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Gases Released from Tissue and Analyzed by Infrared and Gas Chromatography/Mass Spectroscopy Techniques

To help establish the cause of or contributory factors leading to death, gas chromatography (GC) is commonly used to analyze volatile compounds given off as gases from postmortem tissues. Problems seldom arise during confirmatory analyses because available evidence indicates from the start what compounds are likely to be present. When little evidence is available, however, GC by itself is a poor technique for sample identification. Combining a mass spectrometer (MS) and computer system with a gas chromatograph has frequently solved the identification problem. A recent paper [*1*] describes the routine use of such a system to identify drugs and their metabolites in body fluids of overdose victims. In an effort to expand applications in this area, we describe a technique to release gases from tissues and to analyze toxic gases by a GC/MS computer system. Infrared (IR) spectroscopic analyses were also performed on all gas samples, and results were compared with analyses by the more sophisticated and expensive GC/MS computer method.

Materials and Methods

Instrumentation

Infrared Analysis—Infrared spectra were recorded with a Beckman IR-9 high-resolution spectrometer fitted with an adjustable 10-m gas cell. Final gas cell pressure was adjusted to a standard 18 psia (124 kPa absolute) with nitrogen for all calibration-curve and sample gas measurements. Measurement was made over the wavelength range of 2.5 to 25 μm (4000 to 400 cm^{-1}). Wavelength calibration indicates frequencies to be accurate within ± 5 cm^{-1}. Each identified compound was quantitated at the wavelength of strongest absorption where possible. Calibration curves were prepared by standard methods. For example, accurately measured amounts of a pure liquid such as toluene were injected into an evacuated IR cell simultaneously with a small flow of nitrogen. The cell was then pressurized with nitrogen to 18 psia (124 kPa absolute), and the IR absorption intensity of the toluene was measured at an appropriate wavelength free of interference from water, carbon dioxide (CO_2), or other expected gas constituents. Infrared calibration curve points were obtained by diluting the gas standard mix, final pressure

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being held constant. Samples of the original or diluted gas mixes were removed for use as standards in the GC/MS method.

Gas Chromatography/Mass Spectroscopy Analysis—The GC/MS computer system used in this effort consisted of a Varian Model 1400 gas chromatograph, a DuPont Model 21-0491 mass spectrometer, and a DuPont Model 21-094 data acquisition and control system. The data system utilizes an incorporated library [*2*] for mass spectral comparisons. The GC uses a low-volume splitter to divide gas samples between the GC flame ionization detector and the MS. A 3 m by 3.2-mm stainless steel microbore column containing 120–150 mesh Porapak Q (300 ml/min helium flow; −90 to +250°C at 10°C/min temperature programming) was used to effect separations. The identified peaks were quantitated with an Auto Lab IV computing integrator. The MS scanned at 4.5-s intervals over the mass range *m/e* 12 to 230. Each set of analyses was initiated with a preliminary scan through *m/e* 600 to insure that no volatile high-molecular-weight contaminants would escape detection.

Procedure

To minimize loss of volatiles, tissue specimens were bagged and quick-frozen immediately after removal from the victims. The polypropylene bags were heat-sealed and not opened until time for analysis. Transported specimens were packed in dry ice to insure no thawing of tissues would occur prior to analysis. Storage in the laboratory was at 0°C. Immediately before use, tissue samples were divided into suitably sized portions and weighed; they were analyzed untreated or after maceration in an electric blender. The portions were as large as 70 g to insure identification by IR. If the more sensitive GC/MS technique was used alone, as little as 1 g of tissue would be needed. Blood and urine samples were thawed as needed with appropriately sized portions withdrawn and used immediately. In all instances, samples were transferred before significant thawing into a modified 3-litre anaerobic culture jar incorporated in the system schematically illustrated in Fig. 1. The jar was immediately sealed and evacuated to less than 1 mm Hg

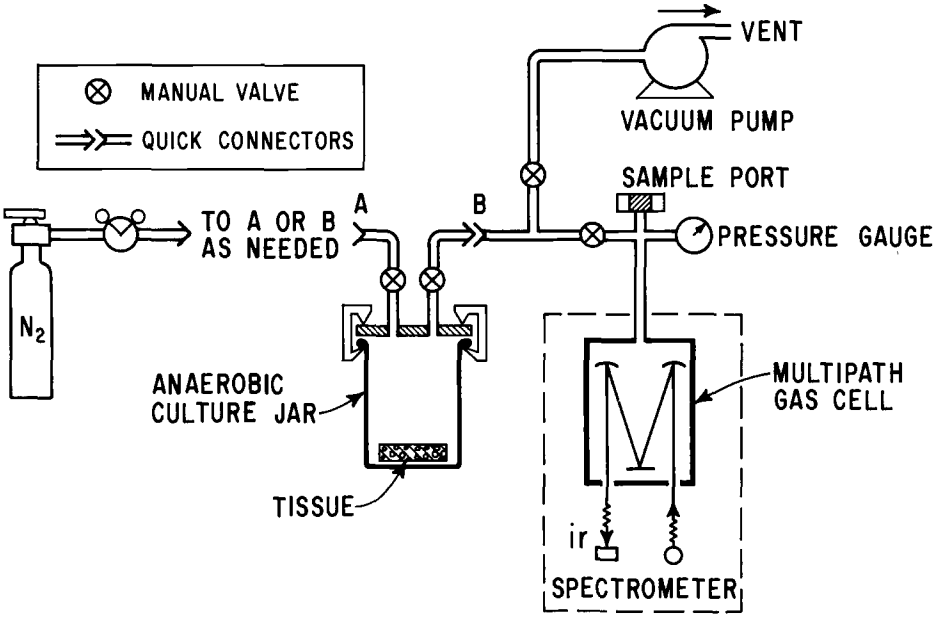

FIG. 1—*Schematic of the apparatus used to collect gases released from postmortem tissue samples.*

within 1 min using a high-volume-capacity vacuum pump. By closing the valve to the vacuum pump and opening the valve to the previously evacuated gas cell, a total evacuated volume of about 6 litres as measured by a spirometer resulted. The release of gases started immediately and was monitored by following the buildup of pressure within the system and by measuring the IR spectrum at 30-min intervals. When no further increases were observed in pressure or contaminant IR absorption intensities, dry nitrogen from a high-pressure cylinder was introduced into the IR cell until the cell pressure reached 18 psia (124 kPa absolute). At this point the sample was ready for analysis.

Infrared spectra were recorded and used to identify contaminants and to verify GC/MS results. Identification was accomplished by comparison with IR spectra found in the Sadtler [*3*] reference or personally collected libraries. Estimates of contaminant concentrations were made from absorption intensity measurements of bands most free of interference from water and other gases present. Examples of absorption bands used are listed in Table 1.

For GC/MS analysis, a 30.0-ml gas sample was removed from the IR cell through a rubber septum/port with a gastight syringe. In each instance, the sample was then pressurized to about 2200 mm Hg (accurately measured by a routinely calibrated pressure transducer) in the GC/MS sample loop (2.987 ml). Helium was used to flush the sample out of the loop and into the GC, where it was separated and its components were identified by MS and quantitated by GC.

TABLE 1—*Infrared absorption bands used for quantitating gases released from postmortem tissue. For a given contaminant, absorption intensity decreases from left to right.*

Contaminant	Absorption Band, cm^{-1}		
Toluene	731	463	1034
1,1,1-Trichloroethane	730	1090	...
Methyl chloride	732	1360	...
p-Xylene	778	485	1520
Acetone	1219	528	1740
Trichloroethylene	851	947	...
Ethanol	1065	880	1400
Isopropanol	960	1150	1370
Methyl ethyl ketone	1175	1375	1745

Results and Discussion

The postmortem tissues analyzed for this study were obtained from three victims of what appeared to be toxic vapor overdoses. The fatalities involved young airmen in three separate incidents. All of the airmen were found dead with plastic bags over their heads. None of the plastic bags or their contents was saved for analysis, so postmortem tissue was the only source of information regarding circumstances responsible for or leading to the deaths.

Case 1 involved an airman found dead in a hospital where he worked. Co-workers indicated that he was abnormally active during the day he died. Since visual and odor inspection of the plastic bag found placed about the victim's head had given no indication of glue or other solvent, it was suspected that he was probably inhaling one of the more readily available hospital gases such as fuel gas, trilene, or nitrous oxide (N_2O).

In this case there was no need to analyze by methods other than IR. The top spectrum in Fig. 2 illustrates typical IR data obtained from gases of both brain and lung tissue at 18 psia (124 kPa absolute) and a 2-m path length. The bottom spectrum is a

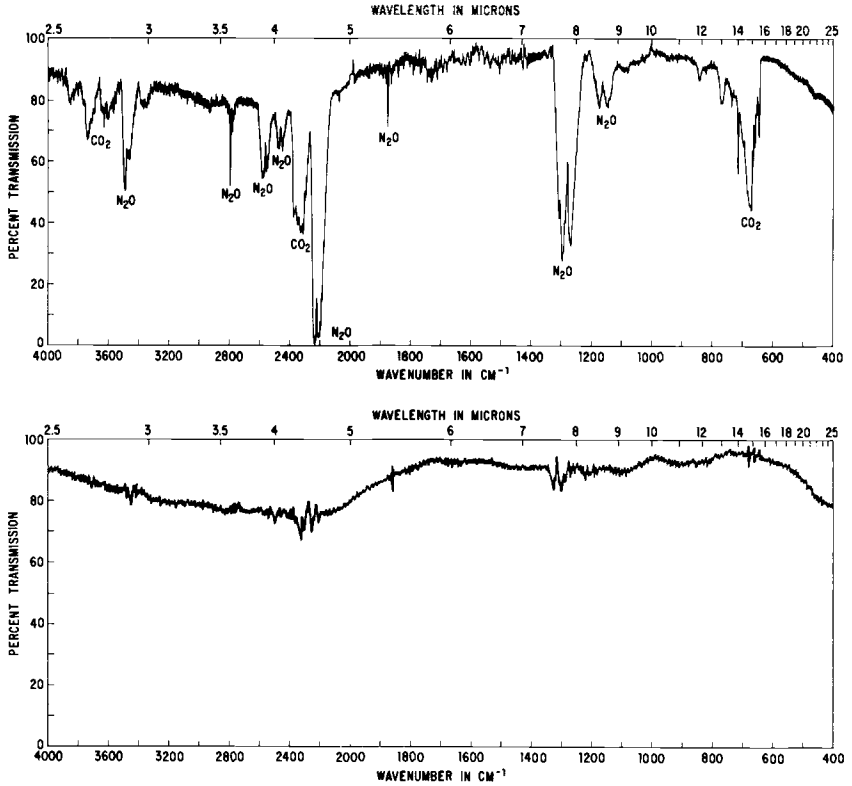

FIG. 2—*Typical infrared spectra during Case 1 analyses*: (top) *gases released from lung tissue and* (bottom) *background scan.*

typical background scan made with contaminant-free nitrogen at the same pressure and path length; it can be used as a guide to isolate and identify absorption peaks arising from contaminants and to provide a baseline for quantitating. The only foreign gas or vapor detected was N_2O; CO_2 is ubiquitous in moist tissue and was present in background scans made from uncontaminated tissues. The relatively low concentration of water and its low level of interference at these gas cell pressures, in this as well as the following analyses, is surprising from a technical viewpoint.

Concentrations of the N_2O are given per unit weight of tissue (Table 2). The listed values (220 mg/100 g of brain; 242 mg/100 g of lung) are minimum values since evacuation of the cell and repetition of the procedure produces additional N_2O, although not in nearly as large an amount. The obtained equilibrium situation (distribution of N_2O between the tissue and gas phases at some constant ratio) contributes to an uncertainty in quantitating that will probably affect the measurement of most gas and vapors with this technique. Slight modifications to the system to permit repeated gas release with an accumulation of contaminants can be easily made. However, the problem does not appear to be severe enough to warrant an adjustment; extremely accurate measurements are not of overriding importance in providing the type of practical information desired in most work of this nature.

Case 2 involved an airman whose death was suspected to be a result of sniffing glue. The gases released from postmortem brain and lung samples were separately admitted into the IR cell system. Complete release of gases required about 4 h in each instance.

TABLE 2—*Minimum concentrations of volatile contaminants observed in postmortem brain and lung tissues obtained from drug overdose victims.*

		Contaminant Concentration			
		Brain		Lung	
Case	Contaminant	mg/100 g	Relative[a]	mg/100 g	Relative[a]
1	N_2O	220	(100)	242	(100)
2	toluene	1.03	(100)	0.43	(100)
	trichloroethylene	0.04	(3)	0.05	(9)
	ethanol	undetected		0.07	(32)
	acetone	0.10	(15)	0.07	(26)
	isopropanol	undetected		0.04	(14)
	methyl ethyl ketone	0.15	(18)	0.12	(35)
	2-butanol	undetected		0.05	(13)
	methyl chloride	0.03	(6)	undetected	
3	toluene	1.23	(100)	0.34	(100)
	1,1,1-trichloroethane	0.55	(31)	0.20	(41)
	methyl chloride	0.03	(4)	0.03	(18)
	xylenes	0.27	(19)	trace	
	acetone	0.04	(5)	trace	
	propane	0.01	(2)	undetected	

[a] Relative concentrations (mole/100 mole of toluene) of the contaminants for a given tissue from a single procedure.

The buildup in contaminant concentrations was monitored by repeated IR scanning at 30-min intervals. No change in contaminant IR absorption intensities suggested the release of gases was complete. The cell was pressurized to 18 psia (124 kPa absolute), an IR spectrum recorded, and a sample removed for GC/MS analysis.

Typical examples of the recorded IR spectrum (brain) and mass chromatograms (lung) obtained from the tissue gases are presented in Fig. 3. Identified contaminants and estimates of their concentrations are listed in Table 2. In this case, concentration estimates were derived from GC/MS data, whereas IR data were used primarily to verify GC/MS identification. The list of substances for Case 2 qualitatively reads like a universal recipe for a glue solvent [*4*]. All contaminant concentrations are given in terms of weight (mg/100 g of tissue) and mole (moles of contaminant per 100 moles of toluene detected) relationships.

Case 3 involved an airman presumed dead as a result of aerosol paint spray inhalation. Analyses of postmortem tissue samples followed the same procedures used in Cases 1 and 2 except that a number of duplicate runs were made to observe the effect of sample pretreatment. Pretreatment consisted of tissue maceration in an electric blender immediately before analysis.

Infrared spectra recorded for gases released from both brain and lung tissue, untreated and pretreated, are qualitatively similar to the top spectrum in Fig. 4; this spectrum is a recording of gases released from macerated brain tissue. Quantitative estimates of the volatile contaminants are listed in Table 2 and are the result of measuring the appropriate IR absorption band intensities (except for propane, which was estimated during the GC/MS analysis). The list for Case 3 (Table 2) reads like a formulation of aerosol paint contents [*4*], with methyl chloride and propane being the propellants and the other organic compounds being the solubilizing vehicles.

Table 3 compares the results of untreated and macerated tissue analyses. Observed

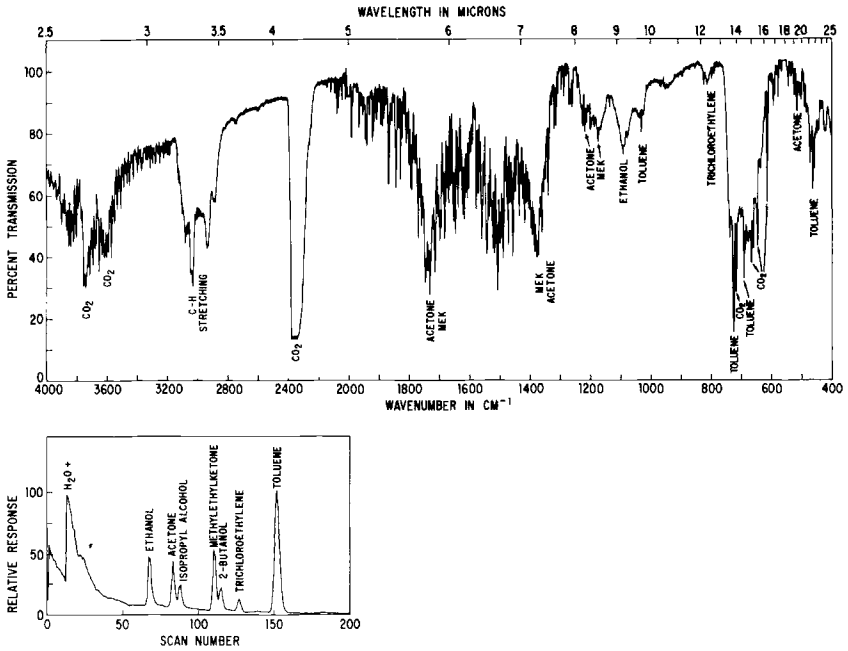

FIG. 3—*Case 2 analyses:* (top) *typical IR spectrum of gases released from brain tissue and* (bottom) *mass chromatogram of gases released from lung tissue.* MEK = *methyl ethyl ketone.*

contaminants and their concentrations were essentially identical, whereas the length of time needed to complete the release of gases differed markedly. That is, reaching maximum contaminant buildup before initiating IR and GC/MS analysis required 4 h. The gases released from macerated tissue reached equilibrium in 30 min, which suggested that a reduction in total analysis time could easily be obtained. The only negative result of tissue maceration was a faster buildup and higher final concentration of water, which could interfere in the IR identification or measurement of certain substances. This problem was not serious in our efforts and is of no concern if analyses are accomplished solely by GC/MS.

Conclusion

Brain and lung tissues were good sources of gases for postmortem assays used to establish death by accidental or intentional inhalation of toxic organic gases and vapors. Liver tissue, blood, and urine samples retained such small amounts of the inhaled volatile contaminants that they could be considered useless for routine assay. Based on our results, we judge brain tissue to be superior to lung.

The GC/MS computer technique proved superior to the IR method except for cost. For analyzing very small gas samples, the GC/MS computer technique appears to be essential. When tissue samples are large enough, however, the IR gas cell method is totally adequate for confirmatory analysis of most gases and gas mixtures. Some difficulties can arise where sample histories are not known and experienced personnel are not available. Overall, the IR method is judged superior to GC and is a practical, more cost-effective alternative to the GC/MS computer method for most postmortem tissue assays.

Our present conclusions are made on a somewhat limited experimental data base, which we plan to expand by performing postmortem analyses on a more routine basis.

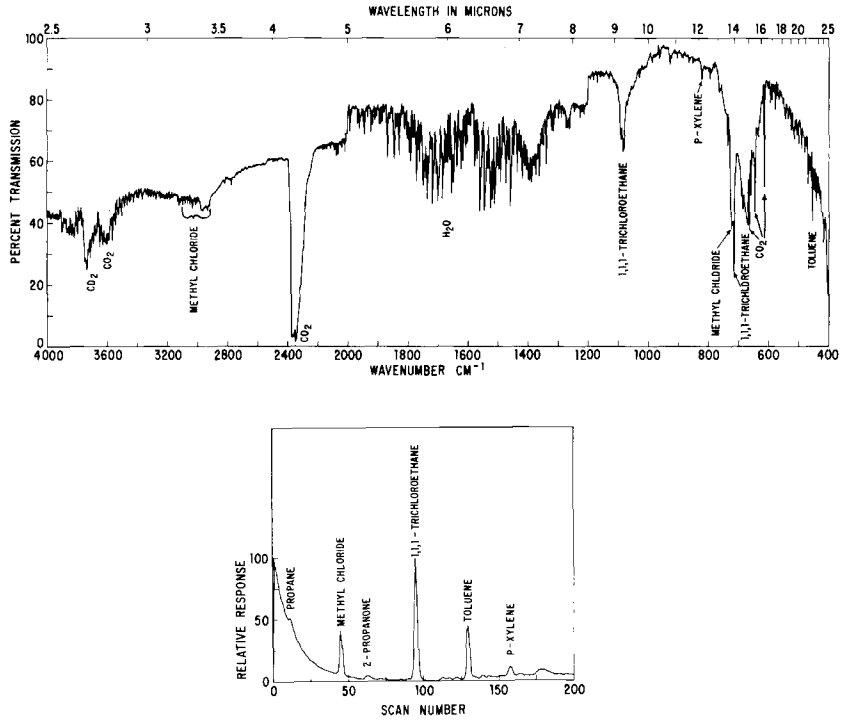

FIG. 4—*Case 3 analyses*: (top) *typical IR spectrum of gases released from macerated brain tissue [18 psia (124 kPa absolute) and 18-m gas cell path length] and* (bottom) *mass chromatogram of nonmacerated brain tissue.*

TABLE 3—*Comparison of contaminant concentrations found in gases of macerated and untreated tissues from Case 3.*

	Contaminant Concentration, mg/100 g tissue			
	Untreated		Macerated	
Contaminant	Brain	Lung	Brain	Lung
Toluene	1.23	0.34	1.16	0.31
1,1,1-Trichloroethane	0.55	0.20	0.49	0.05
Methyl chloride	0.03	0.03	0.02	0.01
Xylenes	0.27	trace	0.34	0.16
Acetone	0.04	trace	0.05	undetected

Summary

Two techniques for analyzing contaminants released as gases from postmortem tissues were described and compared. One technique used gas chromatography/mass spectrometry (GC/MS); the other, infrared spectroscopy (IR). Brain, lung, liver, blood, and urine specimens were obtained from suspected drug-overdose victims whose deaths were contributed to or caused by inhalation of unknown gases or vapors during the period immediately preceding death. Gases from the postmortem tissues and liquid samples were separately admitted into an evacuated IR gas cell, the IR spectra recorded, and

gas samples then removed for GC/MS analysis. Nitrous oxide, glue, and paint solvent constituents were identified and measured. Only the brain and lung tissues contained measurable amounts of inhalants. Both IR and GC/MS methods were adequate for normal confirmatory analyses; the GC/MS system was judged superior for fast routine efforts normally hampered by incomplete sample history.

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