CASE REPORT

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A Fatal Case of Hydrogen Sulfide Poisoning in a Geothermal Power Plant

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ABSTRACT: An adult man entered an oil separator room to remove waste oil from a vacuum pump in a geothermal power plant. He suddenly collapsed and died soon after. Since hydrogen sulfide gas was detected in the atmosphere at the scene of the accident, poisoning by this gas was suspected and toxicological analysis of sulfide and thiosulfate in blood, brain, lung, femoral muscle was made using the extractive alkylation technique combined with gas chromatography/mass spectrometry (GC/MS). The concentrations of sulfide in these tissues were similar to those previously reported for fatal cases of hydrogen sulfide gas. The concentration of thiosulfate in the blood was at least 48 times higher than the level in control samples. Based on these results, the cause of death was attributed to hydrogen sulfide gas poisoning.

KEYWORDS: forensic science, forensic toxicology, hydrogen sulfide, thiosulfate, metabolite, analysis, gas chromatography/mass spectrometry

Hydrogen sulfide poisoning is frequently encountered in the practice of forensic science. Although identification of sulfide can be made in cases of fatal poisoning (1–7), detection of sulfide in nonfatal cases can be difficult because this compound is unstable and is rapidly metabolized within the human body (8–10). There are also problems such as postmortem production of sulfide due to putrefaction of blood and tissues (11). We reported that analysis of thiosulfate, a metabolite of sulfide, in blood, urine, brain and lung, was useful for confirming hydrogen sulfide poisoning in animal experiments (12), and also reported on the usefulness of thiosulfate in identifying this metabolite in human blood and urine in three cases of poisoning (7).

We report here a case of fatal hydrogen sulfide gas poisoning in a geothermal power plant, and also on evaluations of sulfide and thiosulfate concentrations in tissues that were made.

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Case Report

A 53-year-old man entered an oil separator room (length 4.8 m, width 3.6 m, height 2.9 m) to remove waste oil from a vacuum pump in a geothermal power plant. He suddenly collapsed and died soon after. An autopsy was carried out 20 h after death. The body was kept at 0°C until autopsy. The man was 163 cm tall and weighed 57 kg. There were some small bruises on the right arm but there was no other sign of trauma over the entire body. An obnoxious odor of rotten eggs and slight greenish discoloration of the surface of the brain were apparent. There were no other significant findings except for nonspecific congestion of all organs. Blood and tissues were collected and were kept at −20°C until analysis.

The concentrations of hydrogen sulfide and oxygen in the oil separator room were measured one week after the accident under the same conditions as those on the day of the accident. Measurements were made with a gas detector tube (Gastec Type No. 4HH 1000 to 20,000 ppm) and an XP-302II model gas analyzer (Shincosmos Electric Japan), and were found to be 3500 to 5000 ppm and 17 to 19%, respectively. The cause of the accident was as follows: The pipe (9 cm inside diameter, length 36 m) used to release exhaust gas containing high levels of hydrogen sulfide was plugged with dust and mud and the gas flowed backwards to the oil separator tank (length 3.3 m, width 1.3 m) set at the corner of the oil separator room through a drain tank (Fig. 1).

Toxicological Analysis

Procedures Used to Analyze Sulfide and Thiosulfate

Blood samples were analyzed using our published methods (13,14). All tissue samples were minced before analysis. Sulfide was detected as bis(pentafluorobenzyl)sulfide ($C_6F_5CH_2SCH_2$-C_6F_5), as follows: 0.2 mL (or g) of the sample was added to the mixture of 0.5 mL of 20 mM pentafluorobenzyl bromide (PFBBr) solution in toluene, 2.0 mL of internal standard (I.S.) solution (10 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

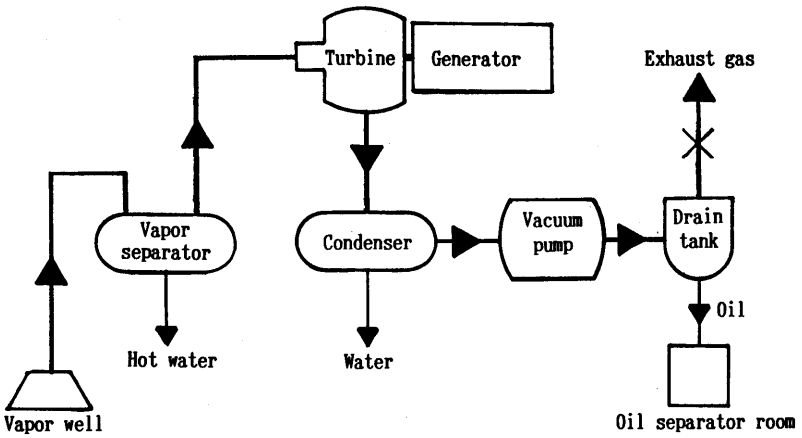

FIG. 1—*Setup of a geothermal power plant.*

again vortexed for 10 s and centrifuged at 2500 rpm for 10 min. An aliquot of the organic phase was injected onto a GC/MS apparatus.

Thiosulfate was detected as bis(pentafluorobenzyl)disulfide ($C_6F_5CH_2SSCH_2C_6F_5$), as follows: 0.2 mL (or g) of the sample was added to the mixture of 0.5 mL of 20 mM PFBBr solution in acetone, 0.05 mL of 200 mM L-ascorbic acid solution and 0.05 mL of 5% sodium chloride. The preparation was vortexed for 1 min, and 2 mL of 25 mM iodine solution in ethyl acetate and 0.5 mL of I.S. solution (40 μM TBB in ethyl acetate) were added to the mixture. The preparation was again vortexed for 30 s and the mixture was centrifuged at 2500 rpm for 15 min and allowed to stand for 1 h. An aliquot of the organic phase was injected onto a GC/MS apparatus.

GC/MS Conditions

GC/MS was carried out on a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA) interfaced to a JEOL Automass 150 mass spectrometer. The column was a Hewlett-Packard fused silica capillary tube of HP-5 (30 m × 0.32 mm inside diameter, 0.25 μm film thickness). A splitless injection mode was selected with a valve off-time of 1.0 min. The initial temperature of the column was held at 100°C for 2 min, then programmed at 10°C/min to 220°C. The injection port, separator and ion source were kept at 220, 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.

Preparation of Calibration Graphs

GC/MS in scan mode was used for both qualification and quantitation of sulfide and thiosulfate. Calibration curve for sulfide was obtained by plotting the peak area ratio of the molecular ion (*m/z* 394) of the derivative of sulfide to that (*m/z* 314) of TBB against the sulfide concentration, using mass chromatography. The calibration curve for thiosulfate was obtained in the same manner using the molecular ion (*m/z* 426) of the derivative of thiosulfate.

Results and Discussion

Figures 2 and 3 show the total ion chromatograms and mass chromatograms of the derivatized extracts for sulfide and thiosulfate obtained from blood samples of the victim. The peaks with retention times of 6.95, 9.75, 11.92 min were identified as I.S., the derivatives of sulfide, and thiosulfate, respectively.

Since we found that pertinent tissues for sulfide analysis were blood, brain, lung and femoral muscle from a site distant from the abdominal area (11), we studied these tissues. We also reported

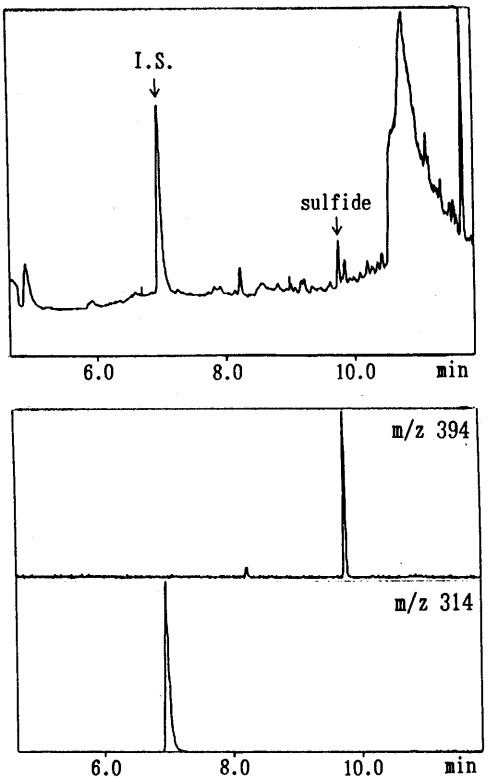

FIG. 2—*Total ion chromatogram and mass chromatograms of the derivatized extract for sulfide obtained from a blood sample of the victim:* (upper) *total ion chromatogram,* (lower) *mass chromatograms.*

that postmortem production of sulfide was inhibited at below 20°C and within 24 h until autopsy (11). In our case, the victim was kept at 0°C until autopsy, which was carried out 20 h after death. Therefore, the postmortem production of sulfide due to putrefaction of blood and tissue was considered to be sufficiently suppressed. The concentrations of sulfide in blood, brain, lung and femoral muscle were 0.014 μM/mL (0.45 μg/mL), 0.085 (2.72), 0.013 (0.42), 0.005 (0.16) μM/g (μg/g), respectively (Table 1). The sulfide concentration in blood was at least 9 times higher than the level in the control samples, 0.0016 μM/mL (0.05 μg/mL) (2,3,11). Sulfide concentrations in the tissues were similar to those reported by Kimura et al. (5) and also noted in our animal experiments (11), as shown in Table 1, except for the level in the brain,

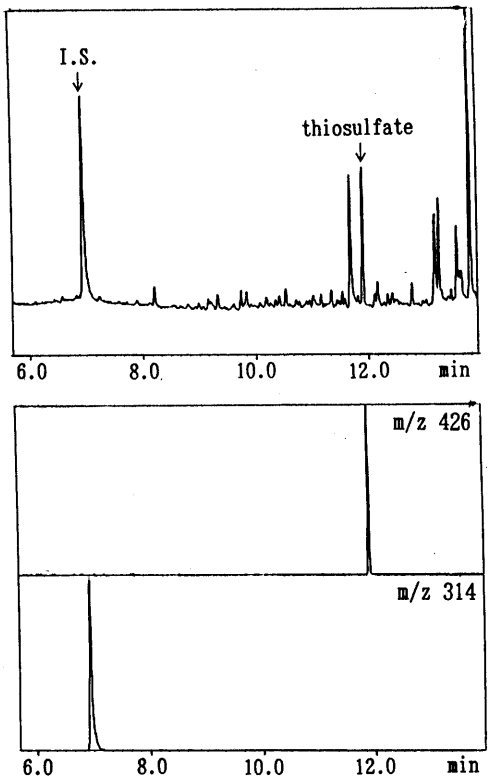

FIG. 3—*Total ion chromatogram and mass chromatograms of the derivatized extract for thiosulfate obtained from a blood sample of the victim:* (upper) *total ion chromatogram,* (lower) *mass chromatograms.*

TABLE 1—*Sulfide concentrations in blood and tissue samples of the victim (μM/mL or g).*

Sample	Victim	Other Victims (5)			Animals (11)
		A	B	C	
Blood	0.014	0.016	0.007	N.D.*	0.012
Brain	0.085	0.006	0.033	0.013	0.010
Lung	0.013	0.021	0.007	0.007	0.019
Fem. muscle	0.005	N.A.†	N.A.	N.A.	0.007

* N.D. = not detected (below 0.003 μM/g).
† N.A. = not analyzed.

which was higher. We find no reports on correlation between sulfide levels in tissues and atmospheric hydrogen sulfide concentrations; however, we think that the high level of sulfide in the brain was probably due to the higher concentration of atmospheric hydrogen sulfide (3500 to 5000 ppm) compared with the level in our animal experiments (550 to 650 ppm).

For thiosulfate analysis, we found blood, brain and lung to be pertinent tissues (12), so these were used for analysis. Concentrations of thiosulfate in blood, brain and lung of the victim were 0.143 μM/mL (16.02 μg/mL), 0.045 (5.04), 0.083 (9.30) μM/g (μg/g), respectively (Table 2). The level in the blood was at least 48 times higher than the level in the control samples, 0.003 μM/mL (0.34 μg/mL) (15). Concentrations of thiosulfate in our subject were similar to data obtained when we exposed rabbits to 500 to 1000 ppm of hydrogen sulfide gas (12).

Thiosulfate concentrations in blood and lung in the victim were 6 to 10 times higher than sulfide concentrations, similar to findings

TABLE 2—*Thiosulfate concentrations in blood and tissue samples of the victim (μM/mL or g).*

Sample	Victim	Animals (12)
Blood	0.143	0.080
Brain	0.045	0.023
Lung	0.083	0.095

in our animal experiments. Therefore, evidence of thiosulfate in the tissues can serve as an indicator of hydrogen sulfide poisoning. Based on these results, the cause of death was attributed to hydrogen sulfide gas poisoning.

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References

1. Winek CL, Collom WD, Wecht CH. Death from hydrogen sulphide fumes. Lancet 1968;1:1096.
2. McAnalley BH, Lowry WT, Oliver RD, Garriott JC. Determination of inorganic sulfide and cyanide in blood using specific ion electrodes: application to the investigation of hydrogen sulfide and cyanide poisoning. J Anal Toxicol 1979;3:111–4.
3. Osbern LN, Crapo RO. Dung lung: a report of toxic exposure to liquid manure. Ann Inter Med 1981;95:312–4.
4. Ikebuchi J, Yamamoto Y, Nishi K, Okada K, Irizawa Y. Toxicological findings in a death involving hydrogen sulfide. Jpn J Legal Med 1993;47:406–9.
5. Kimura K, Hasegawa M, Matsubara K, Maseda C, Kagawa M. A fatal disaster case based on exposure to hydrogen sulfide—an estimation of hydrogen sulfide concentration at the scene. Forensic Sci Int 1994;66:111–6.
6. Imamura T, Kage S, Kudo K, Jitsufuchi N, Nagata T. A case of drowning linked to ingested sulfides—a report with animal experiments. Int J Legal Med 1996;109:42–4.
7. Kage S, Takekawa K, Kurosaki K, Imamura T, Kudo K. The usefulness of thiosulfate as an indicator of hydrogen sulfide poisoning: three cases. Int J Legal Med 1997;110:220–2.
8. Curtis CG, Bartholomew TC, Rose FA, Dodgson KS. Detoxication of sodium ^{35}S-sulphide in the rat. Biochem Pharmacol 1972;21: 2313–21.
9. Bartholomew TC, Powell GM, Dodgson KS, Curtis CG. Oxidation of sodium sulfide by rat liver, lungs and kidney. Biochem Pharmacol 1980;29:2431–7.
10. Beauchamp RO, Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA. A critical review of the literature on hydrogen sulfide toxicity. CRC Crit Rev Toxicol 1984;13:25–97.
11. Nagata T, Kage S, Kimura K, Kudo K, Noda M. Sulfide concentrations in postmortem mammalian tissues. J Forensic Sci 1990;35: 706–12.
12. Kage S, Nagata T, Kimura K, Kudo K, Imamura T. Usefulness of thiosulfate as an indicator of hydrogen sulfide poisoning in forensic toxicological examination: a study with animal experiments. Jpn J Forensic Toxicol 1992;10:223–7.
13. Kage S, Nagata T, Kimura K, Kudo K. Extractive alkylation and gas chromatographic analysis of sulfide. J Forensic Sci 1988;33: 217–22.
14. Kage S, Nagata T, Kudo K. Determination of thiosulfate in body fluids by GC and GC/MS. J Anal Toxicol 1991;15:148–50.
15. Kawanishi T, Togawa T, Ishigami A, Tanabe S, Imanari T. Determination of thiosulfate in human urine and plasma by high performance liquid chromatography with a dual electrochemical detector. Bunseki Kagaku 1984;33:E295–300.

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