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CASE REPORT

Pathological and toxicological findings in four cases of fatal hydrogen sulfide inhalation

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Abstract Recently in Japan, there have been scattered cases of suicide by inhalation of hydrogen sulfide (H_2S), produced by mixing domestic chemicals containing sulfur and hydrochloric acid. We report four cases of such fatalities with regard to the pathological and toxicological findings. Two cases were male and female suicide victims (cases 1 and 2; about 2 days and 30 h postmortem, respectively), and the other two were a female suicide victim and her husband (cases 3 and 4; about 3 days postmortem). Partial greenish discoloration was observed on the skin in three cases (cases 1, 3, and 4), and also in the airways and lungs and on the brain surface in one case (case 3). The most evident pathology was marked pulmonary and cerebral edema in all cases. Concentrations of H_2S and the metabolite thiosulfate (TS) in the blood varied by case (0.66–85.0 and 0.00–369 μg/ml, respectively), and similar distributions were seen in the viscera; these concentrations were markedly high in a case of visceral discoloration (case 3). Both H_2S and TS contents in the skin and muscle were higher at the sites of discoloration in individual cases. A control study demonstrated an unexpected increase of TS contents along with H_2S production due to putrefaction as well as higher TS content than H_2S. It was difficult to discriminate putrefaction using H_2S and TS measurements, especially in gastric contents, bone marrow, heart, liver, kidney, spleen, pancreas, and intestine. However, vitreous body, pericardial fluid, and lung tissue showed different findings compared with putrefactive changes, and may be used as supplementary or alternative samples. These findings suggest the significant contribution of postmortem exposure to H_2S gas to the pathological and toxicological findings, depending on the circumstances, and the importance of systemic toxicological analysis for determining death due to H_2S intoxication.

Keywords Forensic toxicology · Hydrogen sulfide · Thiosulfate · Postmortem change

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Introduction

Hydrogen sulfide (H_2S) is a colorless, water-soluble irritant gas with a specific gravity of 1.19 (vs. air) and has the characteristic odor of rotten eggs [1]. H_2S is highly toxic, and accidental intoxication can occur due to exposure to natural, volcanic, waste, or industrial gases [2–8], or from ingestion of sulfur products [9, 10]. Recently in Japan, there have been scattered cases of suicide by mixing domestic chemicals (e.g., a liquid bath essence and a toilet bowl cleaner, containing sulfur and hydrochloric acid, respectively) to produce H_2S. In such cases, a delay in the recovery of bodies may interfere with toxicological investigations due to postmortem changes involving the diffusion of H_2S and putrefaction. Exogenous sulfide is, in part, rapidly oxidized to thiosulfate (TS) by hemoglobin and liver enzymes, and TS is excreted through the kidneys [11], while a portion is excreted unchanged by the lungs as H_2S [12, 13]. Previous studies suggested that blood and tissue TS contents are normally very low without any significant influence of postmortem changes involving putrefaction [12, 14]; TS has been believed to be an indicator of antemortem H_2S exposure [2–4, 12, 15–18]. TS in the urine is also considered useful for this purpose [19, 20]; however, H_2S may be oxidized to TS nonenzymatically by

hemoglobin or enzymatically by bacteria during early and late postmortem periods [21, 22].

The present report describes four cases of fatal inhalation of H_2S that occurred after mixing domestic chemicals, and composes the pathological and toxicological findings with a toxicological control study.

Toxicological analysis

Alcohol analysis

Ethanol was analyzed by automated head-space gas chromatography–mass spectrometry (GC–MS): apparatus, Shimadzu QP 5000 GC–MS combined with a head-space gas sampler; column, DB-624 (60 m × 0.32 mm i.d., film 1.8 μm); column temperature, 60–150 °C; injector temperature, 150 °C; carrier gas, He; flow rate, 34 cm/s; split, 1:30; interface temperature, 230 °C. One gram of each sample was mixed with internal standard solution (0.5 mg/ml *t*-butanol, 1 ml), incubated at 60 °C for 30 min, and analyzed by GC–MS with monitoring at *m/z* 45.10 for ethanol and *m/z* 59.05 for butanol. Calibration was achieved using standard ethanol solutions (Wako Pure Chemicals, Osaka). The lower detection limit of ethanol was 0.001 mg/g, and the diurnal and interday precision was ±5 % [23].

Analysis of hydrogen sulfide and thiosulfate

Sample preparation

All samples were analyzed using published methods [9, 24]. Sulfide was detected as bis(pentafluorobenzyl)sulfide ($C_6F_5CH_2SCH_2C_6F_5$), as follows: 0.2 ml (or g) of the sample was added to a mixture of 0.5 ml of 20 mM pentafluorobenzyl bromide (PFBBr) solution in ethyl acetate, 100 μl of internal standard (IS) solution [200 μM 1,3,5-tribromobenzene (TBB) in ethyl acetate], and 0.8 ml of 5 mM tetradecyldimethylbenzylammonium chloride (TDMBA) solution in oxygen-free water saturated with sodium tetraborate. The preparation was vortexed for 1 min, and 0.1 g of potassium dihydrogenphosphate was added to the mixture as a buffer to prevent excessive alkylation by tissue protein. The preparation was again vortexed for 10 s and centrifuged at 2500 rpm for 15 min. An aliquot of the organic phase was injected into the GC–MS apparatus.

Thiosulfate in the heart blood, pericardial fluid, and bone marrow aspirate were quantified by a previously described procedure using gas chromatography–mass spectrometry (GC–MS) [17]. The derivatization of thiosulfate was performed with pentafluorobenzyl bromide: to 0.2 ml of heart blood, pericardial fluid, or bone marrow aspirate, was added a mixture of 0.05 ml of 0.2 M L-ascorbic acid solution, 0.05 ml of 5 % sodium chloride, and 0.05 ml of 0.02 M pentafluorobenzyl bromide in acetone in a 3-ml centrifuge tube. The mixture was vortexed for 1 min, and 2 ml of 0.025 M iodine in ethyl acetate and 0.05 ml of IS solution (0.04 mM 1,3,5-tribromobenzene in ethyl acetate) were added to the mixture. The preparation was then vortexed for 30 s and centrifuged at 2500 rpm for 15 min. An aliquot of the organic phase was injected into the GC–MS apparatus.

GC–MS conditions

GC–MS was carried out on a Shimadzu QP 5000 GC–MS (Kyoto, Japan) with a J&W DB-1 (15 m × 0.25 mm i.d., 0.25 μm film thickness) fused silica capillary column (Agilent, Santa Clara, CA, USA). A splitless injection mode was selected with a valve off-time of 1 min. The initial temperature of the column was held at 100 °C for 2 min, then elevated at 10 °C/min to 220 °C. The injection port, separator, and ion source were kept at 230, 240, and 210 °C, respectively. Helium was used as the carrier gas at a flow rate of 2 ml/min. The ionization energy was 70 eV [17]. The lower detection limits for sulfide and thiosulfate were 0.001 and 0.003 μmol/ml, respectively.

Calibration curve

GC–MS in scan mode was used for both identification and quantitation of sulfide and thiosulfate. The calibration curve for sulfide was obtained by plotting the peak area ratio of the molecular ion (*m/z* 394) of TBB against the sulfide concentration, using mass chromatography. The calibration curve for thiosulfate was obtained in a similar manner using the molecular ion (*m/z* 426) of the derivative of thiosulfate [17].

Case reports

Case 1

Case history

A 32-year-old man, who committed suicide and left a note, was found dead supine on the bathroom floor. There was a bucket containing powder and an aqueous liquid, and bottles of liquid bath essence containing sulfur (610HAP) and toilet bowl cleaner containing 9.5 % HCl (Sunpole) were nearby. An "unidentifiable gas" was detected at a concentration of 230 ppm in the ambient air by police investigators using a gas detector. The postmortem interval was estimated to be about 24 h at the time of discovery, based on

circumstantial and physical evidence. A forensic autopsy was performed about 24 h later (about 48 h postmortem).

Autopsy findings

The body was 170 cm in height and weighed 88.0 kg. Bright red to dark reddish-purple hypostasis was seen on the back, with greenish discoloration on part of his buttocks and lower limbs, surrounding the regions in contact with the floor. There were a few bruises in the regions of the scapula, without any other evidence of injury. The conjunctivae palpebrae and oral mucosa were hyperemic with a few petechiae. The conjunctivae bulbi were also hyperemic. The heart (440 g) contained a large amount of dark reddish fluid blood. Bilateral lungs (left, 630 g; right, 670 g) were inflated and congested with pleural effusion (about 7 ml/30 ml in the left/right). Submucosal hemorrhages were evident in the trachea and bronchi. The brain (1680 g,) was markedly swollen and edematous. There was no pathology other than congestion in other viscera. Discoloration was not seen in the viscera.

On histological examination, the lungs showed marked congestion and edema. Immunohistochemistry of pulmonary surfactant-associated protein A (SP-A) [25, 26] showed intense positivity for the intra-alveolar granular pattern (respiratory distress), the membranous pattern on the intra-alveolar interior surface of alveoli, and on the interface of intra-alveolar effusion (alveolar injury). Cardiomyocytes and cerebrum neurons presented with acidophilic change. The skin and skeletal muscle with discoloration showed decreased H&E staining, and the skeletal muscle showed varying changes. No other pathology, except for congestion, was detected in other viscera.

Toxicological findings

Drug screening proved negative, while low levels of blood alcohol (0.31 and 0.03 mg/ml in the left and right cardiac blood, respectively) were detected, but *n*-propanol was not detected. Carboxyhemoglobin (COHb) and methemoglobin (MetHb) saturation in the left/right cardiac blood, as measured by an OSM3 CO-oxymeter (Radiometer, Westlake, OH, USA) [27], was 6.2 %/5.5 % and 1.3 %/1.3 %, respectively, without significant elevation of sulfhemoglobin (SulfHb) (<1 %).

Using GC–MS, H_2S and TS concentrations in the blood were found to be 30.0 and 3.55 μg/ml in the left cardiac blood, 2.71 and 1.12 μg/ml in the right cardiac blood, and 1.65 and 1.68 μg/ml in the peripheral iliac venous blood, respectively (urine was not available) (Table 1a). Pericardial H_2S and TS contents were 0.48 and 4.42 μg/ml, respectively; cerebrospinal H_2S and TS contents were 0.32 and 5.34 μg/ml, respectively.

H_2S/TS contents in the skin and muscle were higher at the site of discoloration (skin: 0.55 and 5.98 μg/ml; muscle: 13.7 and 2.71 μg/ml, respectively) than at intact sites (skin: 0.21 μg/ml and <quantitative assay limit; muscle: 1.69 μg/ml and <quantitative assay limit, respectively). H_2S and TS contents in other tissues were 0.13–1.46 and 0.00–0.94 μg/ml, respectively. Further details are shown in Table 1a and b.

Case 2

Case history

An 18-year-old woman, who committed suicide and left a note, was found dead prone in a bathtub. A bucket containing a brownish aqueous liquid, and bottles of liquid bath essence containing sulfur (610HAP) and toilet bowl cleaner containing 9.5 % HCl (Sunpole) were found nearby. H_2S was detected at a concentration of 110 ppm in the ambient air using a gas detector. The postmortem interval was estimated to be about 15 h at the time of discovery, based on circumstantial and physical evidence. A forensic autopsy was performed about 15 h later (about 30 h postmortem).

Autopsy findings

The body was 163 cm in height and weighed 62.4 kg. Dark reddish-purple hypostasis was seen on the front, without any greenish discoloration. There were a few bruises on the face, without any other evidence of injury. The conjunctivae palpebrae and bulbi, and the oral mucosa were hyperemic without petechiae. The heart (280 g) contained a large amount of dark reddish fluid blood. The lungs (left, 370 g; right, 450 g) were markedly inflated and congested with pleural effusion (about 5 ml/3 ml in the left/right). There was a small amount of milky white viscous, partly foamy fluid in the hyperemic trachea and bronchi. The brain (1435 g) was swollen and edematous. There was no pathology other than congestion in other viscera. Discoloration was not seen in the viscera.

On histological examination, the lungs showed marked congestion and edema. Pulmonary SP-A immunostaining demonstrated findings of respiratory distress and alveolar injury, as described above. Cardiomyocytes and cerebrum neurons showed acidophilic change. No pathology, except for congestion, was detected in other viscera.

Toxicological findings

Drug screening proved negative, while low blood alcohol levels (0.24 and 0.13 mg/ml in the left and right cardiac blood, respectively) were detected, but *n*-propanol was not

Table 1 Hydrogen sulfide and thiosulfate concentrations in body fluids (a) and tissues (b)

	Hydrogen sulfide (μg/ml)								Thiosulfate (μg/ml)							
	Case no.		Nonputrefactive control		Case no.		Putrefactive control		Case no.		Nonputrefactive control		Case no.		Putrefactive control	
	1	2	C-1	C-2	3	4	C-3	C-4	1	2	C-1	C-2	3	4	C-3	C-4
Postmortem interval	48 h	30 h	24 h	29 h	72 h	72 h	7 days	12 days	48 h	30 h	24 h	29 h	72 h	72 h	7 days	12 days
Ambient temperature (°C)	9	16	31	29	25	25	16	19	9	16	31	29	25	25	16	19
a) Body fluids																
Sample group I																
Vitreous body (left)	0.07	0.00	0.00	0.00	0.55	0.00	–	–	7.05	3.35	0.00	0.00	12.0	1.74	–	–
Vitreous body (right)	0.07	0.00	0.00	0 00	0.54	0.00	–	–	6.71	1.19	0.00	0.00	41.7	2.40	–	–
Heart blood (left)	30.0	0.66	0.05	0.03	59.6	1.52	0.62	0.54	3.55	1.71	1.03	0.09	369	0.00	10.0	4.33
Heart blood (right)	2.71	0.81	0.09	0.06	85.0	3.75	1.27	0.36	1.12	1.54	1.25	0.18	200	2.20	9.32	3.31
Pericardial fluid	0.48	0.38	0.03	–	12.0	1.11	1.85	0.79	4.42	2.16	0.01	–	390	2.74	19.6	3.72
Sample group II																
Iliac vein	1.65	–	0.12	0.05	–	–	–	–	1.68	–	0.82	0.31	–	–	–	–
Bile	–	–	0.01	0.08	0.80	0.04	0.14	–	–	–	1.45	0.49	26.7	0.00	7.85	–
Cerebrospinal fluid	0.32	0.13	0.12	0.00	–	–	–	–	5.34	1.19	0.93	0.02	–	–	–	–
Bone marrow	1.00	3.50	–	–	61.6	1.57	0.23	–	0.77	0.00	–	–	320	0.00	8.26	–
Gastric content	0.00	1.47	0.20	1.29	0.67	1.12	1.45	0.21	0.00	0.00	1.34	8.06	180	0.00	18.6	0.55
Urine	–	0.41	0.11	–	8.47	17.7	1.08	0.49	–	0.00	2.96	–	57.59	7.49	13.0	1.77
b) Tissues																
Sample group I																
Brain	0.51	2.50	0.04	0.32	12.7	4.00	0.52	0.44	0.49	0.83	0.96	0.52	28.0	0.00	18.0	7.64
Lung	0.86	1.25	0.06	0.06	26.0	0.98	1.66	0.64	0.94	1.12	0.27	0.06	480	0.00	41.0	4.33
Sample group II																
Heart	0.93	1.62	0.29	0.19	4.25	2.72	0.62	0.10	0.00	0.35	1.80	0.99	29.0	0.00	4.96	1.39
Liver	0.66	2.56	0.37	0.80	4.54	3.02	0.44	0.71	0.00	0.85	1.86	7.17	9.74	0.00	5.58	2.23
Kidney	1.46	4.24	0.24	–	11.7	2.01	0.95	0.55	0.00	0.80	0.56	–	10.0	0.00	9.45	2.41
Spleen	0.90	–	0.43	0.12	3.53	0.74	0.95	0.33	0.00	–	1.88	0.64	6.55	0.00	30.9	4.71
Pancreas	0.34	2.24	0.07	0.07	0.00	0.00	0.36	0.01	0.00	0.77	0.83	1.03	0.00	0.00	5.27	0.89
Intestine	0.13	–	–	0.26	8.68	0.39	0.70	–	0.00	–	–	2.29	0.00	0.00	7.13	–
Skin																
Normal color	0.21	–	0.03	0.02	0.59	0.60	–	–	0.00	–	0.38	1.54	0.00	0.00	–	–
Discoloration	0.55	–	–	–	2.46	1.02	0.06	0.37	5.98	–	–	–	20.7	10.0	19.0	8.35
Skeletal muscle																
Normal color	1.69	–	1.11	0.13	3.39	0.35	–	–	0.00	–	3.71	0.40	5.70	0.00	–	–
Discoloration	13.7	1.22	–	–	2.58	0.97	0.73	0.20	2.71	1.87	–	–	13.0	7.35	15.0	0.61

detected. COHb and MetHb saturation in the left/right cardiac blood, measured as above, were 3.4 %/3.3 % and 1.6 %/1.5 %, respectively, without significant elevation of SulfHb (<1 %).

Using GC–MS as above, H_2S and TS concentrations in the blood and urine were 0.66 and 1.71 μg/ml in the left cardiac blood, 0.81 and 1.54 μg/ml in the right cardiac blood (peripheral blood was not available), and 0.41 μg/ml and <quantitative assay limit in the urine, respectively (Table 1a). Pericardial and cerebrospinal H_2S/TS contents were 0.38 μg/ml/2.16 μg/ml and 0.13 μg/ml/1.19 μg/ml, respectively. Tissue H_2S and TS contents were 1.22–4.24 and 0.35–1.87 μg/ml, respectively. Further details are shown in Table 1a and b.

Case 3

Case history

A 23-year-old woman, who committed suicide and left a note, was found dead prone in a bathtub. A bucket containing aqueous liquid was found nearby, and bottles of liquid bath essence containing sulfur (610HAP) and toilet bowl cleaner containing 9.5 % HCl (Sunpole) were found in another room. The ambient air was not examined. The postmortem interval was estimated to be about 2 days at the time of discovery, based on circumstantial and physical evidence. A forensic autopsy was performed about 18 h later (about 3 days postmortem).

Autopsy findings

The body was 160 cm in height and weighed 52.3 kg. Dark reddish-purple hypostasis was seen on the front, with dark gray-greenish discoloration on the face, chest, and upper limbs, excluding areas in contact with the bottom of the bathtub. There was no evidence of injury. The conjunctivae palpebrae and bulbi were hyperemic with petechiae. Oral mucosa was dark greenish. The heart (205 g) contained a large amount of dark reddish fluid blood with soft clots. The lungs (left, 395 g; right, 380 g) were markedly inflated and congested with pleural effusion (about 50 ml/20 ml in the left/right), showing dark gray-greenish discoloration, which was also seen throughout the airways with a small amount of dark gray-greenish, partly foamy viscous fluid. The brain (1390 g) was swollen and edematous, with gray-greenish discoloration on the brain surfaces, excluding the brainstem. There was no pathology other than congestion or discoloration in other viscera.

On histological examination, the lungs showed marked congestion and edema. Pulmonary SP-A immunostaining demonstrated the findings of respiratory distress and alveolar injury, as described above. Decomposition was not evident in the airways and lungs. No other pathology, except for congestion, was detected in other viscera.

Toxicological findings

Drug screening proved negative, as did tests for blood alcohol and *n*-propanol. COHb and MetHb saturations in the left/right cardiac blood, measured as above, were 5.3 %/2.1 % and 4.9 %/2.0 %, respectively, with a high SulfHb saturation (>1 %).

Using GC–MS as above, H_2S and TS concentrations in the blood and urine were 59.6 and 369 μg/ml in the left cardiac blood, 85.0 and 200 μg/ml in the right cardiac blood (peripheral blood was not available), and 8.47 and 57.59 μg/ml in the urine, respectively (Table 1a). Pericardial H_2S and TS contents were 12.0 and 390 μg/ml, respectively.

H_2S and TS contents in the skin and muscle were mostly higher at the sites of discoloration (skin: 2.46 and 20.7 μg/ml; muscle: 2.58 and 13.0 μg/ml, respectively) than at intact sites (skin: 0.59 μg/ml and <quantitative assay limit; muscle: 3.39 and 5.70 μg/ml, respectively). H_2S and TS contents in other tissues were 0.00–26.0 and 0.00–480 μg/ml, respectively. Further details are shown in Table 1a and b.

Case 4

Case history

A 25-year-old man was found dead prone in a bathtub, lying on his wife, who had committed suicide (case 3). The postmortem interval was estimated to be about 2 days at the time of discovery, based on circumstantial and physical evidence. a forensic autopsy was performed about 18 h later (about 3 days postmortem).

Autopsy findings

The body was 180 cm in height and weighed 79.0 kg. Dark reddish hypostasis was seen on the front, with greenish discoloration around the right elbow, excluding the area in contact with the bathtub. There were a few bruises in the regions of the chest and right upper arm, without any other evidence of injury. The conjunctivae palpebrae and bulbi, and the oral mucosa were hyperemic with petechiae. The heart (350 g) contained a large amount of dark reddish fluid blood. The lungs (left, 480 g; right, 560 g) were markedly inflated and congested with pleural effusion (about 20 ml each in the left and right). The airways were hyperemic. The brain (1520 g) was swollen and edematous. There was no pathology other than congestion in other viscera. Discoloration was not seen in the viscera.

On histological examination, the lungs showed marked congestion and edema. Pulmonary SP-A immunostaining demonstrated the findings of respiratory distress and alveolar injury, as described above. No other pathology, except for congestion, was detected in other viscera.

Toxicological findings

Drug screening proved negative, as did tests for blood alcohol and *n*-propanol. COHb and MetHb saturation in the left/right cardiac blood, measured as above, was 4.2/3.5 % and 1.9/1.4 %, respectively, without significant elevation of SulfHb (<1 %).

Using GC–MS as above, H_2S and TS concentrations in the blood and urine were 1.52 and 0.00 μg/ml (below the quantitation limit) in the left cardiac blood, 3.75 and 2.20 μg/ml in the right cardiac blood (peripheral blood was not available), and 17.7 and 7.49 μg/ml in the urine, respectively (Table 1a). Pericardial H_2S and TS contents were 1.11 and 2.74 μg/ml, respectively.

H_2S and TS contents in the skin and muscle were higher at the sites of discoloration (skin: 1.02 and 10.0 μg/ml; muscle: 0.97 and 7.35 μg/ml, respectively) than at intact sites (skin: 0.60 μg/ml and <quantitative assay limit; muscle: 0.35 μg/ml and <quantitative assay limit, respectively). H_2S and TS contents in other tissues were 0.00–4.00 and 0.00 μg/ml, respectively. Further details are shown in Table 1a and b.

Toxicological control study

Nonputrefactive control cases

In nonputrefactive control cases, blood alcohol or *n*-propanol was not detected. Using GC–MS as above, H_2S and TS concentrations in the blood and other body fluids for two control cases without any putrefactive changes (postmortem interval, about 24 and 29 h) ranged from 0.00 to 0.12 μg/ml and from 0.00 to 2.96 μg/ml, respectively. Tissue H_2S and TS contents were 0.03–1.11 and 0.06–7.17 μg/ml, respectively. Further details are shown in Table 1a and b.

Putrefactive control cases

In putrefactive control cases, a low level of blood alcohol (0.20 and 0.66 mg/ml in the left cardiac blood, respectively) was detected, and *n*-propanol was detected in the left cardiac blood in one case (0.07 mg/ml in the latter case). For two control cases with evident putrefactive discoloration (postmortem interval, about 7 and 12 days), H_2S and TS concentrations in the blood and other body fluids ranged from 0.14 to 1.85 μg/ml and from 1.77 to 19.6 μg/ml, respectively. Tissue H_2S and TS contents were 0.01–1.66 and 0.61–41.0 μg/ml, respectively. Further details are shown in Table 1a and b. Differences between putrefactive and nonputrefactive control cases were detected for sample group I, but were not apparent for sample group II.

Discussion

Previous case reports of H_2S intoxication showed significant increases of H_2S and TS contents in blood, other body fluids, urine, and tissues to be diagnostic indications [10, 28]; an increase in urinary TS level has been regarded as a sign of antemortem H_2S exposure [19, 20]. Marked pulmonary edema and congestion were common pathological findings [29]. However, several arguments remain with regard to blood SulfHb formation and greenish discoloration of the skin and viscera in the context of differentiation from putrefaction and the postmortem influence of H_2S exposure; SulfHb formation and greenish discoloration of the skin and/or viscera are not so common in H_2S intoxication cases [30].

The present four cases pathologically showed evident pulmonary edema and congestion without any other particular findings of injury or disease, while toxicological investigations demonstrated fatal H_2S and TS concentrations in the blood, other body fluids, and tissues. High concentrations of H_2S were detected in the heart blood and lung in these cases in this study as compared with those of the nonputrefactive control cases, which were as high as those previously reported for H_2S intoxication [31]. Moreover, higher concentrations of H_2S were detected in the pericardial fluid as compared with those detected in nonputrefactive control cases. In addition, TS concentration was also high in the vitreous body, blood, pericardial fluid, and lung. From these findings, the cause of death was established as acute H_2S intoxication. Marked pulmonary edema and congestion, and pulmonary SP-A immunostaining patterns suggested acute pulmonary alveolar injury and respiratory distress due to H_2S inhalation [25, 26]. In one case (case 3), however, a substantial amount of cardiac blood clots suggested a subacute death process.

On toxicological investigation, the present control study using cases without H_2S intoxication or putrefaction demonstrated lower H_2S and TS contents in the blood, body fluids, and tissues, mostly <0.12 and 1.45 μg/ml, respectively. These H_2S and TS contents were similar to those in previous reports [32]. However, the specimens from cases with putrefaction often had higher H_2S contents of >0.5 μg/ml, and also showed an unexpected increase in TS contents of >4.0 μg/ml. Although ambient temperature

above 20 °C can greatly affect putrefaction [21], blood *n*-propanol was not detected in nonputrefactive control cases, suggesting insignificant influence of decomposition. These findings suggest an increase in H_2S contents, accompanied by subsequent conversion to TS, due to postmortem changes involving putrefaction. Thus, increased TS contents cannot immediately be a reliable finding of antemortem exposure to H_2S gas. It was difficult to discriminate putrefaction using H_2S and TS measurements, especially in gastric contents, bone marrow, heart, liver, kidney, spleen, pancreas, and intestine; these materials may be inadequate for toxicological analysis of H_2S. Moreover, collection of cerebrospinal fluid, iliac vein or bile may be difficult in the putrefactive cases. This case report suggested that vitreous body, pericardial fluid, and lung tissue may be used as supplementary or alternative samples.

For the present cases of H_2S intoxication, toxicological investigation showed that H_2S and TS concentrations in cardiac blood varied by case (0.66–85.0 and 0.00–369 μg/ml, respectively), and similar distributions were seen in other body fluids and tissues. These concentrations were evidently high in the airways, and in areas of lung and brain discoloration (case 3). In these cases, higher H_2S concentrations were detected in the heart blood as compared with those in nonputrefactive control cases. TS concentration was also high in the heart blood and lung, except for case 4, although the reason for low TS concentrations in some samples is unknown. This suggests that the victim inhaled H_2S gas in a very high concentration. Nevertheless, cardiac blood clots as a pathological sign of subacute death suggest a substantial period of agony until death, although it is believed that inhalation of H_2S in a high concentration causes almost instantaneous paralysis of the central nervous system followed by an immediate collapse and fatal respiratory arrest [33]. In this case of marked dark gray-greenish skin and visceral discoloration, however, TS content (20.7 μg/ml) was higher than H_2S content (2.46 μg/ml). This difference was similar to that in control cases with putrefaction, suggesting some influence of putrefaction on the toxicological analysis. Case 4 (the husband of case 3) also had increased H_2S contents in the blood, urine, and tissues, but TS content was low, showing a pathological sign of acute death; thus, his survival time could have been shorter than that of his wife. For this victim, however, the lower TS content suggested that the influence of putrefaction was not significant despite a similar postmortem period, as indicated by the circumstantial evidence.

The other two cases (cases 1 and 2) showed similar toxicological findings, suggesting acute death following inhalation of H_2S in a high concentration. For case 1, TS content was lower than H_2S content, without significant findings of putrefaction in accordance with the relatively shorter postmortem interval (approximately <48 h).

Among the four cases, greenish discoloration of the skin was seen in part in two cases (cases 1 and 4) and was observed widely in case 3, which showed discoloration of the airways, lungs, and brain. In these cases, skin discoloration appeared in the lower parts of the body, excluding the regions that had been in contact with the surrounding objects, partly overlapping with hypostasis, and the H_2S contents in the skin and muscle were higher at sites of discoloration than at intact sites. This suggests that discoloration occurs due to the postmortem infiltration of a heavy H_2S gas that accumulates downwards. However, the skin and muscle tissues contained also larger amounts of TS at the sites of discoloration, suggesting that H_2S can be converted to TS in the presence of oxyhemoglobin even after death, before the appearance of putrefaction [34]. With respect to these findings, further detailed investigation is needed in consideration of the difference due to sampling site and procedure [21]. With regard to discoloration of the airways, lungs, and brain surfaces (case 3), a similar postmortem influence of H_2S gas may be considered; thus, nonputrefactive greenish discoloration may indicate the postmortem influence of H_2S gas, but careful interpretation of the toxicological findings is needed for specimens with typical discoloration.

Previous reports have described that SulfHb and MetHb are not produced in measurable quantities in the blood of victims of acute H_2S intoxication [34]. However, some investigations showed that SulfHb could be formed when the victims were exposed to H_2S gas in extremely high concentrations (>4000 ppm) [35]. In the present cases, a high SulfHb result (>1 %) was detected in only one case of a markedly high blood H_2S concentration (case 3) by a CO-oximeter. This victim might have inhaled H_2S gas in a very high concentration, and subsequent postmortem exposure to the gas might have caused typical greenish discoloration of the skin and viscera.

In conclusion, the present cases suggest a significant contribution of postmortem exposure to H_2S gas to the pathological and toxicological findings, depending on the circumstances. A control study demonstrated an unexpected increase of TS contents along with H_2S production due to putrefaction; thus, increased TS content cannot immediately be a reliable indication of H_2S intoxication. The findings of this case report suggest that vitreous body, pericardial fluid, and lung tissue may be used as supplementary or alternative samples for H_2S and TS measurements. Careful interpretation of the toxicological findings is needed for tissue specimens with typical discoloration; indeed, systemic toxicological analysis is needed to determine death due to H_2S intoxication.

References

1. Reiffenstein RJ, Hulbert WC, Roth SH (1992) Toxicology of hydrogen sulfide. Annu Rev Pharmacol Toxicol 32:109–134. doi:10.1146/annurev.pa.32.040192.000545
2. Snyder JW, Safir EF, Summerville GP, Middleberg RA (1995) Occupational fatality and persistent neurological sequelae after mass exposure to hydrogen sulfide. Am J Emerg Med 13:199–203. doi:10.1016/0735-6757(95)90094-2
3. Kage S, Takekawa K, Kurosaki K, Imamura T, Kudo K (1997) The usefulness of thiosulfate as an indicator of hydrogen sulfide poisoning: three cases. Int J Legal Med 110:220–222. doi:10.1007/s004140050071
4. Kage S, Ikeda H, Ikeda N, Tsujita A, Kudo K (2004) Fatal hydrogen sulfide poisoning at a dye works. Leg Med 6:182–186. doi:10.1016/j.legalmed.2004.04.004
5. Knight LD, Presnell SE (2005) Death by sewer gas: case report of a double fatality and review of the literature. Am J Forensic Med Pathol 26:181–185. doi:10.1097/01.paf.0000163834.87968.08
6. Tanaka S, Fujimoto S, Tamagaki Y, Wakayama K, Shimada K, Yoshikawa J (1999) Bronchial injury and pulmonary edema caused by hydrogen sulfide poisoning. Am J Emerg Med 17:427–429. doi:10.1016/S0735-6757(99)90102-X
7. Gabbay DS, De Roos F, Perrone J (2011) Twenty-foot fall averts fatality from massive hydrogen sulfide exposure. J Emerg Med 20:141–144. doi:10.1016/S0736-4679(00)00301-2
8. Bartholomew TC, Powell GM, Dodgson KS, Curtis CG (1980) Oxidation of sodium sulphide by rat liver, lungs and kidney. Biochem Pharmacol 29:2431–2437. doi:10.1016/0006-2952(80)90346-9
9. Kage S, Nagata T, Kudo K (1991) Determination of thiosulfate in body fluids by GC and GC/MS. J Anal Toxicol 15:148–150
10. Nagata T, Kage S, Kimura K, Kudo K, Imamura T (1994) How to diagnose polysulphide poisoning from tissue samples. Int J Legal Med 106:288–290
11. Beauchamp RP Jr, Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA (1984) A critical review of the literature on hydrogen sulfide toxicity. Crit Rev Toxicol 13:25–97. doi:10.3109/10408448409029321
12. Kage S, Kudo K, Ikeda N (1998) Determination of sulfide, thiosulfate and polysulfides in biological materials for diagnosis of sulfide poisoning. Jpn J Forensic Toxicol 16:179–189
13. Dorman DC, Moulin FJ, McManus BE, Mahle KC, James RA, Struve MF (2002) Cytochrome oxidase inhibition induced by acute hydrogen sulfide inhalation: correlation with tissue sulfide concentrations in the rat brain, liver, lung, and nasal epithelium. Toxicol Sci 65:18–25. doi:10.1093/toxsci/65.1.18
14. Kågedal B, Källberg M, Mårtensson J, Sörbo B (1983) Reversed-phase ion-pair liquid chromatographic procedure with electrochemical detection for the analysis of urinary thiosulphate. J Chromatogr 274:95–102
15. Kangas J, Savolainen H (1987) Urinary thiosulphate as an indicator of exposure to hydrogen sulphide vapour. Clin Chim Acta 164:7–10. doi:10.1016/0009-8981(87)90101-X
16. Kage S, Nagata K, Kimura K, Kudo K, Imamura T (1992) Usefulness of thiosulfate as an indicator of hydrogen sulfide poisoning in forensic toxicological examination: a study with animal experiments. Jpn J Forensic Toxicol 10:223–227
17. Kage S, Ito S, Kishida T, Kudo K, Ikeda N (1998) A fatal case of hydrogen sulfide poisoning in a geothermal power plant. J Forensic Sci 43:908–910
18. Kage S, Kashimura S, Ikeda H, Kudo K, Ikeda N (2002) Fatal and nonfatal poisoning by hydrogen sulfide at an industrial waste site. J Forensic Sci 47:652–655
19. Torun M, Bayhan A, Yentür G (1989) Response of allergic and normal persons to sulfiting agents in wine: determination of thiosulfate excretion in urine. Clin Chem 35:1792–1793
20. Voroteliak V, Cowley DM, Florin TH (1993) Improved colorimetric determination of urinary thiosulfate to study intermediate sulfur metabolism in humans. Clin Chem 39:2533–2534
21. Nagata T, Kage S, Kimura K, Kudo K, Noda M (1990) Sulfide concentrations in postmortem mammalian tissues. J Forensic Sci 35:706–712
22. Dubinina G, Grabovich M, Leshcheva N, Rainey FA, Gavrish E (2011) Spirochaeta perfilievii sp. nov., oxygen-tolerant, sulfide oxidizing, sulfur and thiosulfate-reducing spirochete isolated from a saline spring. Int J Syst Evol Microbiol 61:110–117. doi:10.1099/ijs.0.018333-0
23. Maeda H, Zhu BL, Ishikawa T, Oritani S, Michiue T, Li DR, Zhao D, Ogawa M (2006) Evaluation of postmortem ethanol concentrations in pericardilal fluid and bone marrow aspirate. Forensic Sci Int 161:141–143. doi:10.1016/j.forsciint.2006.01.016
24. Kage S, Nagata T, Kimura K, Kudo K (1988) Extractive alkylation and gas chromatographic analysis of sulfide. J Forensic Sci 33:217–222
25. Zhu BL, Ishida K, Quan L, Fujita MQ, Maeda H (2000) Immunohistochemistry of pulmonary surfactant protein A in forensic autopsy: reassessment in relation to the causes of death. Forensic Sci Int 113:193–197. doi:10.1016/S0379-0738(00)00264-4
26. Zhu BL, Ishida K, Fujita MQ, Maeda H (2000) Immunohistochemical investigation of a pulmonary surfactant in fatal mechanical asphyxia. Int J Legal Med 113:268–271. doi:10.1007/s004149900109
27. Wu C, Kenny MA (1997) A case of sulfhemoglobinemia and emergency measurement of sulfhemoglobin with an OSM3 CO-oximeter. Clin Chem 43:162–166
28. Imamura T, Kage S, Kudo K, Jitsufuchi N, Nagata T (1996) A case of drowning linked to ingested sulfides—a report with animal experiments. Int J Legal Med 109:42–44
29. Winek CL, Collom WD, Wecht CH (1968) Death from hydrogen-sulphide fumes. Lancet 18:1096
30. Tangerman A, Bongaerts G, Agbeko R, Semmekrot B, Severijnen R (2002) The origin of hydrogen sulfide in a newborn with sulfhaemoglobin induced cyanosis. J Clin Pathol 55:631–633. doi:101136/jcp558631
31. Yajima Y, Funayama M, Yamamoto Y, Hashiyada M, Nata M, Nishi K (2003) An autopsy case of hydrogen sulfide poisoning in a fish feed processing factory. Res Pract Forensic Med 46:199–202
32. Togawa T, Ogawa M, Nawata M, Ogasawara Y, Kawanabe K, Tanabe S (1992) High performance liquid chromatographic determination of bound sulfide and sulfite and thiosulfate at their low levels in human serum by pre-column fluorescence derivatization with monobromobimane. Chem Pharm Bull 40:3000–3004
33. Kombian SB, Warenycia MW, Mele FG, Reiffenstein RJ (1988) Effects of acute intoxication with hydrogen sulfide on central amino acid transmitter systems. Neurotoxicology 9:587–595
34. Adelson L, Sunshine I (1966) Fatal hydrogen sulfide intoxication. Report of three cases occurring in a sewer. Arch Pathol 81:375–380
35. Adachi J, Tatsuno Y, Fukunaga T, Ueno Y, Kogame M, Mizoi Y (1986) Formation of sulfhemoglobin in the blood and skin caused by hydrogen sulfide poisoning and putrefaction of the cadaver. Nihon Hoigaku Zasshi 40:316–322 (Japanese)