TECHNICAL NOTE

Takeaki Nagata,[1] M.D.; Shigetoshi Kage,[2] B.S.; Kojiro Kimura,[1] M.D.; Keiko Kudo,[1] Ph.D.; and Midori Noda,[1] B.S.

Sulfide Concentrations in Postmortem Mammalian Tissues

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ABSTRACT: Postmortem changes in sulfide concentrations in body tissues were examined in autopsied rats exposed to hydrogen sulfide concentrations of 550 to 650 ppm, and in nonexposed rats and humans. Analyses were made by gas chromatography, following an extractive alkylation. Sulfide concentrations in the blood, liver, and kidneys of rats increased in both the exposed and nonexposed groups, depending on the lapse of time after death. On the other hand, the lung, brain, and muscle showed little or no change in sulfide concentration with elapse of time after death. The data obtained from human tissues were almost the same as those for rats, except data for blood, in which no or little increase of sulfide was observed.

KEYWORDS: toxicology, hydrogen sulfide, extractive alkylation, gas chromatography, mass spectrometry, postmortem change, body tissues, blood level, poisoning

A sensitive method for determining the presence of sulfide in biological materials is presented [*1*]. Using this procedure, sulfide was alkylated by pentafluorobenzyl bromide, and the stable derivative, bis(pentafluorobenzyl) sulfide, was analyzed by gas chromatography (GC) with electron capture detection (GC-ECD). The detection limit was 0.01 μg/g. To assess possible practical application for routine autopsy toxicology, changes in sulfide concentrations in tissues, at various intervals after death and during cold storage, were examined in a series of animal experiments. Human body tissues were also examined.

Materials and Methods

Setup for Exposure to Hydrogen Sulfide

The system used for exposure is illustrated in Fig. 1. Hydrogen sulfide (H_2S) gas was generated from sodium monosulfide (Na_2S) and phosphoric acid (H_3PO_4) in a flask (No.

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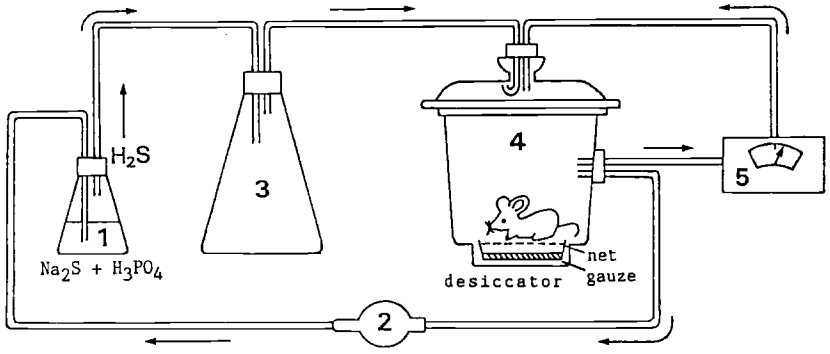
[1]Professor and lecturers, respectively, Department of Forensic Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

[2]Forensic chemist, Forensic Science Laboratory, Fukuoka Prefectural Police Headquarters, Fukuoka, Japan; and guest researcher, Department of Forensic Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan.



1 H_2S generating chamber (0.1 L)
2 Rubber air bulb
3 Trap chamber (3 L)
4 Exposure chamber (10 L)
5 Gas analyzer (Gastec GOH-1A)

FIG. 1—*Apparatus used for exposure of rats to H_2S gas.*

1 in Fig. 1). The mixture was bubbled using a single spray bulb (No. 2). The H_2S produced was introduced into a trap chamber (No. 3) and then into a 10-L volume glass desiccator used as the exposure chamber (No. 4). The concentrations of H_2S and oxygen were monitored by a Gastec GOH-1A model gas analyzer (No. 5), modified to cover a wide range of sulfide concentrations. The concentration of H_2S gas was adjusted to 550 to 650 ppm to produce an acute and fatal poisoning [*2,3*]. The concentration of oxygen in the exposure chamber never fell below 18% during the experiment.

Animal Experiments

Adult male Wistar rats weighing about 300 g were put into the chamber and exposed to the H_2S gas. The carcasses were removed, and dried with air for 30 s to remove any gas remaining on the body surface. The nonexposed control rats were pithed. Their carcasses were put in a box maintained at 20°C, and the tissue samples were collected 0, 4, 24, and 48 h after death. The sulfide concentration in each sample was measured 1, 7, and 30 days after storage at −20°C.

Human Tissue Samples

Control human body tissue samples were obtained from 11 autopsied persons. None of these deaths was related to hydrogen sulfide poisoning. The time intervals after death were 8 to 39 h. Samples from two others, who had died 66 h and about 1 week before, were examined as controls of putrefaction. The blood used for the test was collected from intracranial vessels or the femoral artery. The blood and tissue samples were kept at −20°C from 1 to 13 days until analysis.

Procedure of Analysis

The sulfide concentrations in the samples were analyzed as described [*1*]. About 0.2 g of the biological sample was treated, using the extractive alkylation technique, and subjected to GC-ECD.

GC Conditions—We used a Shimadzu GC-3BE model with a Ni^{63} electron capture

detector and a 2.1-m by 3-mm inside diameter glass tube packed with 5% Apiezon grease L on Chromosorb W [acid-washed dimethyldichlorosilane (AW-DMCS)], 60–80 mesh. The temperature of the column was maintained at 200°C, and nitrogen was maintained at a flow pressure of 0.3 kg/cm^2.

Results and Discussions

Animal Experiments

Sulfide Concentration in Whole Blood—Changes in sulfide concentrations in the whole blood were examined to assess the suitability of the samples for toxicological examination. Five rats were used at each of the postmortem times of 0, 4, 24, and 48 h (20 rats in all).

The sulfide concentration in the blood analyzed immediately after death averaged 0.48 μg/g. This is close to the data reported by Fukui et al. [*4*], who exposed rats to 400 ppm H_2S for 1 h. On the other hand, no sulfide was found in the controls. The sulfide concentrations in both the exposed and nonexposed groups markedly increased with elapse of time after death: the exposed group showed 1.95 μg/g at 4 h, 2.93 at 24 h, and 2.66 after 48 h, whereas the nonexposed group showed 0.55, 1.34, and 1.88 μg/g, respectively. The concentration in the nonexposed group at 4 h after death exceeded the fatal level in the exposed group measured at the time of death. According to the animal experiments, blood appears to be an unsuitable tissue for toxicological examinations.

Sulfide Concentrations in Other Tissues—Postmortem changes in sulfide concentrations in the lung, brain, liver, kidney, and thigh and abdominal muscles were also examined. The samples were collected 0, 4, 24, and 48 h after death and kept at −20°C for 24 h. Table 1 shows data on the samples, listed approximately in the order of increasing sulfide concentration in the nonexposed samples.

The mean values of sulfide concentrations in the lung, brain, and thigh and abdominal muscle tissues collected immediately after death were 0.60, 0.31, 0.21, and 0.22 μg/g, respectively. There was a significant difference between these data and the findings in

TABLE 1—*Changes in sulfide concentrations in autopsied rat tissues with elapsed time after death, in micrograms per gram.*[a,b]

Samples	Hours After Death			
	0	4	24	48
Lung	0.60 ± 0.21 (0.00 ± 0.00)	0.40 ± 0.17 (0.00 ± 0.00)	0.36 ± 0.24 (0.00 ± 0.00)	0.27 ± 0.19 (0.00 ± 0.00)
Brain	0.31 ± 0.14 (0.00 ± 0.00)	0.22 ± 0.16 (0.00 ± 0.00)	0.09 ± 0.11 (0.00 ± 0.00)	0.07 ± 0.12 (0.00 ± 0.00)
Thigh muscle	0.21 ± 0.14 (0.00 ± 0.00)	0.33 ± 0.22 (0.05 ± 0.06)	0.36 ± 0.33 (0.07 ± 0.11)	0.47 ± 0.38 (0.46 ± 0.10)
Abdominal muscle	0.22 ± 0.21 (0.00 ± 0.00)	4.08 ± 1.11 (3.49 ± 1.27)	4.63 ± 1.08 (4.30 ± 0.84)	5.34 ± 1.01 (4.30 ± 1.25)
Liver	1.67 ± 0.37 (0.95 ± 0.12)	2.74 ± 0.48 (2.17 ± 0.35)	3.68 ± 0.88 (4.91 ± 1.49)	2.82 ± 1.11 (2.98 ± 0.24)
Kidney	1.45 ± 0.24 (1.19 ± 0.23)	1.85 ± 0.68 (1.01 ± 0.25)	2.00 ± 0.90 (3.04 ± 0.85)	3.56 ± 0.57 (1.67 ± 0.93)

[a]Each value is the mean (n = 5) ± standard deviation. The period of storage is at −20°C for 24 h.

[b]The values in parentheses are the data on controls.

the nonexposed group, shown in the left column. On the other hand, the liver and kidney data in both groups were close and showed high levels of sulfide. Thus, these two organs were considered to be unsuitable for analyzing for sulfide poisoning.

Since collection of organ or tissue samples at the time of death in humans is rarely feasible, data on samples collected over 4 h after death in rats were examined. The sulfide concentrations in the lungs and brains of the exposed group showed a tendency toward a slight decrease with elapse in time after death, while findings in the nonexposed group were nil. The thigh muscle showed a slight increase of sulfide with lapse of time and could be distinguished from the nonexposed sample, which showed a minimum increase up to 24 h after death. After 48 h, the sulfide concentration in the thigh muscle of a nonexposed rat reached a level equal to that of the exposed one. The sharp increase of sulfide in the thigh muscle with an extended time after death may be accounted for by diffusion of sulfide from the abdominal region. The abdominal muscle showed a marked increase of sulfide, even in the nonexposed sample after 4 h, which suggests postmortem production of sulfide due to putrefaction.

Influence of Cold Storage—The blood, lung, brain, and thigh muscle were used to test the influence of cold storage for up to 30 days on the sulfide concentration. Table 2 shows data on the samples collected 24 h after death. The blood sample was collected immediately after death.

All the samples showed only slight variations in sulfide concentration during cold storage for 30 days, and no sulfide was detected in the nonexposed group of the lungs and brains. Why small amounts of sulfide were present in the nonexposed blood was not determined.

Thus, the lung, brain, and muscle, but not abdominal muscle, should be used to test for sulfide concentrations, and all tissues must be frozen as soon as possible after death. Animal blood can also be used as a testing material, but only when collected and frozen immediately after death.

Sulfide Concentrations in Human Body Samples

The sulfide concentrations in human tissues were examined. The samples were obtained from eleven autopsied persons, immediately frozen at −20°C, and kept at that temper-

TABLE 2—*Changes in sulfide concentrations in rat tissues stored at −20°C with storage time, in micrograms per gram.*[a,b]

Samples	Days of Storage		
	1	7	30
Blood[c]	0.38 ± 0.13 (0.05 ± 0.04)	0.48 ± 0.17 (0.12 ± 0.13)	0.66 ± 0.26 (0.06 ± 0.06)
Lung	0.36 ± 0.24 (0.00 ± 0.00)	0.58 ± 0.24 (0.00 ± 0.00)	0.98 ± 0.20 (0.00 ± 0.00)
Brain	0.09 ± 0.11 (0.00 ± 0.00)	0.03 ± 0.07 (0.00 ± 0.00)	0.07 ± 0.08 (0.00 ± 0.00)
Thigh muscle	0.36 ± 0.33 (0.07 ± 0.11)	0.55 ± 0.32 (0.00 ± 0.00)	0.58 ± 0.49 (0.06 ± 0.09)

[a]Each value is the mean (n = 5) ± standard deviation. The postmortem time is 24 h.

[b]The values in parentheses are the data on controls.

[c]The samples were taken immediately after death.

TABLE 3—*Sulfide concentrations in human tissues (autopsied cases), in micrograms per gram.*

	Group A, Case No.					Group B, Case No.				Group C, Case No.	
Samples	1	2	3	4	5	6	7	8	9	10	11
Blood	0.00	0.00	0.00	0.00	0.00	0.04	0.49	0.00	0.00	2.81	...
Lung	0.00	0.00	0.00	0.00	0.00	0.09	0.10	0.05	0.73	3.79	1.37
Brain	0.00	0.00	0.00	0.00	0.00	0.02	0.11	0.00	0.00	0.91	...
Thigh muscle	0.00	0.00	0.00	0.00	0.00	0.33	0.17	0.05	1.21	5.67	4.27
Abdominal muscle	2.47	7.36	0.09	6.30	0.02	0.66	4.94	0.14	4.05	5.67	3.11
Liver	2.15	2.43	0.55	0.94	0.31	3.59	2.17	3.06	1.29	1.89	7.17
Kidney	0.02	0.19	0.23	0.27	0.80	1.82	0.49	1.36	3.63	5.22	3.98
Postmortem interval, h	9	19	22	32	39	8	15	16	18	66	~1 week
Period of cold storage, day	3	6	8	2	5	1	6	3	13	6	6
Mean atmospheric temperature, °C	9.3	16.3	18.7	19.5	9.1	23.4	22.4	25.2	24.7	14.1	...
Comments				[a]	[a]		[b]			[c]	[d]

[a]Kept in dry ice for about 12 h.
[b]Viscera ruptured.
[c]Beginning of putrefaction.
[d]Advanced putrefaction.

ature until analysis. The analytical results on the sulfide concentrations in the samples are shown in Table 3.

The sulfide concentrations were nil in the blood, lung, brain, and thigh muscle in Group A, which included five bodies, numbered 1 to 5. In Group A, the bodies had been kept reportedly at a temperature below 20°C until autopsy, and the postmortem time was within 24 h. Two bodies, Nos. 4 and 5, with a postmortem time over 24 h, were preserved in dry ice for about 12 h before the autopsy. Presumably the postmortem production of sulfide was thus suppressed. On the other hand, sulfide was identified in the abdominal muscles, livers, and kidneys of these two. In Group B, which included four bodies, numbered 6 to 9, and in which the bodies had been kept at a temperature over 20°C, the sulfide concentrations varied from nil to low levels in the blood, lung, brain, and thigh muscle tissue and were fairly high in other tissues. In Group C, high levels of sulfide were produced during the long postmortem interval.

The data obtained from human samples are in fairly good agreement with data obtained from rats, except data for blood. The appearance of sulfide in rat blood in the early postmortem period is probably due to the diffusion of sulfide from the abdomen into the near heart region. Thus, human blood collected from an intracranial vessel or the femoral artery can serve as a representative sample for sulfide analysis.

Winek et al. [5] reported that the sulfide concentration in a dead human exposed to 1900 to 6000 ppm of H_2S was 0.92 μg/g in the blood, 1.06 in the brain, 0.34 in the kidney, and 0.38 in the liver. The levels of sulfide in the liver and kidney are close to those of our "control" cases, shown in Table 3. On the other hand, the brain level is toxicologically significant when no or very small sulfide concentrations in the control human cases are taken into consideration.

Conclusions

From the results of animal experiments and examinations of human tissues, the authors have determined that the most pertinent materials for sulfide analysis are the blood, lung, brain, and muscle tissue from a site distant from the abdominal area.

Instant freezing is recommended. A marked increase in sulfide concentration was observed in the liver, kidney, and abdominal muscle with elapse of time after death. These materials should not be used as samples for toxicological examinations for sulfide.

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Address requests for reprints or additional information to
Prof. Takeaki Nagata, M.D.
Department of Forensic Medicine
Faculty of Medicine
Kyushu University
3-1-1, Maidashi, Higashi-ku
Fukuoka 812, Japan