A Fatal Case Poisoned by Hydrogen Phosphide and the Simultaneous Separation of Inorganic Gases by Gas Chromatography

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INTRODUCTION

The accident by hydrogen phosphide, phosphine has occured in the different fields. Such the fatal cases have been reported as yet on the wet cement used in lying the groundmaking of road[1], the formation of acetylene gas from calcium carbide[2], the "Delicia" fumigation of grain weevil[3], the welding with phosphate-coated aluminium welding road[4], and the shipment or handling of ferro-silicon[5][6]. However, those due to ferro-silicon were very rare.

Recently, authors performed an autopsy on a male victim who died in a cargo-boat loaded with ferro-silicon. On the basis of various toxicological examinations, the victim was elucidated to be poisoned to death by hydrogen phosphide gas generated from it. Furthermore, taking this opportunity, we attempted the simultaneous separation of the eight inorganic gases which frequently become a daily task in the medico-legal toxicology.

CASE REPORT

This fatal accident happened in a small cargo-boat loaded with ferro-silicon. First, without the knowledge of what hydrogen phosphide is evolved from ferro-silicon, we have conducted an investigation concerning the cause of death on the basis of the information of a police man and the advice of a metallurgist. As a result, a long time had been consumed till a conclusion was led.

The member of the cargo-boat was nothing but three of a couple of elder brother and one's brother. The couple slept in a steering room was nothing unusual, but their brother, victim in this case, slept in a

hatch died unfortunately. He once got up at 6 in the morning owing to urinate, while at that time, brother did not find out his abnormal symptoms. Subsequently, when he again got up at about 8, he had complained of headache, vertigo, vomiting, and abdominal pain, and then he went into coma and died on the way to a hospital. First, the cause of death might be considered to be the food poisoning due to the lobsters which was eaten last night together with them, or the suffocation under the lack of oxygen.

At once, fire officers searched the leak of liquefied petroleum gas into the cabin, but no evidence for it was obtained.

On the other hand, the abnormal signs were not observed on the digestive tract macrospically in the autopsy. Furthermore, in the microscopical findings, there was not any remarkable change with the exception of congestion in the various organs and fatty degeneration in the liver.

MATERIALS AND METHODS

Materials

The blood, the stomach contents, the air collected in a hatch, and ferro-silicon (iron alloy).

Test for hydrogen cyanide

A test was carried out on the basis of the method of Schönbein-Pagenstecher.

Test for heavy metals

A series of test was performed for Hg, As, and Sb, according to the Reinsch's method.

Determination of methemoglobin and sulfhemoglobin[7)8)]

Phosphate buffer (M/15, pH 6.6): One point nine g of anhydrous Na_2HPO_4 and 2.72 g of anhydrous KH_2PO_4 were dissolved with water in a 500 ml volumetric flask and the solution diluted to mark.

Phosphate buffer (M/60, pH 6.6): M/15 phosphate buffer was dilluted with water to fourfold volume as needed.

Neutralized cyanide solution: A neutralized cyanide solution was prepared within 1 hour of use by mixing equal volumes of 10 per cent sodium cyanide solution and 12 per cent acetic acid.

Twenty per cent potassium ferricyanide solution, 10 per cent sodium cyanide solution, and concentrated ammonia water.

1. One fifth ml of the blood was pipetted into 10 ml of M/60

phosphate buffer. The solution was allowed to stand for 5 minutes and centrifuged. The supernatant was measured the optical density against water blank at the wavelength of 630 mμ (A_1).

2. One drop of neutralized cyanide solution was added to the 10 ml from step 1. The solution was mixed well, allowed to stand for 2 minutes, and read again at the wavelength of 630 mμ (A_2).

3. One drop of concentrated ammonia water was added to the blood solution treated as the same in step 1. The solution was mixed well and 2 minutes later, measured the optical density at the wavelength of 619 mμ (A_3).

4. The 2.0 ml from step 3 was added to 8.0 ml of M/15 phosphate buffer containing 1 drop of 20 per cent potassium ferricyanide solution, mixed well, and allowed to stand for 2 minutes. One drop of 10 per cent potassium cyanide solution was added to this, mixed well, and allowed to stand for 2 minutes. The solution was measured the optical density at the wavelength of 540 mμ (A_4). The calculations were as follows.

Total hemoglobin g/100 ml$=36.8\times A_4$

Methemoglobin g/100 ml$=23.4\times(A_1-A_2)$

$$\text{Methemoglobin concentration } (\%) = \frac{\text{Met Hb g/100 ml}}{\text{Total Hb g/100 ml}} \times 100$$

$$\text{Sulfhemoglobing/100 ml} = 6.5 \times \frac{(\text{Total Hb} - \text{Met Hb})\ \text{g/100 ml}}{205} - \frac{\text{Met Hb g/100 ml}}{84}$$

$$\text{Sulfhemoglobin concentration } (\%) = \frac{\text{Sulf Hb g/100 ml}}{\text{Total Hb g/100 ml}} \times 100$$

Color reaction I[9]

Five per cent lead acetate-impregnated cotton and a strip of 20 per cent silver nitrate-impregnated paper.

The powders of ferro-silicon were put into a flask, and then moistened with a little amount of water. A small test tube was installed to the mouth of the flask. In the middle part of the test tube, 5 per cent lead acetate-impregnated cotton was inserted for preventing the test paper (silver nitrate-impregnated paper) from coloring by hydrogen sulfide, and the mouth of test tube was tightly closed with a rubber-stopper which was hanged a strip of 20 per cent silver nitrate-impregnated paper.

Color reaction II

Aqua regia: A mixture of one part of concentrated nitric acid and three part of concentrated hydrochloric acid.

Ammonium molybdate reagent; Six point five g of molybdenum trioxide was dissolved in a mixture solution of 14 ml of water and 14.5 ml of concentrated ammonia water. After cooling, the solution was gradually added to a mixture solution of 32 ml of concentrated nitric acid and 40 ml of water, left for 2 days, and was filtered with a glass filter.

A strip of silver nitrate-impregnated paper colored was taken out from an apparatus, followed by immersing into aqua regia in order to convert phophide into phosphoric acid. The solution was submitted to the steam distillation by use of a microdistillation apparatus. Subsequently, to the distillate thus obtained were added several drops of ammonium molybdate reagent.

Gas chromatography

Apparatus: Hitachi Gas Chromatograph Model 063-6056 equipped with a thermal conductivity detector.

Column: U-shaped stainless steel column, 3 mm in inside diameter, 1 and 2 m in length. Column packing: Chromosorb 104, molecularsieve 5A, and carbosieve B. Carrier gas: Helium, 29 and 30 ml/min. Column temperature: 50, 60, 80, and 100°C. Chart speed: 10 mm/min.

Gasification

For the purpose of accelerating the generation of gas from the samples, they were treated as follows. A lump of ferro-silicon was crushed with a hammer as finely as possible. The powders were transferred into a 50 ml-flask, and then moistened with a little amount of water. The flask was tightly closed with a silicon-stopper and allowed to stand at room temperature over night. The blood was put into a 10 ml volumetric bottle. The bottle was tightly closed with a sillion-stopper and allowed to stand in an oven at 100°C for 1 hour.

Formation of standered gases

A variety of standard gases were formed according to the following chemical equations (H_2: $Zn+H_2SO_4 \rightarrow ZnSO_4+H_2$; HCN: $2NaCN+H_2SO_4 \rightarrow Na_2SO_4+2HCN$; CO: $HCOOH \rightarrow CO+H_2O$; NH_3: $NH_4Cl+NaOH \rightarrow NaCl+H_2O+NH_3$; CO_2: $2NaHCO_3 \rightarrow Na_2CO_3+CO_2+H_2O$; H_2S: $Na_2S+H_2SO_4 \rightarrow Na_2SO_4+H_2S$; PH_3: $4P+3KOH+3H_2O \rightarrow 3KH_2PO_4+PH_3$; AsH_3: $As_2O_3+12HCl+6Mg \rightarrow 2AsH_3+3H_2O+6MgCl_2$).

Toxic test of hydrogen phosphide to animal

The large amounts of ferro-silicon powders were stored in a large desicator, 36 cm in inside diameter, and a sheet of filter paper was laid on a plate in the middle part. On it, four mice of C 57BL strain

were maintained and a simple toxic test to animal was carried out.

RESULTS

Hydrogen cyanide and heavy metals

Both tests were carried out in the blood and stomach contents, but hydrogen cyanide and heavy metals were not detected.

Methemoglobin and sulfhemoglobin

Judging from a statement of a police man, we first considered the victim to be poisoned to death by hydrogen sulfide. Therefore, methemoglobin and sulfhemoglobin in the blood were determined by means of the spectrophotometric method. The reaults were −0.86% in the former and 0.0% in the latter, respectivery.

Gas chromatography

The gas chromatography was carried out in order to know the components of gases which were dissolved in the blood, involved in the air collected in the hatch, and formed in the reaction with ferro-silicon and water.

Chromosorb 104 was served as a column packing, three peaks appeared from the gases generated from ferro-silicon. These were identified as air, carbon dioxide, and hydrogen phosphide in order of increasing retention time. Moreover, the same gas chromatographic pattern was obtained from the air collected in the hatch, but hydrogen phosphide was very small so that it could be detected with difficulty. On the contrary, the gas chromatographic pattern from the blood was different from those of ferro-silicon and hatch. A peak lastly revealed was identified not to be hydrogen phosphide, but to be hydrogen sulfide (Fig. 1).

On the other hand, in the case of use of molecularsieve 5A, hydrogen, oxygen, and nitrogen each was elucidated from the gases generated from ferro-silicon. However, from other specimen, hydrogen could not be detected.

Color reaction

When a strip of silver nitrate-impregnated paper was hanged in the flask containing ferro-silicon powders, it gave the dark brown color. However, this color reaction occurs in not only hydrogen phosphid but also hydrogen arsenide and hydrogen antimonide. Therefore, the paper thus colored was immersed in aqua regia. The solution was subjected to distillation, and several drops of ammonia molybdate reagent were added to the distillate. It turned pale yellow.

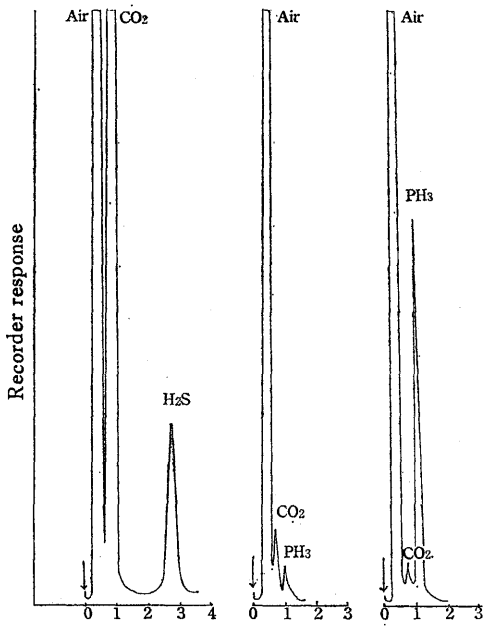


Fig. 1. Retention time, min

Gas chromatograms of gases generated from blood, air sampled in a ship, and gases generated from ferro-silicon. Column: Chromosorb 104 (3mmϕ, 1m), Oven temp. 50°C, Detector temp. 85°C, Detector : TCD, Carrier gas : He,20ml/min, Chart speed : 10mm/min.

Toxic test to animal

When the mice were maintained in the desicator containing ferro-silicon, they became irritated after 20 to 30 minutes lapsed, turned round and round, and vigorously jumped up. Two to 3 hours later, all of them died. In the analysis by gas chromatographic method, hydrogen phosphide was not detected from the blood.

Simultaneous separation of inorganic gases by gas chromatography

The simultaneous separation of inorganic gases was investigated concerning the 8 gases, such as hydrogen, ammonia, carbon monoxide, carbon dioxide, hydrogen cyanide, hydrogen sulfide, hydrogen arsenide, and hydrogen phosphide which frequently become a daily task for accidental death or even suicide by the use of them.

First of all, three column packings of chromosorb 104, molecularsieve 5A, and carbosieve B each was used, and then the analytical conditions were studied. In general, the individual retention times of these gases became more larger along with falling of the flow rate of carrier gas

and the column temperature. The separation was slightly improved. On the contrary, the sharpness of peak became more poor and such a phenomenon was remarkable, especially in the gases having the greater retention time. As a consequence, in consideration of the separation and the shape of a peak, the flow rate of carrier gas and column

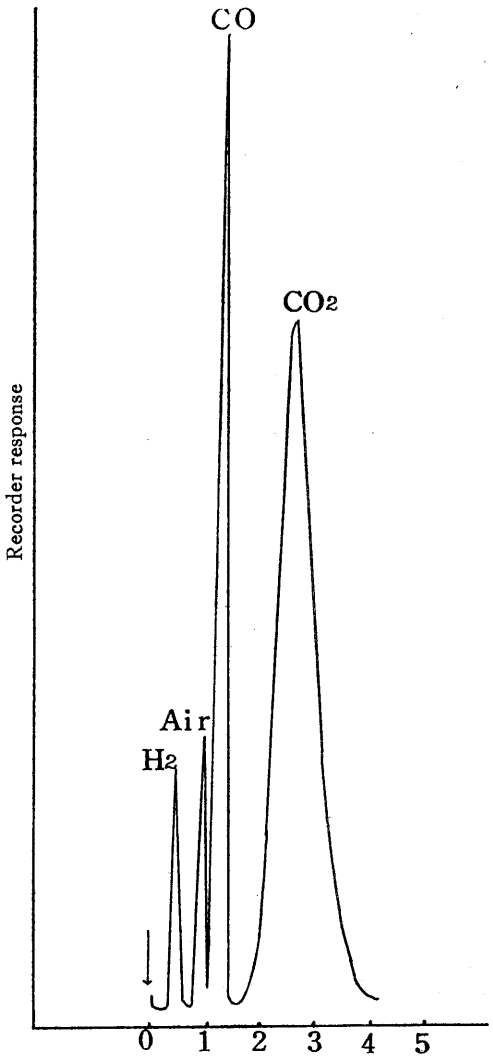


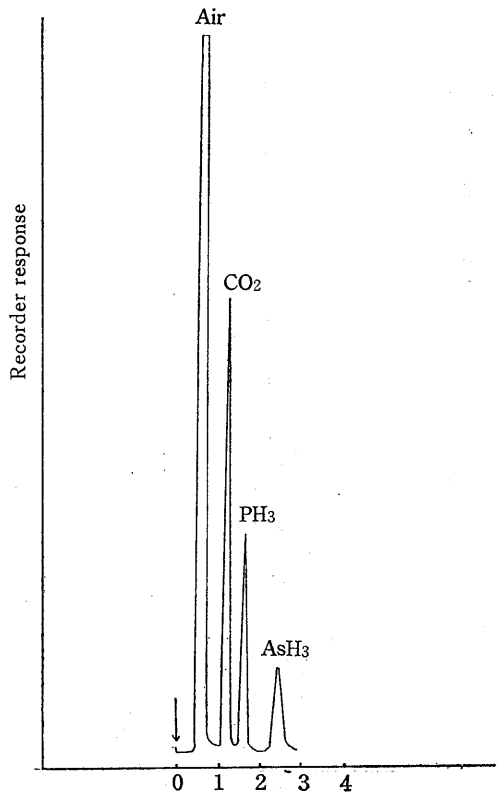
Fig. 2. Retention timn, min

Gas chromatogram of inorganic gases. Column : Chromosorb 104 (3mmϕ, 1m), Oven temp. 80°C, Detector temp. 110°C, Detector: TCD, Carrier gas: He, 30ml/min, Chart speed : 10mm/min.

temperature were considered that 30 ml/min and 80°C for chromosorb 104, 20 ml/min and 50°C for molecularsieve 5A, and 20 ml/min and 60°C for carbosieve B, respectively, were suitable for the simultaneous separation of these gases.

In the case of use of chromosorb 104 as a column packing, 4 gases, air, carbon dioxide, hydrogen phosphide (or hydrogen sulfide), and hydrogen arsenide were successfully separated and their peaks each was very sharp (Fig. 2).

In the case of use of molecularsieve 5A, other gas except hydrogen, oxygen, and nitrogen could not be separated. However, the separation of oxygen and nitrogen was accomplished very satisfactly. In this respect, this column packing is said to be superior to other two column packings.



Fgi. 3. Retention time, min

Gas chromatogram of inorganic gases. Column : Carbosieve B (3mmϕ, 1m), Oven temp. 60°C, Detector temp. 80°C, Detector : TCD, Carrier gas : He. 20ml/min. Chart speed : 10mm/min,

In the case of use of carbosieve B, 4 gases, hydrogen, air, carbon monoxide, and carbon dioxide (or either of hydrogen sulfide, hydrogen phosphide, and hydrogen arsenide) were separated from one another. Of these, carbon dioxide often appeared as a ghost peak, but the shapes of other peaks each was very sharp (Fig. 3).

DISCUSSION

The fatal accident on account of ferro-silicon occurs by the trace amount of calcium phosphide which is contained as impurities in it, may react with moisture in air and give off hydrogen phophide. Hydrogen phosphide, phosphine, PH_3 is a very toxic and colorless gas. Its specific gravity is 1.529 g/l, boiling point −87.7°C, and melting point −133°C. Hydrogen phosphide is only sparingly soluble in ethanol and ether, and it is scarcely a specific odor in the pure state, but has a strong odor resembling acetylen, garlic, or decayed fish in the impure state. Its maximum allowable concentration[10] is 0.05 ppm and a high concentration of 100 ppm is said to cause death within a few hours[1].

The poisonous symptoms by hydrogen phosphide are likely to be very similar to those of hydrogen arsenide, but differenciated from the latter in the point that blood picture is not changed. In general, the poisonous patients complain of the main symptoms, such as headache, vertigo, nausea, vomiting, and abdominal pain. Therefore, most of them are misdiagnosed as mest and fish poisoning or salmonellosis[10,11]. There follows sudden or gradual loss of cosciousness and possibly death by plumonary edema and respiratory arrest. In autopsical findings, congestion and edema are said to be observed on a variety of organs.

Thus, taking the symptoms before the death, the autopsical findings, some toxicological tests, and a few reference on hydrogen phosphide into consideration, the victim was identified to be poisoned to death by hydrogen phosphide generated from ferro-silicon.

Up to date, many works[12,13,14,15] have been reported on the gas chromatographic analysis of various inorganic and organic gases, but a little on simultaneous separation of many inorganic gases. Therefore, taking this opportunity, the authors attempted the simultaneous separation of the 8 inorganic gases, such as hydrogen, ammonia, carbon monoxide, carbon dioxide, hydrogen cyanide, hydrogen sulfide, hydrogen arsenide, and hydrogen phosphide which frequently become a daily task in the medico-legal toxicology. The column temperature and flow rate of carrier gas varied from 50 to 100°C and from 20 to 30 ml/min,

and then the analytical conditions were studied. As a result, the flow rate of carrier gas and column temperature were suitable, respectively, 30 ml/min and 80°C for chromosorb 104, 20 ml/min and 50°C for molecularsieve 5A, and 20 ml/min and 60°C for carbosieve B. Under these conditions, the 4 gases of air, carbon dioxide, hydrogen phosphide, and hydrogen arsenide by chromosorb 104, the 3 gases of hydrogen, oxygen, and nitrogen by molecularsieve 5A, and the 4 gases of hydrogen, air, carbon monoxide, carbon dioxide by carbosieve B, each was separated successfully. The shapes of their peaks were very good except carbon dioxide.

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