Use of Gas Chromatography and Mass Spectrometry to Analyze Underivatized Volatile Human or Animal Constituents of Clinical Interest

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The study of volatilizable components in human and animal samples by gas chromatography and mass spectrometry is reviewed for the years 1970–1975. Only naturally volatile, underivatized compounds are considered, from sources such as body fluids, tissues, and breath. Where possible, the clinical samples are discussed in terms of normal profiling, aberrations associated with disease states, and specific compound analyses. A brief descriptive technical section is included, stressing types of sample preparation currently being used.

I. Introduction

The purpose of this review is to summarize for the clinician the recent advances in the study of human and animal samples by gas chromatographic (GC) and mass spectrometric (MS) methods. Development of sophisticated instrumentation for GC and MS has made it possible to use these tools separately or in conjunction with a computer (COMP) to assess samples quickly and reliably. We have not attempted to give here a technical discussion of instrument design. Excellent descriptions of GC and MS instrumentation are already available and are cited in subsequent sections of this review. We do, however, give a short descriptive technological section which, we hope, will enable the reader to evaluate the papers cited. A noncritical compilation of the systems currently used is provided, together with descriptions of the type of results obtained. The reader is thus provided with an opportunity to evaluate these systems and results with respect to his own specific interests.

This review encompasses the years 1970–1975. Only naturally occurring volatiles are considered, i.e., materials reasonably volatile for direct analysis by GC without decomposition and without further derivatization. The samples examined are all of either human or animal origin. Furthermore, only GC-MS-COMP analysis techniques are discussed, used separately or in combination.

The review is organized to first present a section on technology that is subdivided to include discussions of general GC-MS usage and preparative methods of sample analysis. The results obtained by using these techniques will be discussed in the following sections. A short section of concluding remarks describes in general terms the state of the art as well as future potential.

II. Technology

In this section we shall discuss some general aspects of GC-MS usage that are pertinent to the understanding of the breadth as well as the limitations of this analytical method for the clinician. No attempt will be made to present an in-depth coverage of the technical aspects of GC-MS, since, as cited below, excellent reviews are already available. We would like to especially direct the reader to the recent review by Lawson (1), which is particularly geared toward the interests of the clinical chemist, stresses higher molecular weight compounds, and includes an applications section. A more detailed treatment in the present review is devoted to the preparative methods for sample analyses, because the preparative methods used for the GC-MS approach to analysis are often quite different than those commonly used in the clinical laboratory. Furthermore, the preparative method chosen may be a determining factor in the type of results obtained.

A. General Background and Terminology of Gas Chromatography and Mass Spectrometry

Jellum et al. have stated (2):

It seems reasonable to assume that if one were able to identify and determine the concentrations of all compounds inside the human body, including high molecular weight as well as low molecular weight substances, one would probably find that almost every known disease would result in characteristic changes of the biochemical composition of the cells and of the body fluids. An important problem, therefore, concerns the detection and analysis of the various compounds present in, e.g., the blood and urine.
GC is the most suitable method currently available for resolving into individual components the highly complex mixtures of lipid and carbohydrate compounds encountered in biological specimens. Coupling of a MS to a GC allows spectral data to be collected on each peak eluted from the chromatographic column and vented into the MS. The different means by which MS data can be obtained will be enumerated later. In general, however, information concerning the weight of the ionized molecule and fragment ions into which the molecule can be broken is obtained, along with information concerning the abundance of these ions. This type of information allows for unequivocal identification of the compounds to be made in most instances. Since Holmes and Morrell first coupled GC to MS in 1957, many improvements have been made to make this technology more suitable for the study of biochemical problems, and most recently progress has been directed toward making this technology applicable to clinical problems. Rapid data collection, reduction, and interpretation is a "luxury" in pure research but a necessity for clinical applications. Real-time, on-line data acquisition, treatment, and interpretation afforded by combined GC-MS-COMP systems has made clinical applications a more recognizable goal.

Some excellent recent reviews of MS are available to the interested reader (1, 3-5). Therefore, we have restricted ourselves here to only a brief section dealing with the MS terminology that the reader may encounter in the articles cited in this review. A recently updated selection of MS reference data for drugs and their metabolites has appeared, by Finkle et al. (6, 7).

In the papers we review here GC methods were used to resolve the volatile organics present in body fluids, breath, and tissues. GC basically involves:

(1) introducing the sample into the chromatograph,

(2) resolving the components on the chromatographic column,

(3) detecting the components, and

(4) recording the chromatogram.

The sample is usually introduced into the chromatograph by syringe-injection through a septum into the heated injection port of the chromatograph, where sample volatilization occurs. The sample thus volatilized is swept from the injection port onto the chromatographic column by the carrier gas. Carrier gases vary depending on the type of detector used. Most commonly helium or nitrogen will be used if a flame-ionization detector is used, whereas argon/methane is usually the carrier gas when electron-capture detectors are used. When head-space analysis techniques are used, means of sample introduction other than syringe-injection are often used (vide infra).

Various types of chromatographic columns are used to resolve the components. They are generally made from either glass or stainless steel, although other metals are sometimes used, such as copper, aluminum, or nickel. The column itself should not bond or react with the compounds under study. For this reason glass is often the preferred material, because it is generally less reactive than steel or other metals that possess catalytic activity at increased temperatures. Separation is achieved in the chromatographic column by partitioning the sample between the liquid phase (sometimes referred to as the "stationary phase") and the gas phase (carrier gas). Choice of the proper stationary phase is critical.

Columns may be either of the packed or capillary type. Thus columns fall into four basic structural types: glass or steel packed columns and glass or steel capillary columns. With packed columns the stationary phase is conventionally coated on an inert solid support and packed into the column and held in place with plugs of glass wool. Capillary columns (glass or steel) are generally either wall-coated open tubular columns, where the stationary phase is coated directly on the column wall, or support-coated open tubular, where the stationary phase and a fine-mesh-size inert particle are introduced into the capillary as a slurry. There are some inherent problems associated with the wetability of glass surfaces. Different methods of pretreatment of the glass such as surface corrosion (etching), carbonization, formation of polymer layers on the glass surface, formation of oriented monolayers, and chemical modification of silanol groups have been discussed (8). Glass capillary columns are still prepared by a variety of methods and no one single method seems to have emerged yet as the ideal one.

Four types of GC detectors used most frequently in the studies reported on in this review are: flame-ionization detectors (FID), electron-capture detectors (ECD), flame-photometric detectors (FPD) and thermionic detectors (TID). These detectors vary in mode of operation, sensitivity, linearity, and selectivity of response. Sensitivity and linearity of response of these various detectors differ, so a brief description of each mode of detection is provided together with a few references.

In the operation of the flame-ionization detector (FID), the sample eluted from the chromatographic column passes through a jet into a flame ignited and maintained by the combustion of air and hydrogen. The sample is ionized in the flame and an electric field maintained between the jet and the collector causes the ion to migrate to the detector. Thus the FID is designed to detect a positive increase in current. The FID operates in a sensitivity range of $10^{-10}$ to $10^{-11}$ grams and has a linear dynamic range over a $10^6$ to $10^7$ change of concentration of sample. The FID is a nonselective type of detector with response proportional to effective carbon number. Tables have been published showing responses of various compounds in the FID and their effective carbon numbers (9).

The electron-capture detector (ECD) utilizes either $^3$H (tritium) or $^{60}$Ni as a beta-emitting source. These expelled electrons migrate toward the anode, and thus a standing current in the vicinity of $10^{-9}$ A is maintained. Elution of an electron-capturing species into the ECD results in the decrease of standing current. Thus,
in contrast to the FID, a current decrease is measured. The ECD operates in a sensitivity range of $10^{-12}$ g for high electron-capturing species. The ECD has a small linear dynamic range, operating over only a $10^1$–$10^2$ change in sample concentration, but it is a highly selective detector, the response for polychlorinated organics being $10^6$ times greater than the response for hydrocarbons. Thus calibration for each compound that is being analyzed for is required. Tables illustrating the difference in response for several compounds have been published (9, 10).

The flame-photometric detector (FPD) allows for the nonsimultaneous analysis of sulfur- or phosphorus-containing organic compounds. When subject to combustion in a hydrogen-rich flame, sulfur or phosphorus-containing organics emit light of a characteristic wavelength. The emission spectra at 395 nm (sulfur) and 526 nm (phosphorus) is measured by a photomultiplier tube. Sensitivities in the region of $10^{-11}$ gram for phosphorus and $10^{-10}$ gram for sulfur have been reported. Phosphorus response reportedly is linear in the 0.2–300 ng range, while sulfur response has been reported to be linear in the 0.2–100 ng range (9).

Thermionic detectors (TID) such as the alkali-metal salt detectors have been used for specific detection of nitrogen-, phosphorus-, and chlorine-containing compounds. There are several configurations of thermionic detectors; they usually consist of a combination of an FID block and a ceramic probe or collector coated with an alkali metal. Proper selection of the alkali metal can optimize the detection of a particular hetero-atom. Although the mechanism is not entirely understood, these hetero-atoms exhibit an enhanced response in a flame containing the appropriate alkali metal salt. Sensitivity of the TID has been found to be a function of detector design, alkali-metal salt, hetero-atom selected for, and number of hetero-atoms per molecule. The sensitivity of the TID also diminishes with diminishing alkali-metal salt concentration. This makes it difficult to report the general sensitivity of the TID. Likewise, linear dynamic ranges of $10^1$–$10^5$ have been reported in the literature, depending on the design of the detector (9). It seems that sensitivity is variable with the TID and careful calibration is required in quantitative studies.

When combined GC-MS is used, the column effluent, instead of entering a detector, is transferred into the MS. GC-MS interfacing is another subject, beyond the purpose of this discussion.

For the person unfamiliar with MS, a few definitions of terms and some basic concepts might prove useful in understanding this paper, as well as those cited.

MS basically involves:

1. converting neutral molecules to ions, and ionized fragments,
2. separating these ions on the basis of their mass/charge ratios,
3. detecting and amplifying the signal generated by these ions, and
4. recording the mass spectrum.

Each of the above phases of analysis can be achieved in one of several ways. The different forms of ionization will be discussed because they affect the type of mass spectrum generated and have different sample-size requirements. There are primarily three methods of ionization that have been applied in organic analyses of clinical interest: electron-impact ionization, chemical ionization, and field ionization. In electron-impact MS the molecules under study are bombarded with electrons (usually of a 70 eV ionizing voltage, although a "milder" 20 eV ionizing voltage is sometimes used), and the resulting inelastic collisions between the molecule of interest and the electrons results in the formation of two types of ions, molecular or parent ions, and fragment ions. This ion formation is done at pressures in the range of $133 \times 10^{-6}$ to $133 \times 10^{-7}$ Pa ($10^{-5}$–$10^{-7}$ Torr). The parent ion is the singly-positively-charged species of the molecule resulting from the loss of one electron, and thus it has the same molecular weight as the un-ionized molecule under study. Fragment ions are produced by breakage of certain bonds in the molecule with concomitant ion formation. Sometimes rearrangement of primary fragment ions occur, resulting in formation of secondary fragment ions. The mass spectrum, consisting of the molecular and fragment ions produced, is generally characteristic of the compound under investigation. With electron-impact MS sometimes a molecular ion is not obtained when there is a minimal amount of sample (less than 50 ng) because some of the molecules are "broken down" into fragment ions and do not contribute to the signal generated for molecular ions. Also, some molecules are more subject to fragmentation under electron-impact conditions than others. For example, alcohols generally exhibit very-low-intensity molecular ions. For this reason, when there is a minimal amount of sample available for analysis, alternative ionization techniques such as chemical ionization or field ionization, which generate few fragment ions if any, are desirable.

Chemical ionization MS is not as harsh an ionization technique as is electron-impact, and less molecule fragmentation occurs. Chemical ionization MS results in the formation of quasi-molecular ions, having the formula MH$^+$, where M is the compound under analysis. Chemical ionization occurs at a pressure of about 133 Pa (1 Torr). A reaction gas is ionized by electron impact. The primary gas ions react again with un-ionized gas molecules to form secondary gas ions, which are responsible for the ionization of the compound under study. Again, the spectrum obtained is characteristic for the compound under study.

Field ionization MS also generates spectra with strong molecular ions and fewer fragment ions than electron-impact MS. Ionization is achieved by stripping electrons from molecules in an electrical field of approximately $10^7$ V/cm. For compounds of low volatility, they can be ionized without volatilization by similar techniques, referred to as field desorption MS.

 Whereas chemical ionization MS and field ionization or field desorption MS usually allow easier determina-
tion of an empirical formula because of stronger molecular ions, the decrease in fragment ions results in less structural information about the molecule. Therefore, depending on the type of study and compounds that are being analyzed for, the advantages and disadvantages of the various ionization techniques should be considered. If additional information about ionization techniques or other aspects of MS is required, there are several reference texts (11-13).

Another technique in MS exists, which by increasing sensitivity decreases the amount of sample necessary for detection. These techniques do not involve modes of ionization of the sample, but rather selective detection of the ions. These techniques are referred to as (1) single-ion monitoring and (2) multiple-ion monitoring, also referred to as selective-ion monitoring or mass fragmentography (5). Single-ion monitoring involves focusing the MS in such a way as to allow for the detection of only one ion, which is exclusively characteristic of the compound under analysis. This allows for detection of smaller amounts of sample, primarily for two reasons. First, contributions from column bleed and other compounds present in the sample do not obscure the presence of the ion of interest, because no other ions formed are analyzed for. Second, because it takes less time to record the presence of one ion than to record the whole spectrum of ions formed during an analysis, more time is spent monitoring for the presence of the particular ion of interest. Thus the probability of monitoring the ion when its concentration is at its maximum is increased and overall sensitivity is increased. Between 10 ng and 1 pg have been detected by these procedures (5). Multiple-ion monitoring in principle is the same. Instead of monitoring the whole spectrum of ions produced, two to five ions may be selected for. This can be done in a variety of ways, the most common method for single-focusing instruments is by alternating the accelerating voltage; alternating the accelerating and electric sector voltage in double-focusing MS and analyzer alteration in quadrupoles and dodecapes also achieves the same results (4). A priori, single-ion monitoring and multiple-ion monitoring MS can be used only if specific compounds are being analyzed for.

B. Preparative Methods

Body fluids. Jellum's hypothesis, that biological fluid composition will often reflect diseased-state aberrations, has been substantiated by the characterization of over 40 metabolic defects, mostly in amino acid metabolism, by GC-MS analyses of blood and urine (2, 14). Some excellent publications focus on clinical applications of GC-MS-COMP systems for the study of body-fluid constituents of higher molecular weight (15, 16). Such studies in higher-molecular-weight organic compounds have proceeded more rapidly than studies on body-fluid constituents of lower molecular weight. One of the contributing factors is the inherent difficulty encountered in extraction and analysis of low-molecular-weight compounds, because of their volatility. Several special approaches to extraction procedures and sample analysis have been used lately that attempt to circumvent these inherent problems.

Methods of sample pre-treatment for the analysis of low-molecular-weight volatile organics in body fluids can be classified into three basic categories: solvent extractions, head-space techniques, or direct chromatographic injection. All methods currently used have both advantages and disadvantages. Naturally, each investigator believes that the method he uses is the best for the type of results he is attempting to produce. It is not our intent to judge the relative merits of the various procedures currently in use, but to summarize them and direct the reader to representative studies with these various methods, so that independent evaluations can be made.

Solvent-extraction techniques represent a frequently encountered method of sample preparation. Described below is a generalized, composite sample preparation method, utilized with a few variations by a number of individual investigators for examination of volatile organics in blood (17-23), urine (23-27), and cerebrospinal fluid (28).

Many investigators add an internal standard as an initial step of the procedure. The sample is usually either acidified (to facilitate extraction of acidic metabolites) or made basic (to facilitate extraction of basic components), depending on the nature of the compounds to be analyzed for. Next, the aqueous fluid of adjusted pH is extracted by shaking with an appropriate organic solvent. It is necessary to centrifuge the sample in most instances, to separate the organic and aqueous layers. The organic phase is often then back-extracted with an aqueous solution of the appropriate pH. Concentration of the organic extract for direct chromatographic injection is the final step in the procedure. This is done in many ways, most commonly by evaporating the solvent under a stream of nitrogen, although one investigator reports a procedure for concentration by use of a fractionation column (20). Because of the similarity in the volatility of the solvent to be removed and the compounds being analyzed for, sample concentration is a critical step of the procedure, to be conducted with caution. In one major deviation from this generalized scheme of solvent extraction, the extract was shaken with Dowex resin as part of the procedure (27). Most of the studies involving blood require 1-3 ml of plasma or serum for analysis; one study requires 15 ml of plasma (20). Analyses of urine covered a broader range of sample requirements, from 2-4 ml of urine (26, 27) to 450-500 ml (24, 25).

Various methods of head-space analysis techniques represent the most frequently used method of sample preparation. Three major types of head-space techniques are described: direct head-space analyses, total-trapping head-space analyses, and selective-trapping head-space analyses.

Direct head-space analysis usually involves equilibration of the sample (blood, urine, etc.) with the head-space in the container and direct injection of a
portion of this head-space gas for chromatographic analysis. One investigator using these techniques has reported accurate measurements in the ppm range of volatile organics (29).

Total-trapping generally involves sparging the heated sample with inert helium gas, which sweeps the thermally extracted components from the sample head-space and concentrates them in a trap held at liquid-nitrogen temperatures. Heating the sample and purging it with a gas facilitates forcing the equilibrium of compounds in the sample in favor of the head-space. These volatile components concentrated in the trap are next “flushed” (by means of a heat gun) from the cold trap onto the chromatographic column (30). Pauling et al., borrowing from methods used in the flavor-analysis industry, used total-trapping head-space analysis to study urine vapor and breath (30). However, water vapor in the sample eventually freezes, causing plugging of the trap and the termination of the collection process. Thus, in subsequent studies, this research group introduced a Chromosorb-101 trap into the system prior to concentration of the organics in the cold trap, in an attempt to reduce problems caused by the water vapor (31). An in-depth description of several purification precautions that should be taken with gases used in head-space analysis is given by the same investigators (32). Upward of 100 components in urine can be resolved chromatographically on samples prepared from a few hundred milliliters of urine by total-trapping head-space techniques. We found no current reports in which total-trapping head-space techniques were used. Evidently this was an evolutionary stage in the development of head-space techniques and has currently been replaced by selective-trapping head-space analysis (vide infra).

Selective-trapping head-space analysis techniques have been used to study volatile components in urine (33-40), blood (41-44), and cerebrospinal fluid (36). Organics thermally displaced from the aqueous sample are bound on a poly(p-2,6-diphenylphenylene)oxide (Tenax-GC) adsorbent. The performance of several polymers was tested to find one with high thermal stability, low affinity for water, and high affinity for organics. Tenax-GC was found to be the adsorbent best suited for these purposes. Because of its low affinity for water, water vapor in the sample does not present a problem as it does in total-trapping. The Tenax-GC with adsorbed organics is placed directly into the injection port of a GC, where desorption occurs. The desorbed organics are concentrated in a cryogenically cooled precolumn and then “flushed” (by elevating precolumn temperature) onto the chromatographic column. By use of selective-trapping head-space techniques, nearly 200 components have been detected in urine in 100-ml samples. Approximately 65 of these components have been identified by MS (34). About 100 components have been detected in 10-ml plasma samples (40, 41, 44), and about 40 of these have been identified by MS.

“Direct” chromatographic analysis has not been used as frequently as some other methods. It entails injection onto a chromatographic column of the untreated body fluid sample. Direct injection of blood has been used to measure the concentration of methanol, ethanol, acetone, isopropanol, and low-boiling hydrocarbons associated with glue sniffing (45). Another investigator analyzes directly for methanol, ethanol, and acetone by direct injection of the blood sample into a boat-shaped vessel, where volatilization occurs. The unevaporated residue left in the boat is removed after each run. Reported, 150-200 blood samples could be processed in an 8-h work day, with ±5% accuracy (46).

Tissues. Solvent extraction techniques have been used by several investigators (see, e.g., 47-51) and generally involve treatment of the minced or homogenized tissues in a way similar to that just described for body fluids. The nature of the organic solvent(s) chosen is often dictated by the drug under investigation (48, 50) or by the detector used for the GC instrument (47).

Using selective-trapping head-space techniques (see section II.B above) the GC profiles obtained from approximately 4-6 g of liver, brain, and lung tissue from rats (43) have been studied. In this study, the required tissue specimens were removed immediately upon killing the animal and stored frozen. All steps in the tissue work-up (washing, mincing, and homogenization) were done at 0°C, to minimize loss of volatile materials.

One investigation used direct probe insertion into the MS of a 2-3 mg dehydrated sample of skeletal muscle to determine concentrations of specific compounds in the μg/g (ppm) range (52). High-resolution MS and multiple-ion monitoring techniques were used.

A report on the determination of few-nanogram amounts of barbiturates in tissues by use of multiple-ion monitoring has appeared (21). Details of sample pretreatment were not given.

Breath. Collection of volatile effluents in breath presents certain advantages as well as inherent difficulties. Breath, as a source for analysis of volatile organic components, offers the advantage of causing no patient discomfort, thus enabling more frequent sampling than is possible in the case of blood analysis, for example. On the other hand, breath-sample collection presents some unique problems, which will be discussed here.

One particular analytical problem encountered in the collection of breath samples is the introduction of impurities in the air inspired by the individual, or from components contributed by the collection apparatus. Ellin et al. (53), in their analysis of volatile human effluents, discuss at length the purification of air sources supplied to the sample donor. They also describe in detail the precautions taken in the construction and cleaning of collection chambers.

Several problems inherent in breath analysis are discussed by Dubowski (54, 55). Dead-space air (that volume of expired air that has not engaged in any gaseous exchange across membranes) does not reflect the body’s metabolism, but rather only the inspired room

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air. Because the dead-space volume differs from individual to individual, it is difficult to predict when expiration of dead space volume ends and expiration of alveolar air commences. Preliminary data (54) indicate that breath temperature measurements can be indicative of when the alveolar plateau is reached and breath sampling should begin. If inferences are to be made concerning circulating levels of compounds from their levels in exhaled breath, then more detailed preliminary studies are required to determine exactly what portion of expired air best reflects circulating concentrations of a given compound.

Dubowski (55) tried to circumvent the problem of dead space air by utilizing a dual-bag collection device. He was analyzing breath samples only for their content of alcohol. The first bag to be filled during expiration was assumed to contain only dead-space air and was discarded. The second bag to be filled during expiration was assumed to contain alveolar air and was subsequently analyzed by infrared absorbance at 3.39 μm for alcohol content.

III. Applications

A. Body Fluids

Body fluid analyses, for purposes of normal profiling and disease detection as well as analyses for specific compounds, are areas of great potential. Routine analyses of blood and urine by chemical methods have been used for a long time, but the examination of these and other body fluids by GC-MS-COMP systems is still a relatively new concept that has not been exploited to its fullest capacity. A number of general articles and reviews that stress the various aspects of the GC-MS-COMP approach to body-fluid analyses have appeared in the literature.

A recent comprehensive report focuses on the advantages of the GC-MS-COMP approach for the identification of drugs and their metabolites in the body fluids of overdose victims (23). The authors point out the speed and generality of the method and its advantages in an emergency situation. With a computer-searchable collection of 300 mass spectra of drugs and their metabolites as well as normal body-fluid constituents, this approach is especially suited for the identification of multiple drug ingestion or the presence of illicit drugs. The body fluids investigated included gastric contents, blood, urine, cerebrospinal fluid, and bile. A few specific cases are discussed in detail to illustrate the practical applications. This approach has also been described by Nau and Biemann (56).

Vesel and Passananti (57) also reviewed the field of drug monitoring in body fluids, especially in blood. They emphasize the importance of recognizing individual differences in ability to absorb, redistribute, metabolize, and excrete drugs. It is pointed out that drug dosages prescribed on the basis of initial criteria such as body weight or surface area and subsequently modified on the basis of patient reaction are inadequate and often detrimental to the well-being of the patient. Information on concentrations of a drug or combination of drugs in blood would provide a much better estimate of the most desirable dosage range, whereby the pharmacological activity of the drug would be at a maximum and the toxic effects at a minimum. Further general advantages of the combined GC-MS-COMP systems are discussed by Jain and Cravey (58).

The research group of Jellum, Stokke, and Eldjarn has been quite active in the use of the GC-MS-COMP system, predominantly for the study of inborn errors of metabolism, but with some extension to changes in metabolic patterns associated with disease states (2, 59–61). A combination of GC procedures is utilized, including analysis of both derivatized and derivatized extracts of body fluids. Among the body fluids examined are urine, spinal fluid, and serum. Most of the specific cases discussed (2) illustrate the use of GC analyses of derivatized extracts. These authors point out that they have diagnosed about 40 cases of inborn errors of metabolism by using the GC-MS-COMP system and have discovered four new metabolic diseases.

An extensive review of MS, including application to GC-MS-COMP systems, has been presented by Burlingame et al. (3). It encompasses the period December 1971 to January 1974, and includes sections dealing with GC-MS techniques as well as GC-MS-COMP applications. The analytical applications in biomedicine are also pointed out, with special reference to the screening of body fluids and tissues for metabolic and other disorders, to neurobiology, and to pharmacology. Their references include analyses for both derivatized and undervatized compounds. An updated sequel to this review, covering literature up to December 1975, has appeared recently (4).

In the subsequent sections on specific body fluids, we have attempted to select papers that illustrate the applications and potential in the use of GC, MS, and COMP methods. Where possible, the material has been arranged in subsections dealing with general profiling of the normal state, disease-state detection, and analyses for specific compounds. When pertinent, the use in preventive medicine, non-invasive diagnosis, and worker exposure hazards has been pointed out.

Blood. In this section we will include the analysis of whole blood, plasma, and serum. As discussed earlier, these fluids have been examined for the purpose of establishing the normal pattern of volatiles, the aberrations associated with disease states, and determinations of specific compounds. Several reviews of blood alcohol methods have appeared (e.g., 62); consequently, this specific aspect of blood analysis will not be further discussed here.

Profiles of volatile metabolites in normal serum and plasma by selective-trapping head-space analysis involving adsorption on a solid polymer support followed by desorption and analysis by GC-MS have been recently reported by Novotny (36), Zlatkis (44), Dowty (41) and their co-workers. The Novotny group utilized coated-glass capillaries and obtained a profile for normal human serum. They note that the serum GC is
considerably simpler than that of urine, but that the components of these fluids are not mutually inclusive. Another group (44) used coated nickel capillaries and obtained spectra for normal human serum and plasma. It was noted that at 95 °C a sampling time of 1 h and sample size of 5–10 ml will give a GC profile sufficient for screening purposes. A considerable number of the volatile constituents in serum were identified by using reference mass spectra of authentic compounds and spectra from the literature. Among the characteristic components of normal serum are: ethanol, 4-methyl-2-pentanone, hexanol, 1-butanol, 2-hexanol, 2-heptanone, and benzoaldehyde. The Laseter group identified five halogenated compounds in pooled normal human plasma. Tetrachloroethylene, carbon tetrachloride, and three isomers of dichlorobenzene were identified in the plasma. A correlation was noted between the presence of the first two halogenated hydrocarbons in the drinking water of the plasma donors and the presence of these compounds in the plasma. Coated steel capillaries were used in this work. Packed glass columns and flame ionization detection was used by Stoner et al. (20) to examine normal plasma as well as plasma of patients with the uremic syndrome. They found that similar chromatograms were obtained when blood was drawn from the same subject at intervals up to several weeks. The procedure used here involved solvent extraction and subsequent concentration of the plasma volatiles. In normal plasma, about 23 volatile components could be partly resolved by this method. Some complications caused by column-coating elution (column bleed) were encountered. Routine quantitative of free fatty acids (myristic, palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic) in plasma have been reported by Sampson and Hensley (63). Concentrated solvent extracts of the plasma were directly injected into the GC equipped with FIDs. The method has been applied to investigation of metabolic disorders involving fatty acids, such as Refsum's disease.

A general discussion of the application of GC-MS in the clinical laboratory, including analysis of serum from patients with special problems, has been given by Eldjarn et al. (60). The advantages of an on-line data system as well as an off-line library for MS are discussed. A study of low-molecular-weight organics in the plasma of patients before and after hemodialysis has been done by Dowty et al. (42). The plasma donors were patients with renal disease, who were routinely undergoing dialysis. A host of compounds was identified by MS in pre-dialysis patients' plasma and in pooled normal plasma. They found a much higher relative concentration for most of the organic constituents in the former than in the latter group. Certain compounds, such as methyl mercaptan, were observed to decrease in concentration in the plasma after dialysis. However, some higher-molecular-weight compounds (e.g., cyclohexane) were found to increase, thus revealing a selective influence of hemodialysis on the concentration of volatile organics present in the plasma. Selective-trapping head-space analysis followed by compound separation on a coated steel capillary GC column was used. The compounds were identified by coupled GC-MS-COMP methods. Uremic patients on hemodialysis therapy exhibited high concentrations of benzyl alcohol in their plasma as compared to normal subjects. GC-MS methods were employed, with use of both electron-impact and chemical ionization MS (63).

Jellum, Stokke and their co-workers (2, 65) report the identification of considerable quantities of free propionic acid in the serum of a patient suffering from severe metabolic acidosis. The serum was obtained, shortly after death, by heart puncture. A "Porapak P" column was used for the GC.

The determination of specific compounds has received considerable attention. These may be compounds administered either legally or illicitly as drugs (detected as drug metabolites), intoxicants, anesthetics, or compounds that have accumulated in the system owing to occupational exposure to various chemicals. Detection of drugs and their metabolites in the blood is of great importance, especially in cases where the patient is incapable of reliable communication, as well as in forensic practice. Bonnichsen and co-workers (21, 66) used GC-MS-COMP techniques to establish the presence of sympathomimetic amines as well as barbiturate sedatives in the blood. The samples were treated by solvent extractions. Packed glass columns were used, and they point out that small volumes of blood sample as well as samples containing very small amounts of drugs can be used. They also note that the sample purification procedure is simple, because the separation is achieved in the GC. Specific drugs such as amphetamine and phenmetrazine, as well as phenobarbital, amobarbital, and cylobarbital, were identified in human blood. The characteristic high-resolution MS fragmentation peaks are discussed, and the spectra were automatically evaluated and plotted by a computer. Underivatized barbiturate sedatives and derivatized phenytoin in extracts of serum have also been determined by Levy and Schwartz (19). Therapeutic concentrations of nine underivatized sedatives in serum, such as allobarbital and butabarbital, were determined by using packed steel columns and FID. The authors remark on the simplicity and speed of this method; for example, the complete analysis time for sedatives and phenytoin is 1.5 h.

Hall and Risk describe an electrolytic conductivity detector for rapid and specific GC analysis for free barbiturates in serum and urine at all concentrations of clinical interest (47). Barbiturate concentrations of 0.1 mg/liter may be determined by using simple solvent extraction without extensive sample clean-up. A specific nitrogen FID was used for GC determination of some derivatized anticonvulsant and barbiturate drugs in diethyl ether extracts of plasma (67). Again, the rapidity and generality of the method is stressed. In earlier work by the same laboratory (68) FIDs were used to determine concentrations of the anticonvulsants carbamazepine, diphenylhydantoin, phenobarbitone, and primidone in plasma. A nitrogen detector has also been.
reported superior for the analysis of cocaine in human plasma. Solvent extracts of the plasma were examined
(69).

Brunslen and Nash obtain specific and sensitive determinations of codeine in human plasma using an FID
(70). A GC analysis of theophylline in human serum was reported by Chrzanski et al. (18). No interference from
theophylline metabolites or ephedrine, hydrocortisone, caffeine, or their metabolites was observed, in contrast to
some ultraviolet spectrophotometric methods. After solvent extraction of the sample, a packed glass column and an FID were used. The authors note that previously reported assays for theophylline require concentrations higher than the 10–20 mg/liter normally found in serum after a single therapeutic dose of the drug. The described GC procedure is sensitive to amounts as low as approximately 1 mg/liter and is reasonably rapid (about 24 determinations in 8 h). The advantages of fluorometric and GC determinations of procaainamide in human serum have been studied (71). The authors give some guidelines as to the sorts of cases for which each method may be superior.

Intoxicants or volatile compounds introduced into the body from chemical misuse, for example glue sniffing, can be subsequently detected in blood. Direct injection of blood, with use of a packed steel column and an FID, has been used by Jain (45). Methanol, ethanol, acetone, isopropanol, and low-boiling hydrocarbons associated with glue sniffing were detected in amounts of less than 10 mg/liter. Another GC method for direct injection of volatile constituent slurries has been reported by Liesztnr et al. (46). The apparatus described was developed for the purpose of rapid methanol, ethanol, and acetone determination; however, the authors point out its applicability to other volatile compound analyses.

A method for the measurement of distribution of ventilation-perfusion ratios based on the simultaneous
pulmonary clearance of several inert gases has been reported by Wagner et al. (29). The direct head-space method (Section II.B) is suitable for monitoring individual anesthetics and trace concentrations of anesthetics in combination in the blood. FID was used in the determination of methane, ethane, cyclopropane, acetylene, fluoroxene, halothane, diethyl ether, and acetone. ECD was used for sulfur hexachloride. Accurate measurements were reported possible even when concentrations in micrograms per liter were present in blood, which are well below the usual anesthetic concentrations. A packed steel column was used in all studies. A short section on the sampling of gases from liquids—in particular, from blood—appears in the book
Biochemical Applications of Mass Spectrometry
(72).

Local anesthetics such as etidocaine, lignocaine, and prilocaine, in concentrations near 0.15 mg/liter of
plasma, were determined in solvent extracts of plasma by using a nitrogen detector (17). The advantage of using the nitrogen selective detector was that extraction procedures could be simplified and there was negligible contribution to the spectrum from normal plasma constituents. A packed glass column was used in this study.

Lidocaine and its pharmacologically active metabolite monoethylglycine xylidide were measured by GC-mass
fragmentography in seven patients being treated with the drug for cardiac arrhythmias (22). Another new antiarrhythmic agent, disopyramide, can be determined in the serum by GC, by using a nitrogen detector (73). The authors also point out that simple extraction procedures can be used without time-consuming sample clean up when the nitrogen detector is used. The same advantage of speed in conjunction with the use of the nitrogen detector, has been reported by Gifford et al. (74) for determination of tricyclic antidepressants in plasma. The sensitivity of the system to secondary and tertiary amines allows for routine analyses of imipramine and amitriptyline at therapeutic concentrations.

The psychotropic drug, trazodone, can be detected in rat plasma at concentrations of 20 ng by GC and at 200 pg by the mass-fragmentographic procedure (75). Solvent extraction techniques were used to extract the trazodone from plasma. With relation to analysis of fenprazone at therapeutic concentrations in human plasma, Berry (76) has pointed out the importance of blood-level monitoring of drugs, to ensure that patients are taking the drug and that they are doing so at the appropriate dosage. The fenprazone was isolated from plasma by solvent extraction and analyzed by GC fitted with an FID. Determination of serum concentrations of anticonvulsants in general has been related to the ability of these drugs to control seizures. Thus, Booker and Celesia (77) investigated relationships between serum concentrations of diazepam and interictal epileptiform discharges. They suggest that the failure of oral diazepam treatment in subjects responsive to intravenous diazepam administration is due to the lower serum concentrations attained with the usual oral doses. Serum solvent-extraction techniques were used and the samples analyzed on a GC with an FID. In a study to examine the pharmacologic effects of acetyl methylalcohol, a drug used in the treatment of opiate dependence, GC was used for its quantitative determination and that of its two major biotransformation products, noracetylmethadol and dinoracetylmethadol (78). Both plasma and urine extraction procedures are given.

Urine. Pauling et al. (30), using a total-trapping head-space analysis technique, obtained chromatographic profiles of about 280 components present in 200-ml urine samples. To resolve such highly complex mixtures, steel capillary columns were used. Subjects participating in this study were placed on a specially formulated diet ("Vivonex-100"), which eliminated intestinal flora, in an effort to standardize individuals and reduce individual variations. Other work (vide infra) demonstrated that diet had little effect on urinary profile patterns. Pauling and his group (31) later used a selective-trapping head-space analysis technique to identify 42 volatile constituents in urine by using combined GC-MS techniques. Twenty-two of these
compounds had not been previously identified in urine. This added significantly to the data base of compounds identified in normal urine profiles. A 1973 publication by the same group (32) discusses in detail the design of an automated apparatus for the quantitative analysis of volatile compounds in urine vapor and breath. An in-depth description is given of several purification precautions that are necessary for gases used in association with head-space type analyses. The type of COMP system used for peak integration and retention time determination was also discussed. Robinson and Pauling (79) later described the type of statistical analysis used to determine variations from normal profiles of various test groups.

Zlatkis and Liebl (24) independently arrived at the same conclusion that Pauling et al. had: that normal healthy individuals exhibit a characteristic profile of low-boiling constituents in urine. Using a solvent extraction technique coupled with distillation of the low boiling components, they identified 43 compounds by GC-MS. They examined 30 urine samples, from 10 different adults, and showed representative chromatograms of urinary components from both men and women. The concentration of compounds detected ranged from 10 ng to 100 μg per 24-h urine specimen. Again, it was observed that diet played only a minimal role in profile variations; individuals were sampled over a three-month period, in which time their diet must have varied, yet it was reported that the chromatograms of the urinary components did not reflect significant variations. Their preliminary studies on individuals maintained on Vivonex diets support these findings. Some of the components found in high concentrations were dimethyl sulfone, 4-heptanone, and 2-pentanone. Other compounds such as pyrrole and allyl isothiocyanate were found to vary much more in their concentrations. It was reported that pathological states such as diabetes mellitus are reflected in urinary component profiles. Recently, a fully automated GC system has been described that provides for repetitive sampling and analysis, with use of a glass capillary column and simultaneous FID and nitrogen-sensitive detector. Using the selective-trapping head-space analysis, the system has been applied to analysis of human urinary volatiles (80).

With a selective-trapping head-space analysis technique, the differences in profiles obtained from healthy and diabetic individuals was illustrated (35). Healthy individuals gave consistent urinary profiles for samples collected on different days. An FID was employed. Differences by FPD in the profiles of urinary volatile organic-sulfur compounds from normal and diabetic individuals (34) indicated that urinary volatile profiles vary from individual to individual, but that an individual profile remains constant from day to day. A study of 40 samples from patients with diabetes mellitus showed that several types of profiles for diabetics were prevalent, representing different types of therapy that the individual was on. Diabetics on insulin showed different profiles from diabetics not on insulin or those receiving sulfonylurea treatment. Some of the urinary constituents characteristic of normal individuals were 2-butanone, 2-pentanone, 4-heptanone, dimethylsulfide, several alkyl furans, pyrrole, and carbone. Individuals with diabetes mellitus under insulin treatment exhibit high concentrations of pyrazines, cyclohexanone, lower aliphatic alcohols, and octanols.

Another study reports on the urinary profiles from patients with diabetes mellitus, in which a selective-trapping head-space analysis was used in conjunction with single-ion monitoring MS techniques (39). Twenty-five patients were used in this study. Patients treating their condition with insulin, sulfonylurea, biguanide, or by special diet were included in the investigations. Abnormally high concentrations of alcohols in patients with diabetes mellitus were reported. In 85% of the cases increased ethanol and propanol concentrations were found; in 60% of the cases increased isobutanol and n-butanol were found; and in 25% of the cases increased isopentanol concentrations were found. Variations from day to day and individual to individual in the degree of elevation were noted. No obvious correlation with type of therapy was noted. Mass fragmentograms based on the presence of m/e 31, corresponding to the H2C=−*OH ion of primary alcohols, were generated. Various ions that would be characteristic for some of the urinary constituents are discussed (37). More details of the experimental conditions are given in a separate study of the same nature (40).

Chalmers et al. (27), using a solvent extraction procedure on 4-ml samples of urine pooled with a Dowex resin treatment to remove metal ions and other cations, reported detection of propionic acidemia, β-methylcrotonylglycinuria and methylmalonic aciduria from normal individuals in the resulting urinary GC profiles. This method can detect 10 mg of a given component per liter of urine. Thus for abnormal organic acidurias, where several hundred milligrams of organic acids are excreted daily, this procedure could be clinically useful. An analysis of the urinary organic acid GC profile of a nonketotic hyperglycinemic patient revealed the non-specific occurrence of free benzoic acid, caused by a β-streptococcus infection (81). The excretion of free benzoic acid was eliminated after penicillin G treatment. The urinary acids were collected by solvent extraction and analyzed on a GC with an FID.

Specific compounds were analyzed for by using a solvent extraction preparative method. Two-milliliter aliquots of a 24-h urine sample were analyzed for methylmalonic acid by a GC assay method based upon the spontaneous quantitative decarboxylation of methylmalonic acid at 225 °C to propionic acid (26). The total assay time was 2 h and the procedure was suitable for measuring propionic acid excretion at or above 0.5 mg/liter of urine and 5 mg/liter of urine for methylmalonic acid.

A comparative study of radioimmunoassay, thin-layer chromatographic, and GC methods for detection of barbiturates in human urine discusses the advantages and disadvantages of the various methods (82). With
a 5-ml urine sample and a solvent extraction preparative method, 0.2 μg of secobarbital/ml of urine could be detected. GC gave reliable results up to 52–76 h after drug ingestion and for analyzing for several different drugs was found to be slightly more economical than radioimmunoassays. GC was reported to be more reliable and sensitive than thin-layer chromatography.

The drug Darvon (Lilly) and its metabolites were identified by a combined GC-MS-COMP system in the urine extracts of a comatose patient suspected of having ingested an overdose of chlordiazepoxide (83). A comparison of the mass spectrum obtained for the major GC-separated peaks with the computer-library-storage spectra permitted the determination of the nature of the drug. This line of approach for drug identification has been continued, as noted in section III (29).

The simultaneous identification of acetone and isopropanol in urine as well as blood has been described (84). A sample of the biological fluid is diluted with aqueous n-propanol/water and directly injected into a GC equipped with an FID. The determination of acetyl methadol and its active biotransformation products (78) and the determination of free barbiturates in urine as well as blood have already been referred to in the sections on blood (47). Urine and bile are reported to be the body fluids of choice for postmortem isolation and identification of morphine (48). A comparison of the concentrations of morphine in urine and bile indicate that chronic poisoning results in a higher concentration of the opiate in urine whereas acute poisoning results in a relatively higher concentration in the bile. Solvent extraction followed by GC analysis with use of an FID was employed in part of this study.

Miscellaneous body fluids. The normal profile of the more volatile components of cerebrospinal fluid has been examined by Novotny et al. (36), using the selective-trapping head-space analysis technique and coated-glass capillaries. They found that the pattern of volatiles in cerebrospinal fluid was simpler than the pattern for urine, obtained by the same method. Drug overdose, as reflected in enhanced concentrations of glutethimide and associated compounds, can be determined in cerebrospinal fluid as well as in serum (28). The sample was treated with solvent extraction and a packed steel column was used with an FID. The authors noted the presence of peaks other than glutethimide in the serum and cerebrospinal fluid of patients who had taken an overdose. Serum extracts of individuals not taking drugs showed no corresponding peaks. The authors note that they find no corroborative evidence to support a previously proposed hypothesis (85) that one of these auxiliary peaks might play an important role in maintaining coma.

A method for estimating concentrations of nitrosamines in human gastric juice as low as 0.1 μg/liter (0.1 ppb) has been reported by Lane et al. (86). They used solvent extraction and separated the components in a packed glass column. An alkali flame detector coupled to a MS was used, and they discuss the advantages of this analytical tool. It has been suggested (87) that nitrate and secondary amines may react during gastric digestion of nitrate-treated foods to yield N-nitrosamines, which, because of their extreme toxicity and carcinogenicity, are of great interest.

Quantitative GC determinations of short-chain fatty acids (C₃–C₅) in stool water directly after anion-exchange chromatography have been described by Collin et al. (88). Detection of changes in total short-chain fatty acid concentrations may reflect pathological disorders, and the identification of the specific acids present in enhanced concentrations could aid in the determination of these disorders. Packed glass columns and an FID were used to detect acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids in stool water.

A report by Hagenfeldt and Hagenfeldt (89) describes the presence of lactate, pyruvate, 3-hydroxybutyrate, succinate, 2-oxoglutarate, and citrate in normal amniotic fluid as analyzed by GC-MS methods. Direct chromatographic analysis was used. They point out that hippuric acid, the major organic acid component of urine, is not detected in the amniotic fluid. Little experimental detail is given; however, the value of this type of analysis for antenatal diagnosis of hereditary metabolic disorders is discussed.

A combination of GC-microcoulometric titration was used (90) for specific detection and measurement of sulfur-containing volatiles in saliva, separated on a packed-glass column. Total-trapping head-space analysis of putrified saliva was used and the effects of a mouth wash on the amounts of hydrogen sulfide, methyl mercaptan, sulfur dioxide, and dimethyl sulfide in the saliva was noted.

B. Tissues

As in the cases of breath and body-fluid analyses, the analysis of tissue for volatile constituents characteristic of normal as well as of disease or the drugged condition has great potential. Considerable amounts of work have been published relating to the presence of drugs and their metabolites in various tissues; however, in the vast majority of cases, the tissue extracts have been derivatized and subsequent chromatography and spectroscopy geared to the identification of a specific compound or compounds. The techniques of analyses for underivatized volatiles, described in detail in the section on technology have been, to date, relatively unexploited. However, there are indications that workers in the field are becoming increasingly aware of the clinical potential of these methods for tissue analysis.

The potential of the GC-MS methods in analysis of kidney, liver, fat, and epidermis biopsy samples has been mentioned by Jellum et al. (2), but is not pursued further in that paper. Snedden and Parker (59) investigated the presence of altered purine metabolites—such as hypoxanthine, xanthine, uric acid, allopurinol, and oxipurinol—in skeletal muscle of normal and gouty individuals before and after allopurinol therapy. Multiple-ion monitoring MS (Section II.A) was used for the simultaneous determination of these compounds.
Politser et al. (43) examined the normal volatile constituents of profiles of lung, brain, and liver tissues of rats. A coated steel capillary column was used in the GC analysis of the volatile compounds collected by the selective head-space trapping technique. A comparison of the volatile patterns for the tissues examined showed marked differences. It appears that certain constituents may be characteristic of the particular tissue and therefore be useful in discerning aberrations associated with a disease state. A preliminary report purporting a micro-scale method for determination of volatile metabolites in biological samples, including dog liver, has recently appeared (49). A rich chromatogram of volatiles was obtained.

Specific compounds have been examined by Toseland et al. (67), who described the GC identification of phenobarbital, primidone, and phenytoin in a brain sample obtained from a unilateral temporal lobe resection. These underivatized anticonvulsant drugs were chromatographed on a packed column with a nitrogen detector. Another report on the identification of two barbiturate derivatives in liver tissue has appeared by Bonnichsen et al. (21). Few-nanogram concentrations of these barbiturates were detected by multiple-ion monitoring MS techniques (Section II.A). A somewhat bizarre case in forensic practice involved the identification of the same barbiturates in the autopsied liver tissues of a man and a dog (21). A packed GC column was used coupled with a MS. The authors point out the value of such clinical findings in legal matters.

The isolation, identification and quantitation of methaqualone before and after hydrolysis have been described by Christopoulos et al. for liver, kidney, and brain tissue as well as some biological fluids (97). The same laboratory has also reported the isolation and identification of morphine from human postmortem tissues (48). The value of this method for determination of morphine in forensic material is discussed, and although the authors find urine and bile to be the specimens of choice, liver, and less preferably kidney, can be used. Spleen and brain tissue were not found to be acceptable, because extremely large quantities were required. In both of the above studies, minced or homogenized tissue was solvent-extracted. Packed columns were used for the GC study. The presence of morphine in various rat tissues has been examined by diacetylation of this compound to heroin (50). The procedure described therefore is also applicable for the determination of heroin. The rats received intramuscular injections of morphine base and exhibited drug concentrations in decreasing order in the kidney, lung, liver, muscle, and blood. The differing morphine distribution pattern for these rat tissues as compared to the postmortem human tissues described by Christopoulos et al. (48) is notable. Tissues were homogenized and solvent extracted for the GC analysis of drug content.

The current widespread use of methadone prompted Dickson and Palmer (51) to undertake a comparison of isolation procedures for determination of this drug in human liver. Extraction efficiency and specificity were compared for the alkaline-, acid- and the ammonium sulfate-digestion procedures. It was found that only the acid and alkaline procedures were satisfactory for the low methadone concentrations that frequently are encountered in postmortem investigations. Further discussion is given regarding the method of choice with consideration of other drugs possibly present.

C. Breath

Breath analysis and its application to the clinical chemistry laboratory is a relatively modern concept that has, in the past, been customarily exploited only on a limited scale (e.g., breath alcohol determinations) but carries considerable potential as a general diagnostic tool for the future. An excellent recent review article on this subject, covering both present applications as well as future potential of breath analysis has appeared (54). In it, Dubowski points out that breath should reflect the concentration of substances capable of transfer across the alveolar/capillary membrane—an area of about 70 m². Thus, many normal body constituents, metabolic products as well as exogenously introduced compounds, should be available for analysis by the noninvasive, nontraumatic breath-sampling method. Teranishi et al. (92) have pointed out the potential of breath analysis, not only for diagnostic purposes, but for orthomolecular medicine as well. There are, of course, inherent and experimental limitations in using breath as a physiological specimen. Many of the difficulties and limitations have been summarized by Dubowski (54) and include such features as limitations on types of compound seen, sampling difficulties to avoid contamination, and sensitivity of instrumentation (see section II.B). However, with the rapid advance in GC technology, especially when coupled with MS and COMP systems, many of these limitations may soon be overcome or mitigated. Breath alcohol determinations have been recently reviewed elsewhere (93) and will not be discussed.

Respiratory air profiles from normal, healthy subjects have been recently reported by Lelievre (94) and by Teranishi et al. (92). Both groups observed a rich GC pattern of volatiles in the breath samples, but did not give specific compound identifications. Lelievre, using packed columns, found traces of 27 organic compounds in respiratory air. Teranishi et al. used coated, 300-meter stainless-steel capillary columns and a GC with a flame detector. They note that an advantage of the FID is the feasibility of partial saturation of the carrier gas with water vapor, thus reducing tailing and losses caused by water adsorption on the column. The problems encountered because of the large amounts of water in breath samples are also discussed. Total human effluents, including breath, have been studied by Ellin et al. (53), with GC-MS systems incorporating packed steel columns with FID, ECD, and thermal conductivity detectors. More than 135 effluents were identified, including alcohols, ketones, ethers, esters, unsaturated, branched, cyclic and aromatic hydrocarbons, sulphydryl, cyano, and heterocyclic compounds. Five compounds
commonly found in all human subjects were isoprene, acetone, ethanol, butanol, and toluene. It is to be noted, of course, that this study was on total volatile human effluents, not exclusively on breath samples.

Disease-induced aberrations in the breath of patients with cirrhosis of the liver were manifested as enhanced concentrations of volatile short-chain fatty acids as well as of mercaptans and dimethyl sulfide (95, 96). The normal values established for acetic and propionic acids were 790 ± 300 and 230 ± 160 μg/liter of condensed vapor, respectively. Increased amounts of these acids, as well as of isobutyric, butyric, and isovaleric acids, were often associated with cirrhosis of the liver. Volatile fatty acids are produced by the metabolic action of intestinal bacteria upon carbohydrates and amino acids. Altered bacterial ecology of the intestine in concert with altered hepatic blood flow in the cirrhotic patient can explain increases in volatile fatty acids in breath. It is noted that under ordinary circumstances, the cirrhotic patient has more circulating volatile fatty acids than normal. However, significantly abnormal values for volatile fatty acids in the breath can be detected before such aberrations are detectable in the blood. Indeed, by the time blood volatile fatty acid values are altered, complete loss of liver tissue has often occurred.

Enhanced concentrations of dimethyl sulfide, methanethiol, and ethanethiol were identified in the breath of cirrhotic patients in hepatic coma or cirrhotic patients fed methionine (96). These studies were performed with packed GC columns and a flame detector. Mercaptans were also detected in the breath of normal subjects, presumably primarily from hepatic metabolism of sulfur-containing amino acids, primarily methionine. Most of the mercaptan thus formed is readily metabolized, but a small amount is released in the breath. The two principal sources of increased mercaptan concentrations in cirrhotic patients are probably decreased liver metabolism as a result of altered hepatic blood flow and the inability of the liver to further metabolize mercaptans formed by the cellular thiol metabolism mentioned earlier.

MS studies have been used to evaluate ventilation-perfusion abnormalities in patients with respiratory distress syndrome (97). A correlation was indicated between the ability to achieve alveolar-arterial differences for CO₂ of <1.33 kPa (<10 mmHg) by age 7–10 days and potential for pulmonary survival. An MS system capable of simultaneous, continuous measurement of respiratory gases was used. A general discussion of MS methods in functional analysis of respiration and circulation can be found in *Biochemical Applications of Mass Spectrometry*, in the chapter by Muyzers and Smidt (72).

### IV. Concluding Remarks

As illustrated in the preceding sections, the state of the art of GC-MS-COMP analysis has reached a stage where the potential usefulness to the clinical chemistry community has been demonstrated. We believe that making diagnoses from aberrations in the normal profiles of biological specimens needs more study and documentation before it could be a matter of routine in an average clinical laboratory. On the other hand, the detection of specific compounds, whether introduced exogenously or arising from a known disease state, has been developed to the point where incorporation as a routine method in a laboratory is quite feasible. The advantages of speed, quantitation, and dependability of this technique for the analysis of volatile materials have been stressed. Thus, with appropriate selection of instrumentation and personnel, limited numbers of such assays can be performed routinely, easily, and rapidly.

The value of determining blood concentrations of drugs for purposes of prescribing proper treatment has already been pointed out. However, the potential of this method in regular monitoring of occupational health hazards has not been fully recognized. Volatile compounds such as trichloroethylene and other monomers used in manufacture of industrial polymers, are easily assayed by the GC-MS-COMP method without further derivatization. With the growing awareness and concern regarding safe worker-exposure limits, this method holds considerable promise for extended application.

Our survey of the literature has revealed a rapid increase in use of the GC-MS-COMP techniques for clinical studies. Whereas some years ago a GC might have been considered a rarity in the average clinical laboratory, its acceptance as a useful "work-horse" for certain determinations has now been well established. It seems safe to assume, therefore, that with increasing incorporation in clinical training programs, the future clinical chemist will also consider the GC-MS-COMP system as a routine part of laboratory instrumentation.

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### References