43	1-4	+	16.1 18.2	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C3-C9+	Bez, Tol, Xyl, Sty, C4-C9+	Kerosene / gasoline
B 16	M/56	30	1-2	+	7.2 6.8	Bez, Tol, Xyl, C5-C7	Bez, Tol, Xyl	Bez, Tol, Xyl, C5-C7	Gasoline + gas oil
B 17	M/64	48	1-3	+	6.2 5.8	Bez, Tol, C4	Bez, Tol, Xyl, EtOAc, Sty	NT	Thinner
C 18	M/1	40	(3)-4	+	41.2 39.1	Bez, Tol, Xyl, Sty, C3-C7	Bez, Tol, Xyl, Sty, C3-C5	Bez, Tol, Xyl, Sty, C3-C9+	Kerosene / gasoline
C 19	M/51	17	(3)-4	+	24.1 [§]	Bez, Tol, Xyl, EtOH	Bez, Tol, Xyl, EtOH	NT	Ud [¶]
C 20	F/44	18	(3)-4	+	3.9 ^{§§}	Bez, Tol, Xyl	Bez, Tol, Xyl	NT	Ud

* , See text; [†]PI, postmortem interval (hour); [‡], volatiles' analyses in the blood and clothes were performed by headspace - GC/MS described in the text; [§]Volatile hydrocarbons (accelerants), volatile hydrocarbons (accelerants) used show kind(s) of petroleum components, which were found by police investigation and/or volatile-hydrocarbon analysis by this method; ^{**}, volatile compounds such as benzene (Bez), toluene (Tol), xylene (Xyl), styrene monomer (Sty) and other aliphatic hydrocarbons (Cs) were detected; EtOH, ethanol; EtOAc, ethylacetate; [¶]NT, not tested; ^{¶¶}Tol, EtOH, toluene and ethanol were only analyzed; ^{¶¶¶}(3), partially burned at the third degree; ^{¶¶¶}ND, not detected; ^{¶¶¶}Ud, Undetermined; [§], heart blood mixed; ^{§§}, femoral vein blood.

Three persons (Cases 2, 5 and 10) were found dead in different rooms of a burned house. A bottle of LP gas was found in a room, and kerosene was detected from the debris in some rooms. A woman (Case 15) committed suicide by starting fire in a car, and then she was found dead just near the car in which a boy (Case 18) was found as a severely burned body. Two persons (Cases 19 and 20) were found as severely burned bodies in a car burned with explosion in a park.

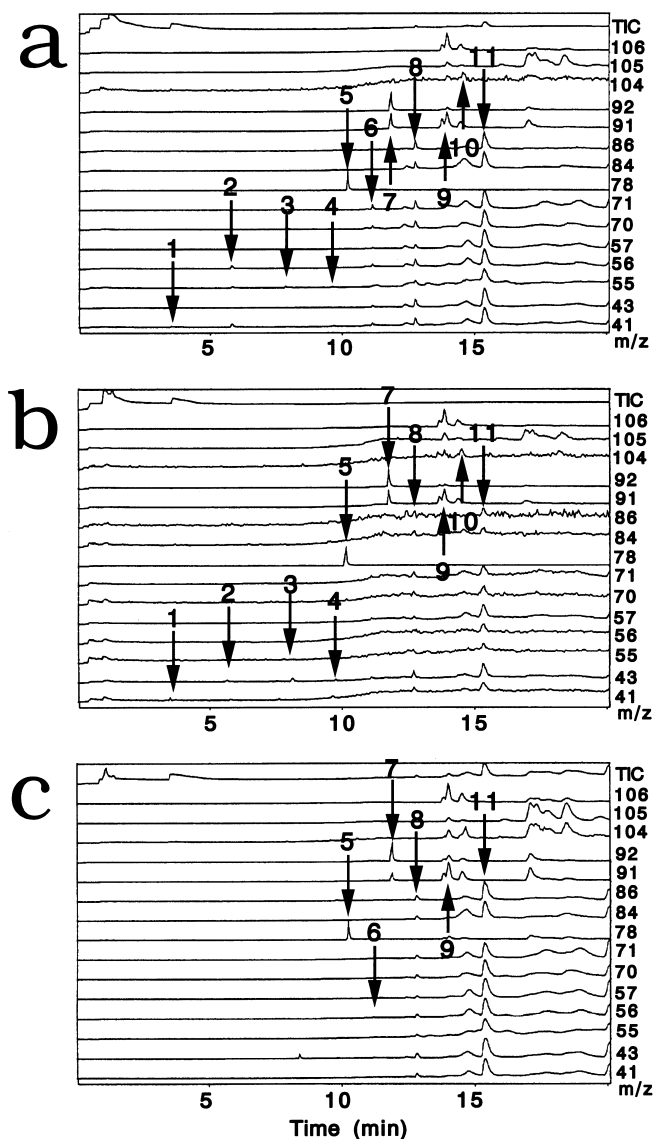


FIG. 2—Total ion and mass chromatograms of volatile substances in the intratracheal gas (a), heart blood (b), and a piece of clothing (c) in Case 4 (Group A). 1—propylene (C3); 2—butane and butene (C4 hydrocarbons); 3—pentane and C5 hydrocarbons; 4—hexane and C6 hydrocarbons; 5—benzene; 6—heptane and C7 hydrocarbons; 7—toluene, 8—octane and C8 hydrocarbons; 9—xylene; 10—styrene monomer; 11—nonane and C9 hydrocarbons. In the analysis of clothing (c), intensities of C3 to C6 aliphatic hydrocarbons were extremely low compared with those of C9 hydrocarbons. Therefore, they are not shown by arrows.

The peak height intensities of the mass chromatograms using m/z 41 for C3 and C4 hydrocarbons, m/z 42 for C5 hydrocarbon, m/z 43 for C6 and C7 hydrocarbons and m/z 57 for C8 and C9 hydrocarbons were drawn on a logarithmic scale by a radar chart in each case (Fig. 6) as well as each standard gas oil. These results showed that in the liquid petroleum (LP) gas cases, the peak height intensities of C3 and C4 hydrocarbons were relatively higher than those of the other hydrocarbons, because C3 and C4 were main components of LP gas (data were not shown). In gasoline cases the peak height intensities of C5 and C6 hydrocarbons were slightly higher than the others in the analytical conditions (Fig. 6a). Further, in cases where kerosene or gas oil was used, the peak height

intensities of the C8 and C9 hydrocarbons were relatively higher than the others (Fig. 6b).

Discussion

It is well known that gasoline and gas oil are used as motor car fuels. Kerosene is the most popular fuel oil for heating of houses in Japan. Moreover, thinner components are characterized by their content of alcohols (i.e., isopropanol), acetates (i.e., ethylacetate) or ketones (i.e., acetone) as well as toluene and xylene. LP gas chiefly contains propane, propylene, butane and 1-butene (C3 and C4 hydrocarbons), and is used in many houses for cooking fuel.

These volatile accelerants have often been involved accidentally or deliberately in fire cases (1–13). Therefore, it is useful for forensic science and police investigation to classify these accelerants. The identification of volatile substances is performed by GC/MS

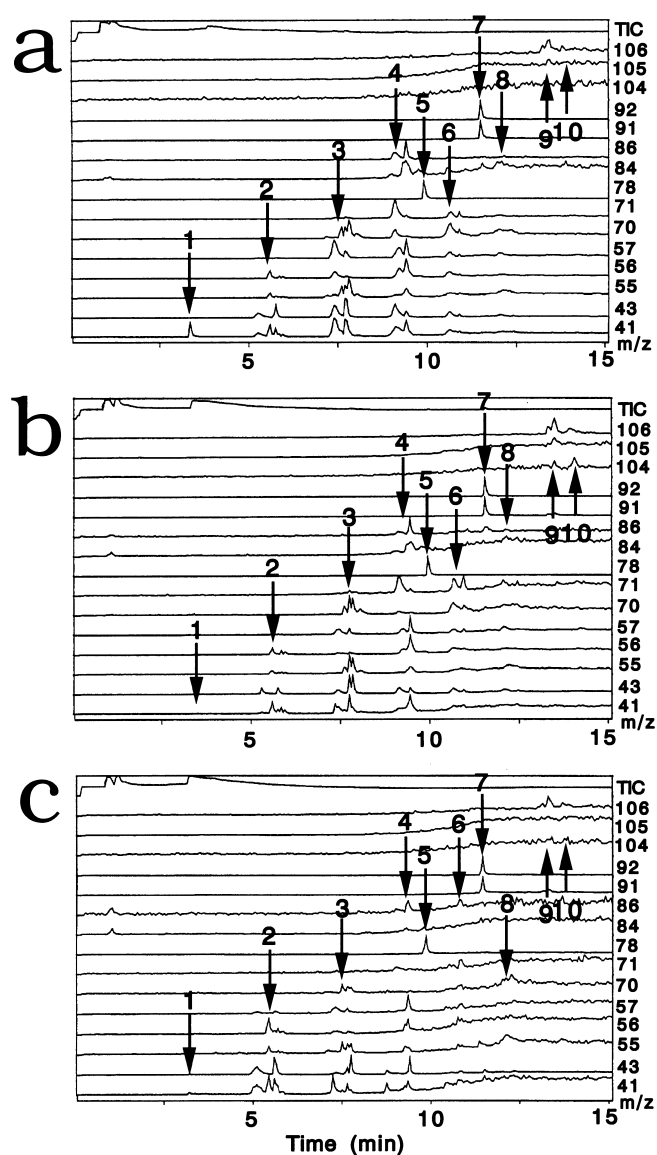


FIG. 3—Total ion and mass chromatograms of volatile substances in the intratracheal gas (a), heart blood (b), and a piece of clothing (c) in Case 15 (Group B). 1—propylene; 2—butane and butene (C4 hydrocarbons); 3—pentane and C5 hydrocarbons; 4—hexane and C6 hydrocarbons; 5—benzene; 6—heptane and C7 hydrocarbons; 7—toluene; 8—octane and C8 hydrocarbons; 9—xylene; 10—styrene monomer.

with some pretreatment techniques—for example, headspace (5,15), charcoal adsorption (17), pulse heating (2,18), solid-phase microextraction (19,20), off-line (2,7) and on-line (21) thermal desorption, and purge-and-trap (22).

In the present study, the headspace method was utilized for the analysis of volatile hydrocarbons, since it has been known as a reliable method without solvent interference for volatiles below C6, despite a limitation for higher molecular weight hydrocarbons (e.g., C9<).

Volatile accelerants are well classified by GC/MS analysis (3). Gasoline contains C5 to C14 hydrocarbons, and C9 to C11 aromatics are particularly useful to distinguish between different samples of gasoline (3). Medium petroleum distillates are characterized by a “bell-shaped” chromatogram with *n*-nonane, *n*-decane, *n*-unde-

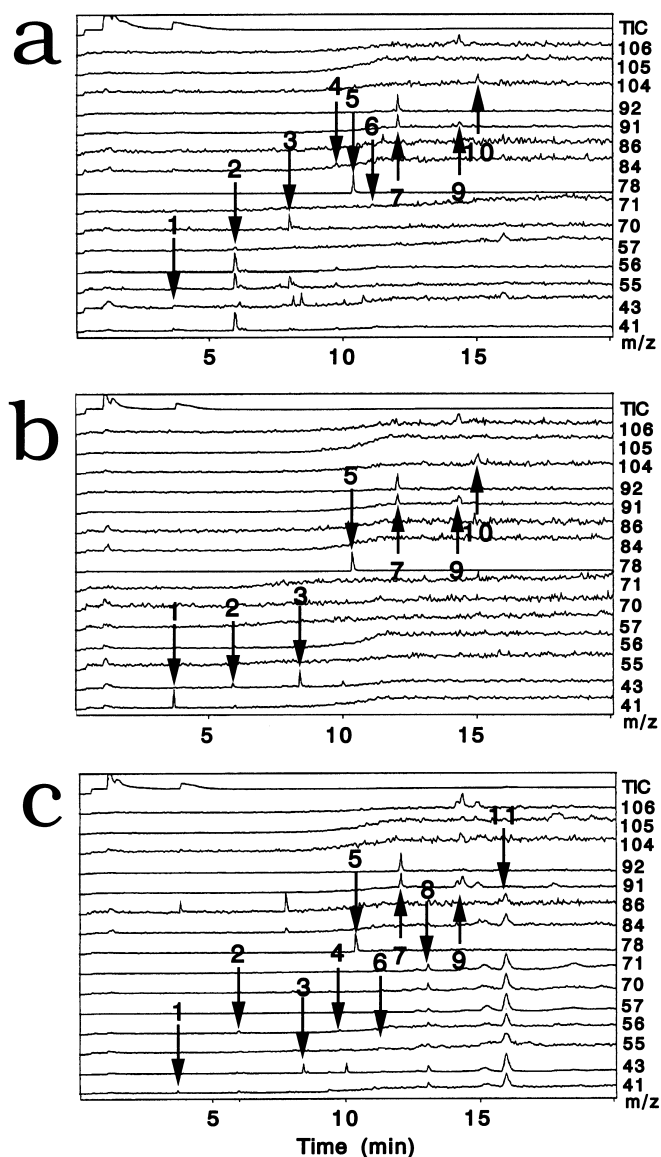


FIG. 4—Total ion and mass chromatograms of volatile substances in the intratracheal gas (a), heart blood (b), and a piece of clothing (c) in Case 18 (Group C). 1—propylene; 2—butane and butene (C4 hydrocarbons); 3—pentane and C5 hydrocarbons; 4—hexane and C6 hydrocarbons; 5—benzene; 6—heptane and C7 hydrocarbons; 7—toluene; 8—octane and C8 hydrocarbons; 9—xylene; 10—styrene monomer; 11—nonane and C9 hydrocarbons.

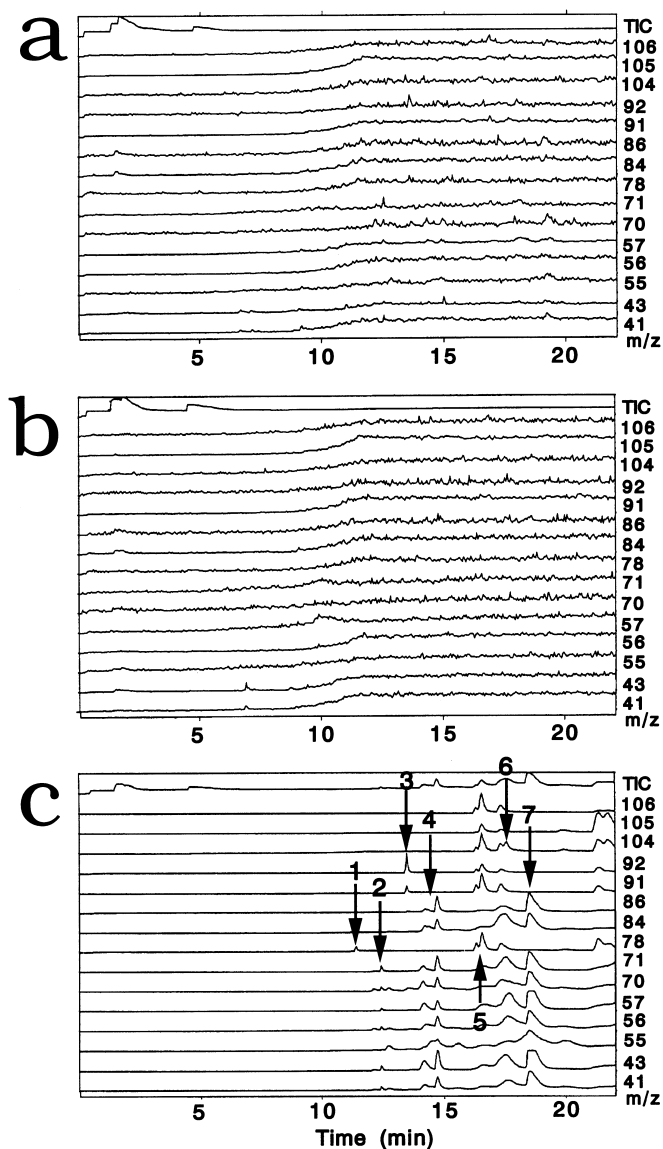


FIG. 5—Total ion and mass chromatograms of volatile substances in the intratracheal gas (a), heart blood (b), and a piece of clothing (c) in Case 12 (Group A). 1—benzene; 2—heptane and C7 hydrocarbons; 3—toluene; 4—octane and C8 hydrocarbons; 5—xylene; 6—styrene monomer; 7—nonane and C9 hydrocarbons. In the analysis of clothes (c), intensities of C4 to C6 aliphatic hydrocarbons were extremely low compared with those of C9 hydrocarbons. Therefore, they are not shown by arrows.

cane and sometimes *n*-dodecane peaks. Moreover, light petroleum-based products (many lacquer thinners and commercial solvents) contain components such as alcohols, acetates, or ketones, which have been added to the distillate fraction.

In our experimental conditions, the pattern of gasoline components showed chiefly C4 to C7 hydrocarbons and those of kerosene and petroleum distillates (gas oil) mainly presented C7 to C9+ hydrocarbons (Figs. 2–4, Table 2). From the data processing using mass chromatograms, radar charts for intensities of C3 to C9 aliphatic hydrocarbons in four cases were drawn as seen in Fig. 6. Gasoline (C4 and C5) and kerosene (C8 and C9) characteristically had relatively higher intensity than those of the other hydrocarbons in the analytical conditions. The pattern of the mixture of LP gas and kerosene in Case 10 showed relatively high intensities of C3,

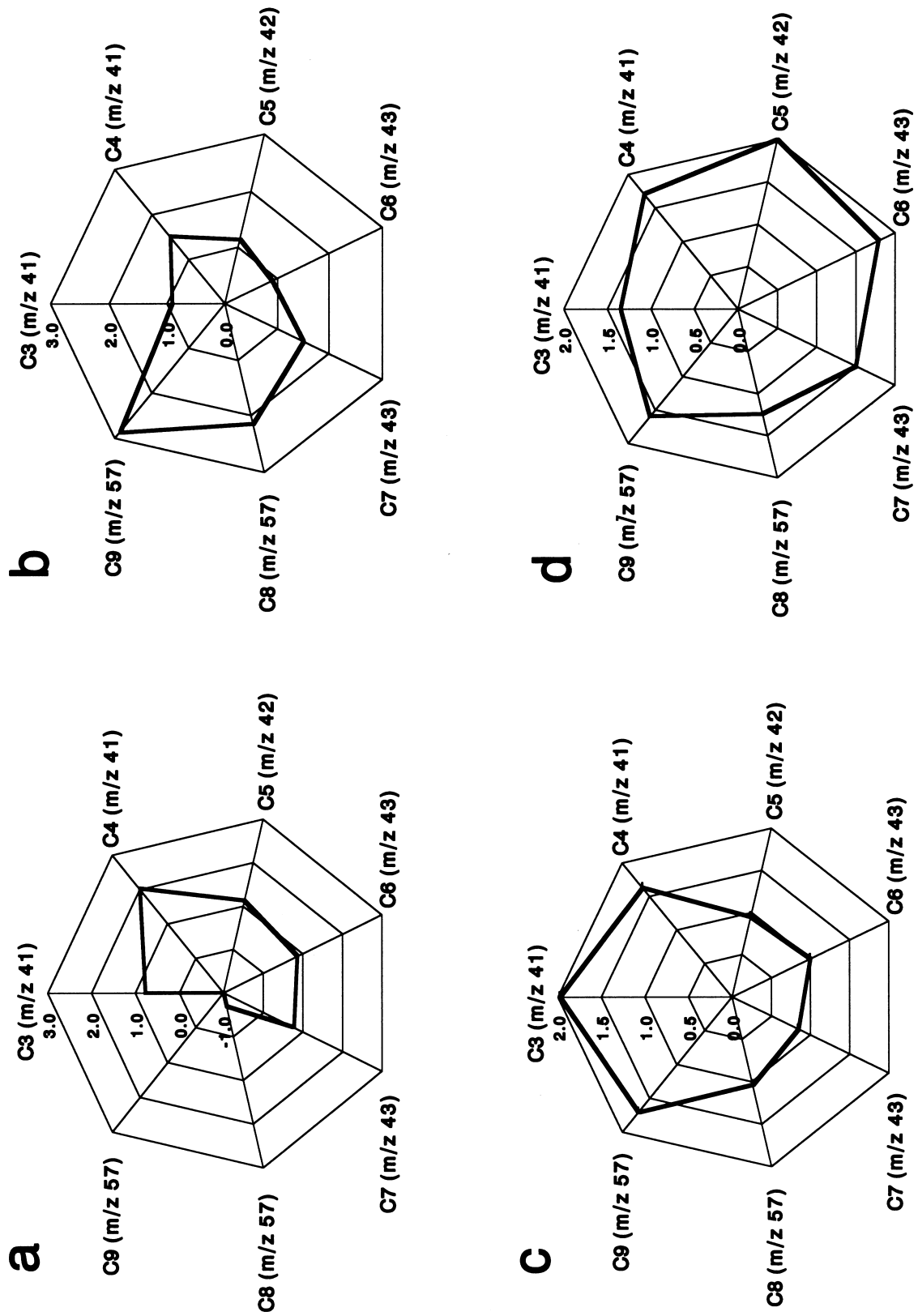


FIG. 6—Radar chart patterns for peak height intensities of aliphatic hydrocarbons (C3 to C9) in the intratracheal gas by GC/MS analysis. Peak height intensity is shown in logarithmic scale. (a), Case 1 (gasoline); (b), Case 4 (kerosene); (c), Case 10 (LP gas + kerosene); (d) Case 14 (gasoline + gas oil). Detailed data in each case are shown in Table 2.

C4, C8, and C9 (Fig. 6c). Therefore, the characteristic pattern of the mixture of LP gas and kerosene was almost the same as the total amount of radar chart pattern of each component. The hydrocarbons detected in the trachea of fire victims, however, were sometimes not accelerants (deliberately added to increase the fire intensity) but the residues of accidental exposures to volatiles (Table 2). From these results, intratracheal gas analysis by GC/MS made it possible to identify volatile hydrocarbons to which the dead body had been exposed just before death.

In our experience, the intensities of C3 to C9 greatly varied when using specimens of clothes. Also, the relatively small hydrocarbons (C3 to C5) tend to be detected with less intensity than large hydrocarbons (C7+) when using clothes because the small molecules evaporate more readily in the open air. Since the intratracheal space was closed in all the burned bodies tested, the components of the intratracheal gas were considered to have undergone little change within 48 hours. Therefore, intratracheal gas may prove to be one of the most important resources in the analysis of volatile substances in a fire scene.

Currently, signs of the victim's vital reaction are used, as described in the introduction of Ref 14. Especially, detection of volatile substances in the blood has been studied for the identification of accelerants and diagnosis of vital reaction (9–13). Nagata et al. (10) first described the detection of gasoline in the blood. Morinaga et al. described its usefulness for detection of petroleum components and styrene in fire victim cases (7). Further, Matsubara et al. (12) described how concentrations of petroleum components in the left heart blood were higher than those in the right in eight fire victim cases.

Kojima et al. (11) described two murder cases as follows. In one case, in which paraffinic hydrocarbons were detected in the left heart blood, the victim had been set on fire before death and burning had been the cause of death. In the other case, paraffin hydrocarbons were not detected in the left heart blood but were detected in a hair sample, so the victim had been burned after death and the cause of death was suspected to be asphyxia (11).

Further, Iwasaki et al. (13), following examination of 73 cadavers found in wreckage after fires, found that accelerants in the blood seemed to be helpful in determining the cause of death and whether volatile hydrocarbons were present.

In one of our cases (Case 12), no volatile substance was detected in the intratracheal gas, nor was there any CO-Hb in the blood, and intratracheal soot was not present. However, large amounts of petroleum components were detected on the clothes. Therefore, it was deduced that the woman was burned with kerosene after her death. From these results, including Case 12, the results of intratracheal gas analysis by GC/MS were consistent with the signs of vital reaction. Therefore, intratracheal gas analysis could be a useful indicator of vital reaction.

In addition, intratracheal gas analysis was capable of being applied to cases of intoxication by volatile substances. For example, chloroform could be detected in the intratracheal gas of a murder case (23).

In conclusion, intratracheal gas analysis provides a supportive method for diagnosing the cause of death in burned bodies, and yields for at least 48 hours valuable information on volatile hydrocarbons (detected in a deliberate or accidental fire cases) to which the body had been exposed just before death.

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