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Sudden Death from Glue-Sniffing

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Abstract This case deals with an 18 year-old young male who suddenly died while amusing himself by glue-sniffing together with a friend in a very narrow toilet beneath a stairway in the company where he worked. Therefore, for the purepose of elucidating the cause of death, gas chromatographic analyses were carried out on the lacquer thinner which was discovered at the scene and on the variety of tissues of the cadaver.

As a result, five ingredients (acetone, ethyl acetate, n-butanol, toluene, and n-butyl acetate) were identified from the lacquer thinner and three ingredients (acetone, n-butanol, and toluene) from the body tissues, respectively. In addition, the detected concentrations of toluene fpr each tissue sample were 2.84μl/g in the adipose tissue, 0.64 μl/g in the subcutaneous fatty tissue, 0.14μl/g in the liver, 0.051μl/g in the lungs, 0.1μl/ml in the heart blood, 0.11 μl/g in the brain, 0.1 μl/ml in the gastric fluid, and 0.1 μl/ml in the urine. The concentrations of hippuric acid and o-cresol in the urine, which are extensively used as the indexes of exposure to toluene vapor, were 10.2 μg/ml and 0.105 μg/ml, respectively.

Key words : Death, While, Glue-sniffing

Introduction

Recently, lacquer thinneer, usually used as a solvent, has been widely used for recreation[1,2] and such behavior has a bad influence on society.

In general, the lacquer thinner on the market consists of several kinds[3] of organic ingredients such as acetone, methyl ethyl ketone, methyl isobutyl ketone, alcohols, acetate esters, toluene, and so forth. However, the main ingredient is toluene and the volume of toluene occupies more than 60% of total volume. Accordingly, the typical symptoms of poisoning by lacquer thinner are likely to be attributable especially to toluene among these ingredients. The toxic level of toluene is around 0.5 to 1.0 g/kg and the principal symptoms[4,5] of acute poisoning include dizziness, weakness, euphoria, headache, nausea, vomitting, tightness in the chest, staggering, and coma. In addition, repeated exposure to toluene is said to bring about anemia, petechia, abnormal bleeding, and finally, abrasion of the bone marrow.

Case report

This cadaver was an 18-year-old male and after working hours, he carried lacquer thinner from the company where he was employed and amused himself by glue-sniffing

together with one of his friends in a very narrow toilet beneath a stairway. The next morning, he was discovered lying on the floor of the toilet already dead. Therefore, in order to elucidate the cause of death, a legal autopsy was performed.

He was about 170 cm in height,and his physical constitution and nutritional grade both were moderate. The congestion in the upper and lower palpebral conjunctiva of the left and the right eyes was prominently advanced and, moreover, there were a few petechial hemorrhages as large as fleas. The cornea exhibited medium grade turbidity, but the pupil could be seen through without difficulty. There were ten petechial hemorrhages as large as fleas or needle heads on the chest wall. He had a butterfly pattern and the letters "route 191" tattooed on the inside of the left leg and, in addition, had a maple leaf tattooed in front of the right leg, and one part had already been cicatrized.

There were several petechial hemorrhages as large as flease on the inner surface on the scalp, but fleeding was not observed. Furthermore, fleeding was found in the temporal muscle.

The cadaver's heart was about 1.5 times as large as his fist and its weight was approx. 250g. Under the outer membrane of the heart, a slight degree of lipid storage was noticed. In the lungs, the edema was strongly developed and in the bronchia, a large amount of yellowish brown liquid containing foam remained. Under microscopic examination, the postmortem degeneration had remarkably progressed in a variety of tissues. In addition, fatty degeneration of hepatic cells, acute tubulonecrosis of the kidneys, congestion and edema of the lungs etc were observed.

Methods

1) Analysis gaseous ingredients in lacquer thinner and in tissues

Tissues such as the brain, adipose tissue, subcutaneous fatty tissue, liver, and lungs were minced as finely as possible with scissors. The slices were transferred to 25 ml vials, which were tightly sealed with caps and immersed in a water bath at 70℃. Twenty minutes later, 1.0 ml of the headspace gas was sampled with a 2.5 ml injection syringe and submitted to gas chromatographic analysis. Following this, 1 drop of the lacquer thinner which was left at the scene was added in a 25 ml vial and, thereafter, it was treated in the same manner.

The gas chromatograph and operating conditions were as follows. Gas chromatograph: Hitachi 163 Gas chromatograph equipped with flame ionization detector and 833 recorder; columns: 3

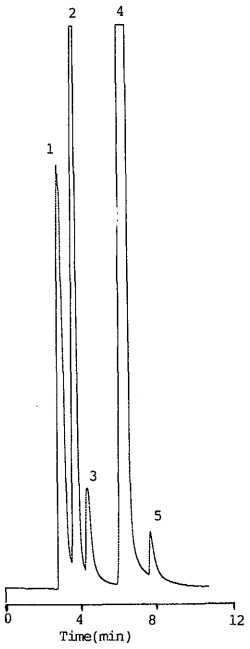

Fig. 1

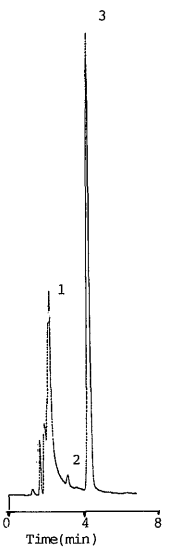

Fig. 2

Fig. 1 Chromatogram of lacquer thinner
Gas chromatographic conditions: column, 3mm×5m glass column packed with 2% Silicone SE 30 coated on Chromosorb W(80-100 mesh, AW, DMCS); column temperature, 70℃; injection port temperature, 120℃; carrier gas and flow rate, nitrogen and 30ml/min; range, 10^{-2}. Peaks: (1) acetone; (2) ethyl acetate; (3) n-butanol; (4) toluene; (5) n-butyl acetate.

Fig. 2 Chromatogram of lacquer thinner's ingredients derived from heart blood
Gas chromatographic conditions: column, 3mm×4m glass column packed with 2% Silicone OV 1 coated on Chromosorb W(80-100 mesh, AW, DMCS); column temperature, 60℃; injection port temperature, 120℃; carrier gas and flow ratw, nitrogen and 30 ml/min; range, 10^{-2}. Peaks: (1) acetone; (2) n-butanol; (3) toluene.

mm× 5 m glass column packed with 2% Silicone SE 30 coated on Choromosorb W (80-100 mesh, AW, DMSC) and 3 mm × 4m glass column packed with 2% Silicone OV 1 coated on Chromosorb W (80-100 mesh, AW, DMSC); column temperature: 70℃ in the former column and 60℃ in the latter column; injection port temperature: 120℃ in both columns; carrier gas: nitrogen, 30 ml/min, respectively.

2) Determination of toluene in tissues

1,2,3,4,5, and 6μl of toluene were transferred to 25 ml vials, followed by addition of 5 μl of n-butyl acetate as internal standard. The vials were thoroughly closed with caps and were treated in the same way as that in the above experiment. The Calculation curve showed a good linear relationship over the range of 1 to 6 μl. The equation of the regression line was given by: Y=0.6691 X-0.1939 ($0.6381<\beta<0.7001$, $\alpha=0.05$) and the correlation coefficient was 0.9995 ($0.82<\rho<0.99$, $\alpha=0.01$).

The gas chromatograph and operating conditions were as follows. Gas chromatograph: the same; column: 3mm×4m glass column packed with 2% Silicone OV 1 coated on Choromosorb W (80-100 mesh, AW, DMCS); column temperature: 60℃; injection port temperature: 120℃. Other conditions were the same as those in the above experiment.

The various tissues were cut as finely as possible with scissors and the minced tissues were placed in 25 ml vials. Subsequently, 5 μl of n-butyl acetate as an internal standard were added, the vials were tightly sealed with the caps and, thereafter, there were treated in the same way as those in the above experiment.

3) Determination of hippuric acid in urine

Hippuric acid was dissolved in ether and the working standard solutions were prepared in the range of 5 to 50mg/ml. Following this, as an internal standard solution, a 0.5 mg/ml concentrated ether solution of N-(m-toluoyl) glycine was prepared. Each 1 ml of the working standard solutions of hippuric acid and 1 ml of the ether solution N-(m-toluoyl) glycine as an internal standard were transferred to a test tube. Subsequently, diazomethane gas was passed into the test tube for a while until hippuric acid and N-(m-toluoyl) glycine were completely methylesterified. The reaction mixture was transferred to a distillation flask and evaporated to dryness under reduced pressure. The residue was dissolved in a small amount of ethanal and the solution was transferred to a conical flask. The distillation flask was washed two or three time with a very small quantity of ethanol and all the washing solutions were combined. Finally, the volume of the solution was raised to 0.5 ml with ethanol, the solution was well mixed, and 1 μl of the solution was subjected to gas chromatographic analysis.

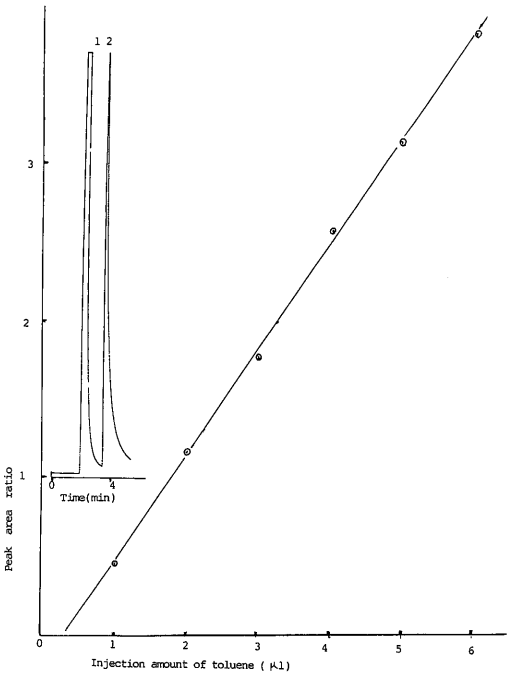

Fig. 3 Chromatogram and calibration curve for toluene
Regression line and correlation coefficient: Y=0.6691 X-0.1939($0.6381<\beta<0.7001$, $\alpha=0.01$); r=0.9995($0.82<\rho<0.99$, $\alpha=0.01$). Gas chromatographic conditions: column, 3mm×4m glass column packed with 2% Silicone OV 1 coated on Chromosorb W(80-100 mesh, AW, DMCS); column temperature, 60℃; injection port temperature, 120℃; carrier gas and flow rate, nitrogen and 30ml/min; range, 10^{-2}. Peaks: (1) toluene; (2) n-butyl acetate(internal standard).

The calibration curve for hippuric acid showed a good linear relationship within the range of 0.1 to 1.0μg. The equation of the regression line was given by: Y=1.2587 X-0.1333 ($1.0875<\beta<1.4317$, $\alpha=0.05$) and the correlation coefficient was 0.

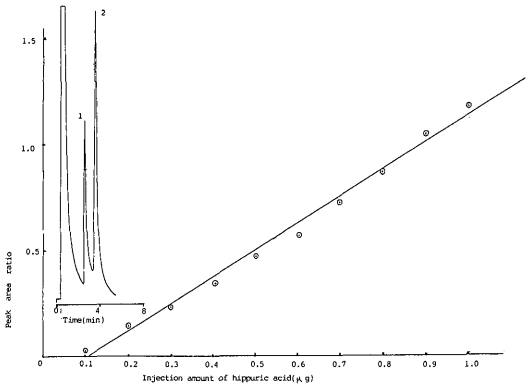

Fig. 4 Chromatogram and calibration curve for hippuric acid
Regression line and correlation coefficient: $Y=1.2587\ X-0.1333$ ($1.0857<\beta<1.4317$, $\alpha=0.05$); $r=0.9961$ ($0.93<\rho<0.99$, $\alpha=0.01$). Gas chromatographic conditions: column, 3mm×4m glass column packed with 2% Silicone SE 30 coated on Chromosorb W(80-100 mesh, AW, DMCS); column temperature, 190℃; injection port temperature, 230℃; carrier gas and flow rate, nitrogen and 35ml/min; range, 10^{-2}. Peaks: (1) hippuric acid methyl ester; (2) N-(m-toluoyl) glycine methyl ester(internal standard).

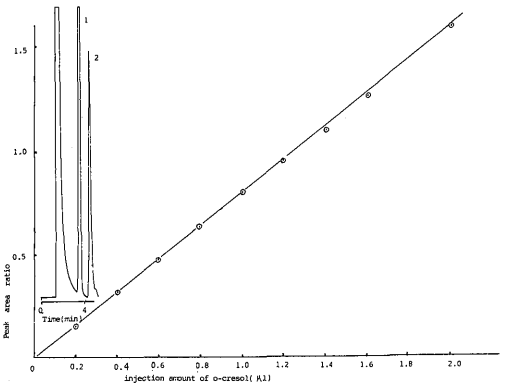

Fig. 5 Chromatogram and calibration curve for o-cresol
Regression line and correlation coefficient: $Y=0.7955\ X-0.0034$ ($0.7815<\beta<0.8095$, $\alpha=0.05$); $r=0.9999$ ($0.92<\rho<0.99$, $\alpha=0.01$). Gas chromatographic conditions: column, 3mm×4m glass column packed with 2% Silicone OV 17 coated on Chromosorb W(80-100 mesh, AW, DMCS); column temperature, 130℃; injection port temperature, 160℃; carrier gas and flow rate, nitrogen and 30ml/min; range, 10^{-2}. Peaks: (1) n-undecane (internal standard); (2) o-cresol.

9961 ($0.93<\rho<0.99$, $\alpha=0.01$).

The Gas chromatograph and operating conditions were as follows. Gas chromatograph: the same gas chromatograph; column: a 3mm×4m glass column packed with 2% Silicone SE 30 coated on Chromosorb W (80-100 mesh, AW, DMCS); column temperature: 190℃; injection port temperature: 230℃; carrier gas: nitrogen, 35ml/min, respectively.

Hippuric acid was extracted from 1ml of the urine with a mixture of ether and ethanol (9:1 v/v)[6]. The extraction was repeated twice. The extracts were combined, dried over anhydrous sodium sulfate, and filtered through dry filter paper. The filtrate was transferred to a distillation flask and evaporated to dryness under reduced pressure. The residue was dissolved in ether and the solution was transferred to a 10ml volumetric flask. The flask was washed several times with a small amount of ether and the washing solutions were combined. Finally, the solution was raised to 10.0ml with ether. One ml of this solution was transferred to a test tube, followed by addition of 1ml of N-(m-toluoyl) glycine ether solution as an internal standard. The methyl esterification was performed by introducing diazomethane gas into the test tube, and the reaction mixture was treated in the same way as that in the preparation of calibration curve.

4) Determination of o-cresol in urine

o-Cresol was dissolved in chloroform and the working standard solutions were prepared in the range of 0.5 to 5.0mg/ml. As an internal standard, the 2.5μl/ml concentrated chloroform solution of n-undecane was prepared. Following this, the mixture of 0.2ml of the working standard solution and 0.1ml of the internal standard solution was transferred to a conical flask and the volume of the solution was raised to 0.5ml with chloroform. The solution was well mixed and 1μl of the

solution was injected into the gas chromatograph.

The cailbration curve for o-cresol showed a good linear relationship over the rang of 0.2 to 2.0 μg. The formula of the regression line was given by: $Y=0.7955\ X-0.0034$ ($0.7815<\beta<0.8095$, $\alpha=0.05$) and the correlation coefficient was 0.9999 ($0.92<\rho<0.99$, $\alpha=0.01$).

The gas chomomatograph and operating conditions were as follows. Gas chromatograph: the same as above; column: a 3mm×4m glass column packed with 2% Silicone OV 17 coated on Chromosorb W(80-100 mesh, AW, DMCS); column temperature: 130℃; injection port temperature: 160℃; carrier gas: nitrogen, 30ml/min, respectively.

Two ml of the urine was transferred to a glass-stoppered test tube and 3ml of 15% hydrochloric acid was added[7]. The test tube was tightly sealed with a glass stopper and immersed in a boiling water bath to hydrolyse the conjugate. One hour later, the test tube was taken out from the water bath and cooled in tap water. After cooling, a small amount of sodium chloride and 1 ml of carbon disulfide were added. The test tube was tightly closed with a glass stopper, and shaken mechanically for 20 minutes, and the carbon disulfide phase was separated out. This extraction procedure was repeated once more and the extrads were combined. The combined extraed was dried over anhydrous sodium sulfate and filtered through dry filter paper. The filtrate was concentrated by being allowed to stand at room temperature for a while, and the concentrated filtrate was transferred to a conical flask containing 0.1 ml of n-undecane chloroform solution as an internal standard. The test tube was washed two or three times with a small amount of chloroform and the washing solutions were combined. Finally, the volume of the solution was raised to 0.5 ml with chloroform, the solution was well mixed, and 1μl of the solution was submitted to gas chromatographic analyses.

Results

The identification of the unknown peaks which appeared was performed mainly through the comparison of their retention times and corrective retention volumes with those the standard ingredients contained in lacquer thinner.

First of all, the analysis of the lacquer thinner which was discovered at the scene was carried out and, as a result, five ingredients such as acetone, ethyl acetate, n-butanol, toluene, and n-butyl acetate were identified.

Furthermore, in the analysis of the ingredients contained in the various tissues, 3 kinds of ingredients such as acetone, toluene, and n-butanol were detected, but the peak of n-butanol was very small.

Quantitative analysis of toluene was then carried out with a variety of tissues and the results were as follows: 0.11μl/g in the brain, 2.84μl/g in the adipose tissue, 0.64μl/g in the subcutaneous fatty tissues, 0.14μl/g in the liver, 0.051μl/g in the lung, 0.1μl/ml in the heart blood, 0.12μl/ml in the gastric fluid, and 0.10μl/ml in the urine.

On the other hand, the measured concentrations of hippuric acid and o-cresol in the urine, which were frequently used as the indexes[8,9] of exposure to toluene vapor, were 10.2μg/ml and 0.105μg/ml, respectively, and neither numerical value differed from that of a healthy adult man. In addition, the detection of methamphetamine and ethanol was attempted, but neither was detected.

Discussion

In the anatomical findings, plumonary edema, which is typical in cases of poisoning by lacquer thinner, had developed to a high degree. However, the degree of central fatty degeneration in the liver was slight and the measured values of hippuric acid and o-cresol in the urine were not different from those of a healthy adult male. Taking these results into account, the cadaver's career of glue-sniffing may be presumed to have not been very long.

On the other hand, the place where the cadaver was discovered was a very narrow toilet beneath a stairway, the capacity of which was only 3.34m^3. In fact, inasmuch as two young males occupied such a very narrow place, it may be considered that the oxygen deficiency quickly occurred and resulted in death.

If a healthy young male who has 64kg body weight were lying on the floor at 20℃, the necessary oxygen demand per 1 hour would be 14.4l[10] and, moreover, if this value is converted into the necessary air demand,

because raito of oxygen and nitrogen in the air is 1:4, the volume of air can be estimated as 72.0 l. Therefore, if two young males occupied such a small toilet for more than 10 hours, the volume percentage of the oxygen consumption reaches 42.0%.

As a result, the narrow toilet rapidly reaches a severe state of oxygen deficiency and such a circumstance may be presumed to accelerate death due to acute poisoning by lacquer thinner.

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