A GAS CHROMATOGRAPHIC DETERMINATION OF METABOLIC PROFILES IN HUMAN BREATH AND URINE

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PREFACE

The objective of the study was to design an analytical system for the high resolution analysis of breath and urine components. This system was then used to study variations in profiles and to correlate the breath and urine components.

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CHAPTER I

INTRODUCTION

Since the conception of gas chromatography by A. J. P. Martin (1941), the technique has been rapidly developed, and it is presently the most widely used analytical tool in the physical sciences. The advent of open tubular columns (Golay, 1958) was a breakthrough for complex mixtures. Chromatographic analysis of complex mixtures with many different classes of trace components is being applied to modern-day analytical problems in fields such as air and water pollution, petroleum and coal research, flavor studies, food products, cigarette smoke, and medicine.

Characteristic chromatograms or fingerprints associated with trace components in body liquids and effluents are termed metabolic profiles. Much could be learned about human metabolism if these metabolic profiles could be correlated, using pattern recognition analysis, with nutritional status, physical exercise, seasonal and diurnal variations, genetic make up, and physiologic and pathologic status (Hutterer et al., 1971), etc.

Urine and breath are two obvious choices for analysis among the body fluids and effluents; their collection does not require the disruption of any bodily processes and does avoid the possible trauma associated with blood or similar samples which may influence the substituents present. Breath offers certain advantages over urine in that it is in

a convenient physical form for analysis by gas chromatography and it gives information about those bloodstream components that readily pass the alveolar-pulmonary membrane in contrast to those filtered by the kidneys.

History

The use of urine examinations (mainly physical properties) in the diagnosis of disease has been known from the first recorded medical practices. Uroscopy became so popular among the physicians of the middle ages that the practice and the practitioners were depicted in art and literature. The eighteenth century marked the beginning of scientific methods in urine analysis (Gershenfeld, 1943). Since that time chemical analyses have progressed prompting the advancement of urine analyses.

In the late eighteen hundreds it was recognized that the physiologic state of man and his environmental conditions were reflected in his expired air (Conkle, 1973). Few advances were made until the advent of gas chromatography because of the lack of analytical technique. Even then analyses were somewhat limited due to the lack of suitable concentration methods (Anonymous, 1960; Eriksen and Kulkarni, 1963; Jansson and Larsson, 1969; Kustov and Tiunov, 1969; Richter and Tonzetich, 1964). In the seventies analytical techniques have progressed to where breath analysis has become a real possibility for correlation with disease. Dubowski (1974) published a review on the state of the art for breath analysis.

Methods for Profile Analysis

Liquid chromatography (LC) and gas chromatography (GC) both have their place in the establishment of metabolic profiles. GC offers high resolution and rapid analyses, but not all substances are volatile or able to be derivatized to a volatile form. It has been estimated that approximately 25% of the several thousand known biologically occurring compounds can be analyzed by GC. For substances such as proteins, peptides, nucleic acids, nucleotides, nucleosides, most coenzymes and cofactors, cytochromes, porphyrins, and bile pigments, GC can not be used (Jellum, Stokke, and Eldjarn, 1971). LC is more suitable for these constituents.

Liquid Chromatographic Techniques

A research group at Oak Ridge National Laboratory published a number of articles using LC for the separation of complex biological mixtures. Members of the group also published two reviews primarily over their work in this area (Scott, 1972; Scott et al., 1973). They developed an automated analyzer for the separation and quantification of ultraviolet (UV) absorbing constituents (Jolley and Scott, 1970). Their system was composed of a heated, high pressure, anion-exchange column, an acetate buffer elution system, and a recording UV photometer. A typical urine sample took 40 hr for analysis and separated more than 100 chromatographic peaks. More than 180 different components were separated from urine that had been concentrated by a sorption process (Mrochek, 1972). Minimum detectable quantities (MDQ's) of less than a microgram were observed for many components. Fifty of the UV absorbing compounds were identified by UV spectral maxima, GC methylene unit

retention values, and mass spectral peaks (Mrochek et al., 1971).

More recently the Oak Ridge group coupled the anion- and cation-exchange columns and achieved higher resolution with up to 17% more UV absorbing compounds (Scott, Chilcote, and Lee, 1972). This advancement resulted in a reduction of analysis time to 14 hr. They later developed a multicolumn, high resolution chromatograph (Pitt, Scott, and Jones, 1972). A design for a multicolumn, high pressure injection valve for the simultaneous injection on parallel columns was presented.

The researchers at Oak Ridge developed a carbohydrate analyzer composed of a temperature controlled, high pressure anion-exchange separation system (Jolley and Scott, 1970). The column eluent was mixed with phenol and sulfuric acid and monitored by a recording, continuous flow colorimeter. The elution system was a borate buffer which made anionic sugar borate complexes. A typical urine sample required a 24 hr analysis time and separated approximately 40 peaks. The sample preparation for both the UV analyzer and carbohydrate analyzer consisted of filtration to remove particulate matter and ultrafiltration for samples such as blood serum which have a high concentration of proteins.

Katz, Pitt and Jones (1973) of the Oak Ridge Research Center also described a detection system for the sensing of oxidizable compounds after anion-exchange chromatography. Detection relied upon the production of fluorescent cerium (III) by the reaction of eluted compounds with cerium (IV) solution. This cerate monitor was used in series with an UV monitor since the UV monitor was relatively insensitive to aromatic acids. For a sample of urine approximately 25 more peaks were detected with the cerate monitor with respect to the UV monitor. MDQ's were in the microgram range. This system was improved by extending the

column elution range and shortening the time required from 2 days to 1 day (Katz, Pitt, and Mrochek, 1975). The improved system was used in examining serum as well as urine components.

Systems for organic acids and amino acids have not been as well developed as the carbohydrate and UV analyzers, but they do show promise. A system for the analysis of organic acids was designed by Rosevear, Pfaff, and Moffitt (1971). The separation medium was activated silicic acid and the eluent was a chloroform, t-amyl alcohol, and water mixture. The eluent was monitored colorimetrically after the addition of an ethanol solution of neutral red. The time required for elution was approximately 6 hr with the separation of more than 40 constituents from urine. MDQ's were less than a microgram for some components.

The analysis of amino acids has been attempted with many different methods. The method of Moore and Stein (1956) is presently in use in most protein and peptide laboratories. Detection of about 30 urinary substances can be done with this low resolution ion-exchange separation of ninhydrin positive substances (including amino acids). Many industrial efforts have been expended in automating and refining the technique (Robinson and Pauling, 1973).

Hamilton (1968) presented a high resolution analysis of amino acids and related compounds. He was able to separate 175 components in human urine with a high pressure, temperature controlled cation-exchange column. Detection was accomplished with a ninhydrin colorimetric system where the ninhydrin positive compounds in the column were reacted and monitored at two wavelengths. A single analysis took as much as 65 hr. Submicrogram MDQ's were demonstrated.

Brown (1973) wrote a book on the use of LC in biomedical applica-

tions. The book contains a breakdown of the instrumentation, experimental methods, and data treatment used in LC. Several references to profiles and other analyses of biomedical interest are given including nucleotides, steroids, vitamins, amino acids, lipids, carbohydrates, drugs and their metabolites, etc.

Young, Epley, and Goldman (1971) studied the influence of diet on the composition of serum and urine. For their analyses they used an Oak Ridge National Laboratory UV Analyzer, an Oak Ridge National Laboratory Carbohydrate Analyzer, and an Amino Acid Analyzer. In general, water excretion tended to increase while quantities of most urinary constituents tended to decrease while the subjects were on a chemically defined, low bulk diet of Vivonex. Further studies needed to be done to establish which compounds were actually from endogenous metabolism and which were due to dietary origin.

Gas Chromatographic Techniques for Nonvolatiles

Jellum, Stokke, and Eldjarn (Eldjarn, Jellum and Stokke, 1974; Jellum et al., 1971; Jellum, Stokke, and Eldjarn, 1972; Jellum, Stokke and Eldjarn, 1973) did research with a large number of patients using a GC-mass spectrometry (MS)-computer (COM) system for the establishment of metabolic profiles. Their work was primarily with urine samples but serum and biopsies were also analyzed for some patients. The system consisted of five GC's equipped with flame ionization detectors (FID's). One of the GC's was interfaced to a single-focusing MS through a molecular separator of the glass frit type. An on-line computer was used for the MS data acquisition and an off-line computer was used for the MS library search. Following is a brief summary of the types of com-

pounds separated by their procedures: (1) ether soluble substances that are volatile without derivation (lower hydrocarbons, alcohols, aldehydes, ketones, short chain free fatty acids, etc.); (2) substances volatile at higher temperatures (higher alcohols, aldehydes, ketones, aliphatic and aromatic hydrocarbons, phenols, barbiturates, and other heterocyclics); (3) acidic metabolites in the form of methyl esters; (4) derivatized amines, aminoacids, and simple dipeptides; (5) derivatized carbohydrates and certain amino sugars; (6) derivatized acidic compounds (e.g., drug metabolites released on acid hydrolysis); (7) total amino acid and amine content, including those liberated by acid hydrolysis. Several patients could be run in one day with the system, and 500 to 1000 total peaks were obtained. They made several observations about such work: (1) the intake of drugs, even seemingly innocuous ointments, complicates the pattern; (2) urine varied considerably from person to person (the difficulty was considerably decreased by using fresh morning urine, which is relatively characteristic of endogenous metabolism) and, (3) large amounts of benzoic acid were regularly encountered which may indicate the presence of bacteria in urine. There are several microorganisms that contain a hippuricase which hydrolyzes hippuric acid into glycine and benzoic acid.

E. C. Horning and coworkers did considerable work in the area of metabolic profiles by GC-MS or GC-MS-COM (Horning and Horning, 1971a; Horning and Horning, 1971b). They published methods for the isolation, derivatization, and analysis of human adult and infant urinary steroids, serum (or plasma) and urinary sugars and sugar alcohols, serum and urinary acids, serum acids of the Krebs cycle and related compounds, and for many acidic and neutral drugs and drug metabolites. Methods for amines were under study. Isolation of the samples was done by either

solvent extraction, ion exchange, column or thin-layer chromatography, or enzymic or chemical hydrolysis. This step can be considered as a low resolution, high capacity method for the separation of classes of compounds. Horning's group discussed several derivatization procedures for the different classes. Their GCs were equipped with packed glass columns with either a FID, a MS, or an electron capture detector (ECD). The ECD was used for analysis of steroids which had been derivatized with a halogenated reagent.

Horning and associates developed a system which utilized glass open tubular columns for the same type of analyses as above (German and Horning, 1972; German and Horning, 1973; Horning et al., 1974). They used a glass injection system which consisted of a packed precolumn and gas phase splitter. The precolumn provided complete sample evaporation before the splitter zone, trapped contaminating nonvolatiles, and prevented decomposition or adsorption of components. They used a coating technique in which the liquid phase contained very fine particles of silanized silica powder.

Rutten and Luyten (1972) worked with steroid analysis using glass open tubular columns. They compared three types of pretreatment for the glass: etching, silanization, and surface-active agents. They found the surface-active agent, used with a static coating procedure, to be the best method. The urine pretreatment consisted of enzymatic hydrolysis, isolation, and purification of the steroids by either a classical extraction technique or an Amberlite XAD-2 resin and Amberlyst A-26 resin, and finally methoxime trimethylsilyl derivatization. Approximately 35 peaks were seen with a FID for the urine of a normal male in about 1 hr.

Hutterer et al. (1971) used a GC-MS-COM system to separate and identify metabolic products of low molecular weight in microliter quantities of physiological fluids. Their samples were first deproteinized and silylated, a process which derivatized and extracted amino acids, sugars, organic acids, drugs, etc. The separation was performed on a packed glass column and the effluent was split to a FID and a MS. In one example of a blood serum sample 46 chromatographic peaks were obtained in about 75 min. The number of peaks was increased by adsorbing the nonpolar components in Amberlite XAD-2 ion-exchange resin and eluting them with an organic solvent.

Amino acid analysis can be done with GC as well as LC techniques. Tucker and Molinary (1973) did this type of analysis using a modified method of Gehrke et al. (1968). The sample (urine, plasma, or cerebrospinal fluid) was first passed through a cation-exchange column and then derivatized to a N-trifluoroacetyl n-butyl ester. The GC was equipped with two packed, glass columns and dual FIDs. The instrumental time involved was about 1 hr.

Chalmers and Watts (1972a, 1972b, 1972c) developed methods for the quantitative extraction and GC analysis of urinary organic acidic metabolites. The acids were extracted with anion-exchange chromatography with subsequent freeze drying of the extracted acids, conversion of the dry acids to volatile trimethylsilyl derivatives, and then GC analysis. Loss of some low molecular weight acids was minimized by forming less volatile derivatives of these compounds before freeze drying. In a later article, Chalmers, Bickle, and Watts (1974) point out that the previous methods were unsatisfactory for short chain aliphatic acids with one to five carbon atoms. These acids were either lost by volatilization on

freeze drying or had trimethylsilyl esters that eluted with the solvent and reagents used. Chalmers et al. suggested an alternate method for these acids using a cation-exchange column to remove metal ions and other cations and to release the free acid from its salt. GC analysis was then performed directly with the liquid. They claimed this method was better than solvent extraction (Mamer and Gibbs, 1973; Zlatkis and Liebich, 1971) or steam distillation (Perry et al., 1970) due to the unquantitative nature of these methods for acids. The acids have an unfavorable partition coefficient between water and organic solvent. Steam distillation resulted in a four to five fold dilution of the acids. This can be avoided but the acid conditions could cause artifact formation.

Gas Chromatographic Techniques for Volatiles

Volatiles are convenient to analyze since they require no pretreatment, yet the above techniques have either not been concerned with volatiles or have not gained as much information from them as is possible. This area has received more attention recently and has been expanded to include breath samples.

Zlatkis and associates published several articles along these lines. Zlatkis and Liebich (1971) used liquid-liquid extraction for preparation of concentrates and GC-MS for the analysis of urine samples. The urine was extracted with ethyl ether, distilled at low temperatures under reduced pressure, and concentrated to a few microliters. The column was a 150 m x 0.5 mm inner diameter stainless steel capillary coated with Dowfax 9N15. A longer column was used in connection with the MS. The GC-MS interface was a two-stage, jet molecular separator of

the Becker-Ryhage type. Approximately 300 compounds were separated, 40 of which were identified with MS. The extraction method was compared to high vacuum distillation of whole urine; very little difference was observed. Characteristic components included dimethyl sulfone, pyrrole, 4-heptanone, allyl isothiocyanate, several alkyl furans, ketones, and lactones. Semiquantitative data was obtained by measuring the peak areas in the chromatograms and by adding known amounts of identified components to samples before isolation. The concentration of the constituents ranged from 10 μg to 100 μg per 24 hr urine. Differences found in chromatograms of all the samples were primarily quantitative in nature. Some components were invariably found in high concentrations while others were much more variable. However, chromatograms from an individual remained fairly constant over a three month period. This indicated that perhaps these variations are characteristic of the individual rather than a result of diet. Zlatkis and Liebich reported that preliminary results from subjects on a chemically defined, low bulk diet (Vivonex) showed that the chromatograms from an individual do remain relatively unchanged.

Later Zlatkis and associates began using an isolation technique from flavor research, i.e., headspace analysis, and adsorption onto the porous polymer, Tenax. (Zlatkis et al., 1973a; Zlatkis, Lichtenstein, and Tishbee, 1973b; and Zlatkis et al., 1973c). Headspace from a 100°C urine sample was swept with helium through a water condenser and trapped onto the Tenax column. In some cases they added $(NH_4)_2SO_4$ to increase volatilization. The Tenax was then transferred to a modified injection port. The components were thermally desorbed to a cooled precolumn. The analytical column was a 100 m x 0.5 mm inner diameter nickel

capillary coated with either Emulphor ON 870 or Emulphor-O; both are polar liquid phases. Detectors used were FID, flame photometric detector (FPD), and/or MS. The analysis time was about 2.5 hr and total time required was less than 4 hr. Multiple sampling, storage condition, and container size evaluations all showed similar profiles. Fifty-one components of urine were identified with MS including ketones, alcohols, aldehydes, pyrroles, and pyrazines. Twenty-five of these compounds were also found using diethyl ether extraction. Many deviations were probably due to increased sample concentration with the adsorption technique. Dimethyl sulfone, a major constituent found using the extraction method, was not detected using the headspace method, whereas a number of isomeric hydrocarbons were.

The system was also used for breath profiles (Zlatkis et al., 1973b). Fifty exhalations from a subject breathing ultrapure air were analyzed on two different days. Profile differences could easily result from substances taken into the mouth as well as decay products from oral bacteria. They did not attempt any identification of the constituents.

Zlatkis et al. (1974) used the same system described above for the analysis of volatiles from serum and plasma. Chromatograms were obtained in approximately 1 hr and were not as complex as urine. Characteristic components in serum were: ethanol, 4-methyl-2-pentanone, hexanal, 1-butanol, 2-hexanol, 2-heptanone, and benzaldehyde.

Liebich and Al-Babbili (1975) studied some of the parameters involved with a system like the one described above. They studied the effect of the temperature of the cooling water, the effect of the desorption temperature, and the effects of the sampling temperatures and time. They recommended the following: cooling water temperature of

12°C, desorption temperature of 300°C, and sampling temperature of 90°C for 1 hr.

Recently Zlatkis and Andrawes (1975) used diethyl ether extraction and concentration on glass wool for analysis of volatiles in serum. Similarly complex chromatograms were obtained with this method as compared to other concentration methods.

Pauling, Teranishi, Robinson, and coworkers analyzed volatiles from urine and breath using high-resolution techniques (Pauling et al., 1971; Robinson et al., 1973; Teranishi et al., 1972). The urine sample was kept at pH 7 by a phosphate buffer. Sample concentration was accomplished with headspace collection followed by a cold trap. Breath exhalations were collected directly in the cold trap. The column was a 1000 ft x 0.03 in inner diameter stainless steel capillary coated with SF 96. The detector was a modified FID. The major change in the detector was a platinum cylinder topped by a platinum-rhodium mesh cone used as an ion collector. This system permitted the quantitative determination of approximately 280 substances from urine vapor in 6 hr and 250 from breath in 2.5 hr. Integration, peak matching, and various statistical pattern recognition procedures were performed by computer. These investigators placed their subjects on Vivonex for several days before analysis. A standard deviation was found for individual peaks on successive days of about 10%, while for a regular diet the fluctuations were several times larger. Pauling and associates described an automatic system controlled by computer (Robinson et al., 1973). They claimed this apparatus was suitable for the routine analysis of about 200 volatile constituents of human urine. Up to 350 compounds actually could be counted on a chromatogram but many of them were: (1) not present in most urine samples, (2)

inadequately resolved, or (3) lost in the urine vapor procedure to where large errors were in their measurement.

Hartigan et al. (1974) designed and used a nitrogen sensitive thermionic detector as well as a FID for the analysis of urine volatiles. Headspace collection and 2 mg of Tenax GC adsorbent were used for collection of the samples. The column was a 55 m x 0.22 mm inner diameter glass capillary coated with SF 96 silicone oil. Analysis time was about 2 hr. Novotny, Lee, and Bartle (1974a) later expanded the samples to include urine, serum, and cerebrospinal fluid. They also analyzed urine samples after treatment with β-glucuronidase and sulphatase which cleave glucuronides and sulfates. Two different liquid phases of differing polarities, Emulphor ON-870 and SF 96, were used. McConnell and Novotny (1975) described the automation of their system and discussed the reporting of retention data. They recommended selecting one peak out of a profile as a reference and reporting the retention times relative to that reference.

Conkle (1973) wrote his dissertation on the GC-MS analysis of organic and inorganic compounds in breath samples. In one series of experiments a rebreathing system was employed which accomplished a two-fold increase in concentrations. The GC columns were packed, 1/8 in stainless steel tubing and the detectors used were FID, ECD, thermal conductivity, and MS. Seventy-two compounds were separated and 69 were identified by MS. Their production rates were also determined. Only four compounds, freon, methyl chloroform, 1-butene, and ethanol, were found in all of the samples and only 14 compounds were found in 80% of the samples. A significant number of components were contaminants from the collection system.

Stoner, Cowburn, and Craig (1975) examined the volatile metabolites in plasma. Solvent extraction was used for concentration. A GC equipped with packed glass columns and a FID was used for separation and detection. In an hour, 21 peaks were eluted, four of which were incompletely resolved from the solvent peak. Recovery of ^{14}C-napthalene, the internal standard, was determined to be 95% with a scintillation counter.

Concentration Techniques

In the methods reviewed for volatiles several concentration methods were used. Many researchers have evaluated these and other techniques; some of their conclusions are given here.

Novotny et al. (1974a) compared cryogenic trapping, freezing of the sample in an empty tube, direct injection onto a capillary column, and adsorbing onto a polymer. The first two methods have problems associated with water interference, solvent impurities, and formation of artifacts. On-column cryogenic concentration is technically difficult because of the large volumes of gas used and is limited to stationary phases which maintain their liquid state at low temperatures. Bellar and Lichtenberg (1974) commented that liquid/liquid extractions with organic solvents provide erratic or low extraction efficiencies. Large solvent responses and solvent impurities could cause serious interferences.

Of the different adsorbents available, most of the common ones, silica gel, alumina, charcoal, etc., have excessive surface activity toward many organic compounds and have high affinity for water. Novotny et al. (1974a) examined Tenax (or Tenax GC), poly-2,6-diphenyl-p-phenylene oxide, for its usefulness as a GC adsorbent. They examined the

effect of water, losses due to irreversible adsorption, and explained qualitative and quantitative changes in chromatograms with different sampling techniques on the basis of frontal and displacement chromatography and selective properties of the adsorbent. They concluded that Tenax was a suitable adsorbent for a wide range of both polar and non-polar organic compounds. However, there was a considerable dependence on sampling technique so that quantitative and sometimes even qualitative data must be reviewed with caution.

Zlatkis et al. (1973a) examined three different adsorbents: Porapak P, Carbon Molecular Sieve, and Tenax. They found Tenax to be the best because of its high temperature stability (375^{o}C limit).

Bellar and Lichtenberg (1974) used a dynamic system where a purge gas was bubbled through the sample rather than headspace for the analysis of volatiles in water. This should quantitatively remove the water insoluble volatiles from the sample and thus provide a direct measure of the original concentration in the aqueous phase. Water soluble compounds were only qualitatively transferred. They found the method applicable to organic compounds less than 2% soluble in water and with boiling points less than 150^{o}C. They tried several adsorbents: silica gel, Porapak Q, Chromosorb 103, and Tenax. Chromosorb 103 and Tenax were found to be most satisfactory but they used Tenax due to its higher temperature stability.

Versino, Groot, and Geiss (1974) cited the following advantages for the dynamic enrichment on adsorption columns: (1) the adsorbed compounds can be thermally desorbed; (2) the adsorption and desorption procedures can be easily automated; (3) the dynamic enrichment allows both the complete adsorption of substances having a retention volume

lower than the sample volume passing through the adsorption column, and for more volatile compounds an equilibrium concentration is established proportional to the concentration in the original sample; and (4) the sampling can be performed in situ. They found Tenax adsorption and desorption efficiencies of better than 90% for different classes of compounds, both hydrophobic and hydrophylic. Columns conditioned at elevated temperatures under a continuous flow of gas had a tendency to produce peaks belonging to the class of biphenyl, n-terphenyl, and triphenylene. This was alleviated by filling the column with N_2, no N_2 flow, at elevated temperatures. This allowed the polymer to form crosslinks.

Butler and Burke (1976) studied Tenax, Chromosorbs 101 and 102, and Porapaks P, Q, R, and T. Their conclusions were that Porapaks Q and R had the best over all sampling capacities as determined with acetonitrile, t-butanol, methyl ethyl ketone, and benzene as molecular probes. They did recommend Tenax for high boiling components because of its high temperature stability and low retention volumes. They claimed work was under way on the use of pellicular beads as adsorbents.

Mass Spectrometry

As seen in the above review MS is used nearly exclusively for the identification of the constituents in a profile. The small sample size required with open tubular columns makes other techniques inpractical. One of the basic problems for GC-MS systems is in interfacing the GC column effluent, which contains primarily carrier gas, and the reduced pressures of the MS. Several methods have been used for this (Plant, 1972; Novotny, McConnell, and Lee, 1974b). For open tubular columns

direct coupling is possible (Leferink and Leclercq, 1974; Grob and Jaeggi, 1973; Schulze and Kaiser, 1971).

Fales et al. (1975) compared various ionization techniques available for use with biologically important compounds. Electron impact is normally used but chemical ionization, field ionization, and field desorption also have their applications. Gordon and Frigerio (1972) discussed the use of mass fragmentography, i.e., the simultaneous monitoring of one or more fragment ions, in biological research rather than the conventional scanning of the total ion spectrum.

Waller (1972) edited a book on biochemical applications of MS. Topics discussed were MS instrumentation including coupling of GC-MS, computerization of MS data, and various applications. Henneberg et al. (1972) and Novotny et al. (1974b) also reviewed the use of computers in GC-MS work.

Open Tubular Columns

Open tubular columns have comparable separation efficiencies per unit length as packed columns but their relatively low pressure drops allow the use of much longer lengths. The longer columns result in greatly enhanced resolution. There are two types of open tubular columns, wall coated (WCOT) and support coated (SCOT) columns. The WCOT columns have a liquid phase coated on the inside of the column whereas the SCOT columns have the walls of the tubing coated with a support material which is then coated with a liquid phase. In practice the SCOT columns give better separation on compounds with low boiling points while WCOT columns are better for compounds with high boiling points. Ettre (1973) predicted this behavior from theoretical considerations.

Inner tubing diameters ranging from 0.01 in to 0.03 in have been used. The larger diameter columns can accommodate larger sample sizes and they reduce the danger of plugging during the coating procedure. However, they do so at the sacrifice of efficiency. Longer columns must be used to obtain comparable resolutions.

Glass may eventually become the tubing material of choice for biological samples; however, it does have problems including fragility, difficulty in the adhesion of a liquid phase, and a short usable life of the liquid phase. Several authors discussed state of the art in the use and preparation of glass open tubular columns (Hartigan and Ettre, 1976; Novotny and Zlatkis, 1971).

Metabolic Profiles

An increasing number of diseases are being recognized as products of a metabolic disorder that is the result of some defect in biochemical processes. Some 150 to 200 of the known genetic disorders are single-gene diseases and are inborn errors of metabolism in the strict sense. About 0.3% of live births show these single-gene diseases. Since a few thousand enzymes and structural proteins are known it is likely the 150 to 200 known inborn errors are only a few of the total number. Inborn errors affecting enzyme activity will usually result in an accumulation of substrate or substrate derivatives and decreased amount of product or substances derived from the product. If the accumulated compounds are small molecules and are water soluble, they will probably show high concentrations in the blood, urine, or breath. Accumulation of high molecular weight compounds that are sparingly soluble in water will usually give rise to storage diseases (Eldjarn, Jellum, and Stokke,

1975). With the high-resolution analyses now available these inborn errors of metabolism can be detected with greater efficiency.

Jellum et al. (1972) used their GC-MS system for: (1) the finding of normal metabolites of unknown metabolic origin, (2) the discovery of new, unknown metabolic disorders, and (3) the diagnosis of known metabolic disorders. They can detect 70 of the known metabolic disorders and they discovered five new disorders (Eldjarn et al., 1975). Many different researchers have studied specific metabolic disorders (Hutterer et al., 1971; Mamer and Gibbs, 1973; Tucker and Molinary, 1973).

Other disease states as well can be determined with these high-resolution systems. These cases may not be as easily diagnosed because relatively small changes in profiles occur. Jellum and associates (Eldjarn et al., 1974; Jellum et al., 1972; Jellum et al., 1973) did work with various types of cancer, hepatic disorders, ketosis (e.g., patients in a diabetic coma), metabolic acidosis and alkalosis, and toxic products produced on sterilization of intravenous nutrition. Horning et al. (1974) characterized normal and pathological states of steroids and studied the developmental changes of steroids in newborns. Zlatkis et al. (1973a) studied patients with diabetis mellitus. Subjects being treated with insulin had high concentrations of pyrazines, cyclohexanone, lower aliphatic alcohols and octanols. Liebich and Al-Babbili (1975) and Liebich (1975) also studied patients with diabetis mellitus under different treatments and found abnormal concentrations of five lower aliphatic alcohols and two ketones. There was, however, considerable variance between different stages and forms of the disease.

These analytical systems could be used for the determination of

toxic or noxious compounds (Stewart, 1974), including drugs (Horning and Horning, 1971a; Horning and Horning, 1971b; Horning et al., 1974; Karasek, 1973) and alcohol (Jain and Cravey, 1972; Witten et al., 1973), and to the analysis of compounds occurring in minute quantities (e.g., hormones) (Eldjarn et al., 1974).

Orthomolecular medicine is defined as the preservation of health and treatment of disease by maintaining the optimum concentrations of essential substances. In the past the practice of orthomolecular medicine has been a trial-and-error procedure. High-resolution systems could make the quantitative determinations of many constituents that are necessary for this kind of treatment (Pauling et al., 1971).

The types of systems described above may at some time become standard equipment in health centers, but there are many problems still to be dealt with. One such problem is the storage of such tremendous quantities of data. Even if the problems bar high resolution systems from becoming common equipment, they will give useful information in research situations about the history of compounds in the body.

Direction of Research

This study was performed to further the development of high resolution human urine and breath analyses. Urine analysis has proven its value in studying bodily processes. The promise of breath analysis is that it could provide information not available from urine analysis about body metabolism. The focus of the project was on correlating volatile urine and breath components using GC. Differences may possibly arise because of several reasons: (1) daily variations may be significant and may affect breath and urine differently; (2) the urine components

are present in much larger quantities; (3) the lungs are more prone to be affected by atmospheric contamination; (4) the breath components are in such small quantities that contamination from the sampling system itself is likely; (5) bacteria in the oral cavity may produce volatile organics which are not necessarily present in urine but may be in breath; (6) variations between individuals are unresolved; (7) diet effects on breath and urine are unresolved; (8) breath analysis reflects those components which readily pass the alveolar-pulmonary membrane in contrast to those filtered by the kidneys; and (9) digestive processes may relatively affect breath more than urine, as has been observed with H_2.

A major portion of the project consisted of instrumental design and modifications. The urine sampling system was designed to provide efficient transfer by dynamic sampling of the volatile components from a specimen to the concentrating and collecting medium as well as to minimize memory effects from its repeated use. The breath analysis system isolated and maximized concentrations of analyte components and minimized contamination. The system was designed to reduce atmospheric contamination by providing the subject with a pure air supply and to reduce contamination from the system by avoiding the use of plastic tubing, etc. Most common breath supply apparatus, such as scuba diving gear, use a rubber diaphragm in the demand regulator. The unique design of the breath supply control utilized a fluidics control circuit to operate a semiautomatic demand regulator. The trace amounts of volatile organics present in breath and urine made a concentration technique necessary. Due to the large amounts of water, a technique which did not concentrate H_2O was needed. The technique chosen was adsorption onto a

porous polymer, Tenax, followed by thermal desorption. Tenax has a high affinity for organics and a low affinity for water (Bellar and Lichtenberg, 1974). The injection system of the GC used in this investigation was redesigned to be compatible with the concentration technique used and to insure reproducible retention times. The injection port on the GC was completely removed. A valve injection system was installed to provide three functions: (1) the thermal desorption of the sample from the Tenax to a cryogenic trap, (2) the isolation of the sample so that it could be heated to re-volatilize the components, and (3) the injection of the sample as a slug onto the analytical column. The resolution required for the analysis of the complex, biological mixtures used in the project made the use of open tubular columns necessary. The GC used for the project was not factory designed for use with open tubular columns: the inherent dead volume caused significant peak broadening. This was reduced by the installation of the redesigned injection system and by the introduction of a make up gas stream at the end of the column. WCOT and SCOT columns were used to resolve the urine and breath samples. An additional detector, a FPD, was installed to provide a convenient elemental analysis for sulfur compounds.

Early morning urine samples have been reported to be most indicative of endogenous metabolism (Jellum et al., 1971). Therefore early morning samples were used in this study for comparison purposes. The variations of these early morning samples for a given individual from day to day and during the day were studied. The differences between individuals were examined. Early morning breath and urine components were compared to see to what extent the same constituents were present. The question of diet variations as they affect urine volatiles was

examined with a brief study using the ingestion of asparagus. For approximately 50% of the population asparagus produces a characteristic odor in urine. White (1975) reported the components responsible to be two sulfur compounds, S-methylthioacrylate and S-methyl 3-(methylthio)-thiopropionate. This phenomenon was used to determine whether relatively large quantities of two sulfur compounds appeared in urine.

CHAPTER II

EXPERIMENTAL

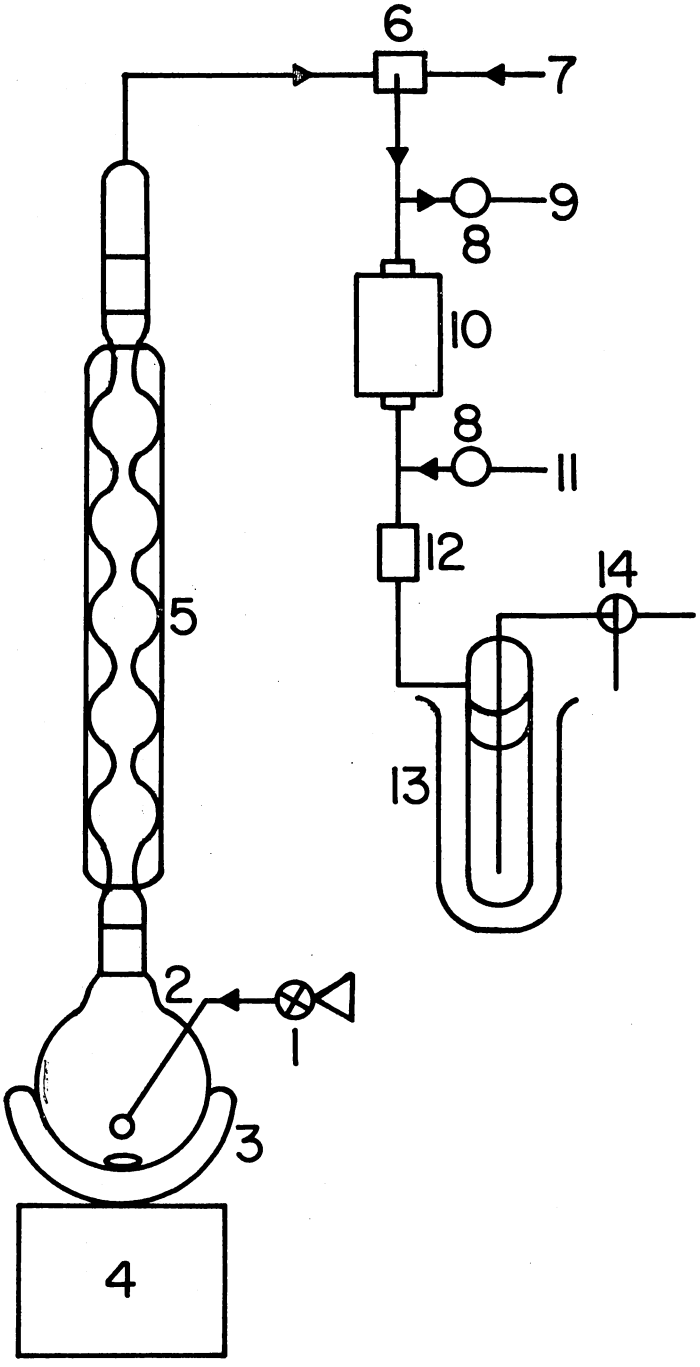
The analytical system used for these samples was designed to keep the differences in sampling procedures between breath and urine at a minimum.

Urine Sampling System and Procedures

The urine sampling system consisted of glassware apparatus which allowed the volatiles from urine to be flushed into a gas stream. A coarse diffusion tube was inserted into a 250 ml round bottomed flask (O.S.U. Chemistry Glass Shop) to allow helium to be bubbled through the stirred and heated urine sample. (See Figure 1.) A tap water cooled condenser was used to minimize the amount of water vapor transferred. Capillary tubing, kept at 70-80^{o} C with heating tapes, connected the glassware to a three-way ball valve (Whitey, Model SS-44XF4). The three-way ball valve selected either the urine or breath sampling systems. The volatiles were then swept downstream through the Tenax adsorption trap at ambient temperature. The helium flow was vented through a liquid nitrogen trap and out into the room with the three-way vacuum stopcock.

Urine samples were collected in a 250 ml glass-stoppered bottle and refrigerated until analysis. Thirty grams of ammonium sulfate was added to a 100 ml sample of urine to increase volatilization. Larger

Figure 1. Schematic of Urine Sampling System: 1. Flow Controller, 2. Diffusion Tube in 250 ml Flask, 3. Heating Mantle, 4. Magnetic Stirrer, 5. Water Condenser, 6. Three-Way Ball Valve, 7. Breath Sample Inlet, 8. Shut-off Valve, 9. Pathway to Injection Valves, 10. Tenax Column in Aluminum Heating Block, 11. Helium Supply, 12. Two-Way Ball Valve, 13. Liquid Nitrogen Trap, 14. Three-Way Vacuum Stopcock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samples would sometimes foam excessively. Helium was swept through the 105°C sample at 50 ml/min for 1 hr.

The glassware was cleaned between runs with chromic acid and then stored in a 90°C oven. The connecting capillary tubing was flushed with helium for several hours between runs.

Blank runs were made to check for contamination from extraneous sources. The same procedure described above was used without a sample in the 250 ml flask.

Breath Sampling System and Procedures

The breath analysis system had a modular design. The air supply control and the air supply and breath collection apparatus comprised the two modules.

Fluidics Control Circuit

Fluidics is a technology which uses fluid flow much as electronics uses electron or current flow. In this application the fluid is air. The basic principle behind operation of fluidic devices is that a turbulent flow issuing from a jet in a confined region will draw air in from its surroundings. (See Figure 2.) This jet is unstable and flips back and forth rapidly. When the jet veers close to one wall this air flow is interrupted and a decrease in pressure occurs. (See Figure 3.) This holds the jet stream against the wall until some outside force disturbs it. The phenomenon is known as wall attachment or Coanda Effect. To control the jet stream control ports are cut in the confined region. (See Figure 4.) Thus the jet stream can be switched back and forth at

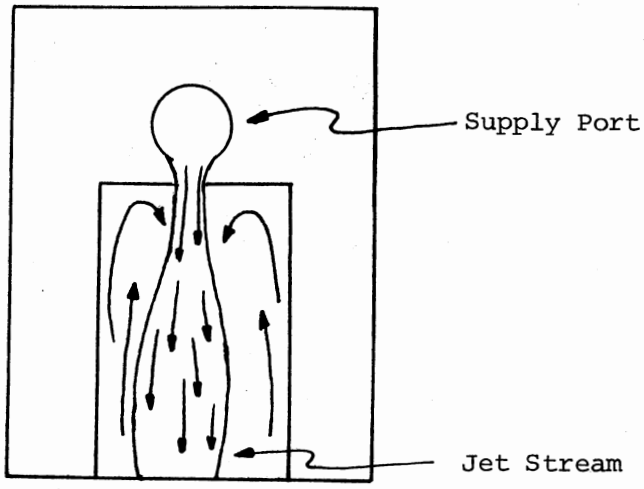


Figure 2. Representation of Jet Stream Drawing Air From Surroundings

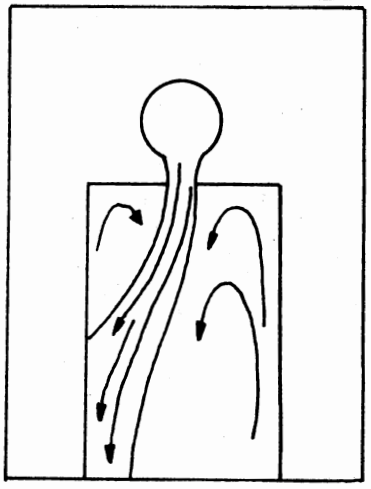


Figure 3. Representation of Wall Attachment Phenomenon

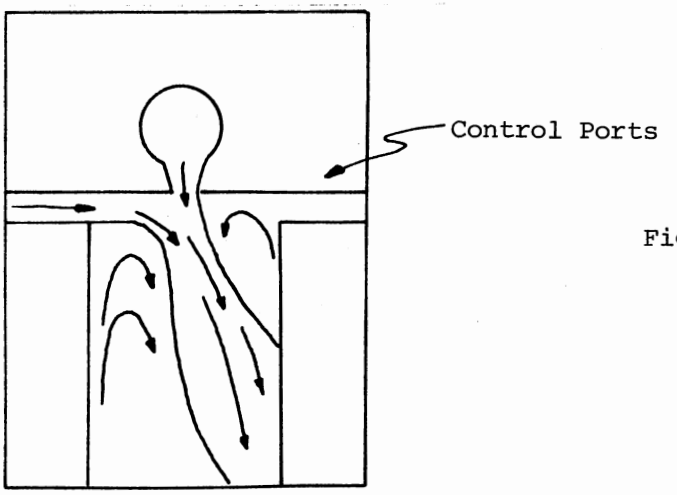


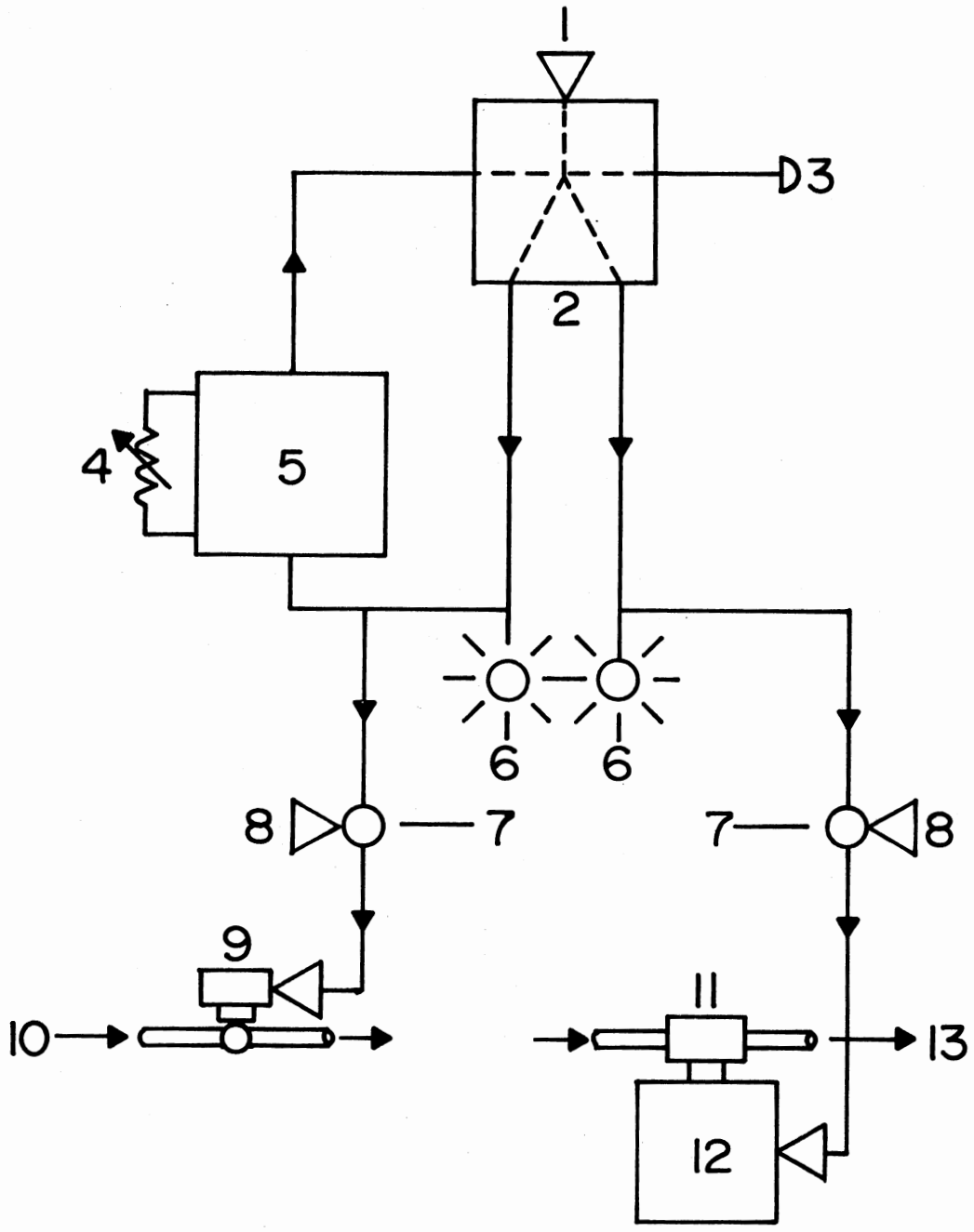
Figure 4. Representation of Control of Wall Attachment Phenomenon

will (Norgren, 1974).

The breath control circuit contained this basic component, the flip-flop (Corning, Model 191454), to supply breathing air to the subject while simultaneously closing the exhalation port on the inhale cycle. Conversely, it shut off the supply air and opened the exhalation port during the exhalation cycle. Figure 5 gives a diagram of the control circuit. The jet stream supply was maintained at 5 psi with a Norgren Model B06-102-MlAA regulator. The valves and interface valves were normally closed in the absence of a pressure pulse. The time delay relay (Corning, Model 191466) and the flip-flop were mounted on a manifold (Corning, Model 191724) which had two of the gates capped (Corning, Model 191654). All of the fluidic devices were mounted on a chassis box to facilitate operation.

During exhalation the jet stream was attached to the right wall which opened the right visual indicator (Norgren, Model 5VS-010-BR0) and the interface valve (Corning, Model 192621). The interface valve opened and a supply air pressure opened the valve on the exhalation port of the mouthpiece. When the pulse button (Norgren, Model 5PB-010-AR0) was pushed, the jet stream flipped to the left side which opened the left visual indicator (Norgren, Model 5VS-010-BG0) and interface valve. The valve on the inhalation port of the mouthpiece opened, supplying air to the subject. The time delay relay acted as a capacitor and when the pressure built sufficiently, the jet stream switched back to the right side. The time delay relay was adjustable with the needle valve which restricted the rate of pressure build up.

Figure 5. Diagram of Fluidics Control Circuit: 1. Air Supply, 2. Flip-Flop, 3. Pulse Button, 4. Needle Valve, 5. Time Delay Relay, 6. Visual Indicator, 7. Interface Valve with Atmospheric Vent, 8. Nitrogen Supply, 9. Air Actuated Bellows Sealed Valve, 10. Inhalation Supply, 11. Air Actuated Two-Way Ball Valve, 12. Air Actuator, 13. Exhalation Collection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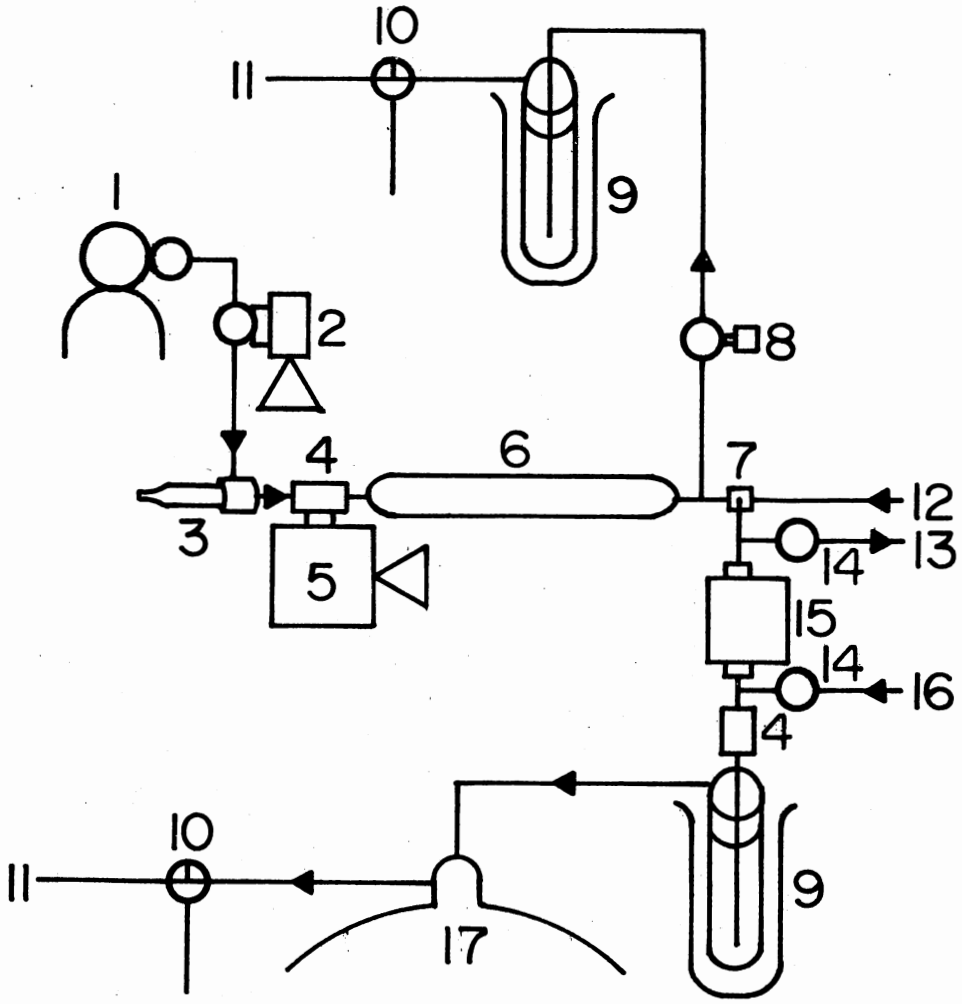
Apparatus for Air Supply and Breath Collection

The air supply used was ultra pure air from Linde which had a minimum purity level (with respect to hydrocarbon impurities) of < 0.1 ppm. The regulator used was a high-purity, corrosion-resistant Linde regulator, Model UPC-3-30-1340. The air supply was turned off and on by an air operated bellows sealed valve (Nupro, Model SS-4BK-NC). (See Figure 6.) The mouthpiece was custom machined (O.S.U. Chemistry Instrument Shop) in two parts from stainless steel. A cap was also machined to substitute for the mouthpiece so that the system could be closed for cleaning and leak checking. The exhalation collection system was built to maintain a minimum back pressure as well as minimum contamination. The exhalation valve was a two-way ball valve (Whitey, Model B-44F4) equipped with an air actuator (Whitey, Model MS-133SR). The small ballast was a 500 ml sample cylinder (Whitey, Model 304-HDF4-500) which helped to decrease the pressure drop. As shown in Figure 6, the system was divided with one side vented into the room through a cold trap and the other connected to the Tenax adsorption trap in series with a vacuum manifold. These two paths will hereafter be designated as flushing and sampling paths, respectively. The breath was moved through a nitrogen trap in the flushing path by a vacuum pump. The liquid nitrogen trap prevented pump exhaust from permeating the apparatus and simultaneously protected the pump intake from water vapor. The needle valve (item 8, Figure 6) controlled the amount of suction from the vacuum pump. The sampling side was opened or closed with use of the three-way ball valve (item 7, Figure 6). A 12 l round-bottomed flask was modified so that it could be used as a ballast (item 17, Figure 6) (O.S.U. Chemistry

Figure 6. Schematic of Breath Sampling System: 1. Ultra Pure Air Supply, 2. Air Actuated Bellows Sealed Valve, 3. Mouthpiece, 4. Two-Way Ball Valve, 5. Air Actuator, 6. 500 ml Ballast Volume, 7. Three-Way Ball Valve, 8. Needle Valve, 9. Liquid Nitrogen Trap, 10. Three-Way Vacuum Stopcock, 11. Vacuum Pump, 12. Urine Sample Inlet, 13. Pathway to Injection Valves, 14. Shut-Off Valve, 15. Tenax Column in Aluminum Heating Block, 16. Helium Supply, 17. 12 l Ballast Volume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Glass Shop). The vacuum pump evacuated the glass ballast. After pump-down the vacuum pump was turned off at the three-way vacuum stopcock (item 10, Figure 6). A monitor of the pressure with a mercury manometer allowed the volume of breath pulled through the Tenax to be determined.

Early morning breath samples were used before the subject ate, brushed his teeth, etc. In order to minimize the amount of volatiles resulting from oral bacteria, the subject rinsed his mouth thoroughly with salt water before analysis.

The subject adjusted the pressure of the supply air (typically 40 psi), the length of inhalation, and the amount of suction from the vacuum pump until he felt comfortable. The subject's nose was clamped during sampling. A 10 min flushing period was chosen mainly because of the practical time limitations of the system.

The ballast tank would normally be filled twice for one sample. This corresponds to about a 20 l sample. The method of calculation of the volume of breath sampled is shown in Equations 1-4.

$$P_1 = P_B - \Delta R_1 \tag{1}$$

and likewise for P_2, where P_1 and P_2 were the pressures in the ballast before and after sampling, P_B was barometric pressure, and ΔR_1 was the change in the manometer readings. From the ideal gas law,

$$\Delta n = \Delta P V_o / RT \tag{2}$$

where Δn was the change in the number of moles of gas, ΔP was $P_1 - P_2$, V_o was the volume of the ballast (12 l), R was the gas constant, and T was the temperature which was constant. Equation 3 was used to calculate the volume of breath sampled at the barometric pressure and temper-

ature of that day.

$$\Delta V = \Delta nRT/P_B \quad . \tag{3}$$

Substituting Equation 3 for Δn gives

$$\Delta V = \Delta PV_o/P_B \tag{4}$$

The breath system was cleaned by capping the mouthpiece, opening the exhalation and inhalation valves, and flushing with nitrogen.

Blank runs were made capping the mouthpiece and pulling approximately 20 l of the supply air through the Tenax.

Sample Concentration Apparatus and Procedures

Once the sample (either urine or breath volatiles) was collected onto the Tenax column the three-way and two-way ball valves were both closed; thus isolating the Tenax (60-80 mesh, Applied Science Laboratories, Inglewood, Calif.). (See Figures 1 and 6.) The shut-off valves (Whitey, Model B-1KS4) were opened to allow helium to sweep the thermally desorbed components to the injection system. The Tenax column was contained in a aluminum heating block which was controlled with one of Hewlett-Packard (HP) 19120A circuits. The glass wool plug in the Tenax column was silanized by refluxing the glass wool in a 5% solution of trimethylchlorosilane in toluene. The Tenax column was made from 0.25 in stainless steel tubing and silanized by aspirating trimethylchlorosilane through the tubing.

In order to thermally desorb the volatiles, the Tenax, 50 mg for urine and 10 mg for breath, was heated to 300^{o}C and flushed with helium at 50 ml/min for 30 min. Blockage of the coil in the liquid nitrogen trap was minimized by flushing the Tenax at room temperature for 30 sec.

This decreased the amount of water trapped. Also the trap was positioned so that the sample entered the coiled side of the trap first. When blockage did occur it was corrected by blowing over the top of the trap with a heat gun.

Injection Apparatus and Procedures

In order to inject the components as a slug at the front of the column, a sampling valve injection system was designed. The syringe injection port on the GC was completely removed and a six-port sampling valve (Valco, Model V-6-HPa) and a four-port sampling valve (Valco, Model V-4-HPa) were used. Figure 7 shows a diagram of their functions and Figure 8 shows the relative orientation of the valve system to the rest of the GC. During thermal desorption of volatiles from the Tenax the valves were in position 1. The components were trapped with a liquid nitrogen bath surrounding the coil (dashed line in Figure 8) on the four-port valve. The coil was then isolated (position 2) and heated with a heat gun. The six-port and four-port valves were switched (position 3), respectively, which injected the sample onto the column. The dead volume of the injection system was minimized by using 0.01 in inner diameter tubing for all connections except in the coil which was 1 ft x 0.03 in inner diameter tubing. The total length of the tubing between the coil and the column was approximately 18 in. The valves were enclosed and heated to 75^{o}C using one of the HP 19120A circuits for control. After injection the valves were left in the inject position for 1 min.

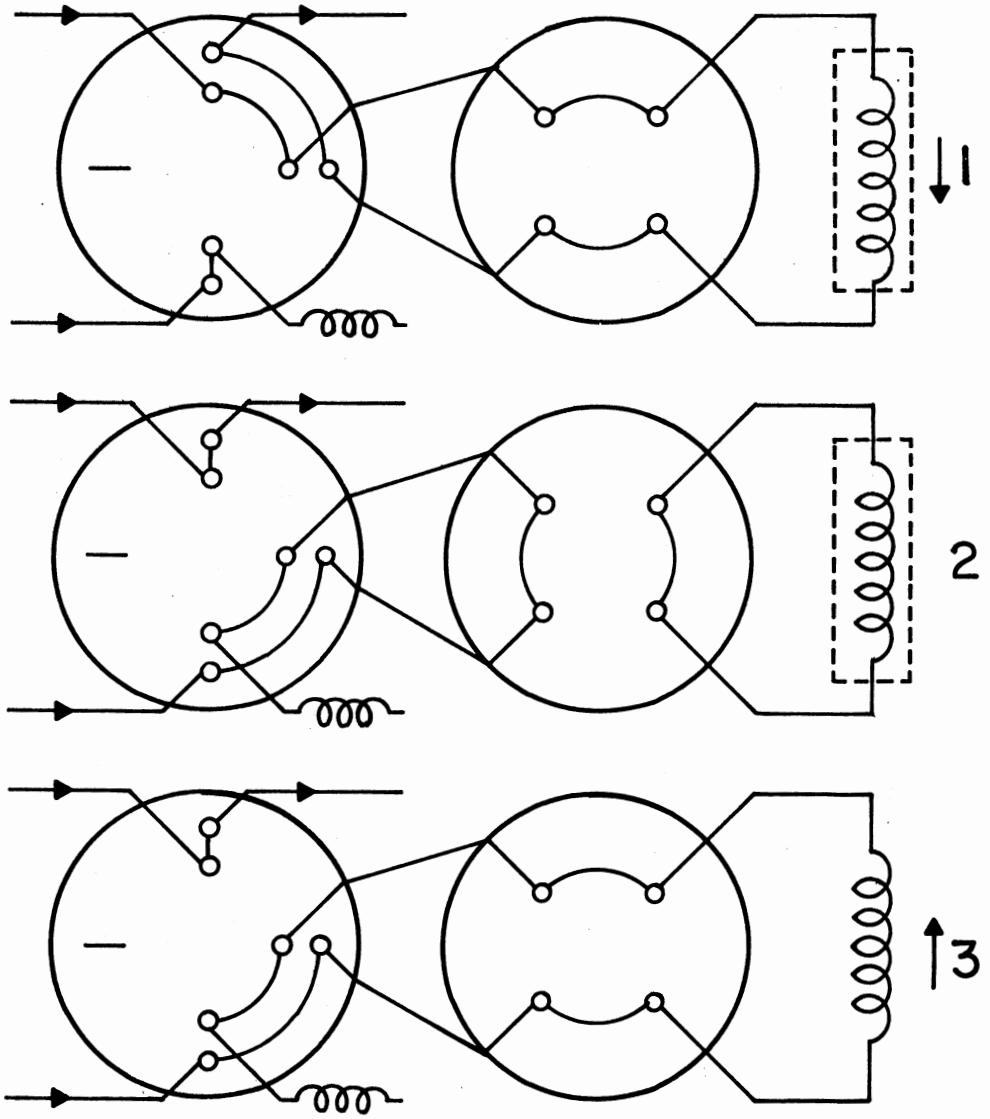
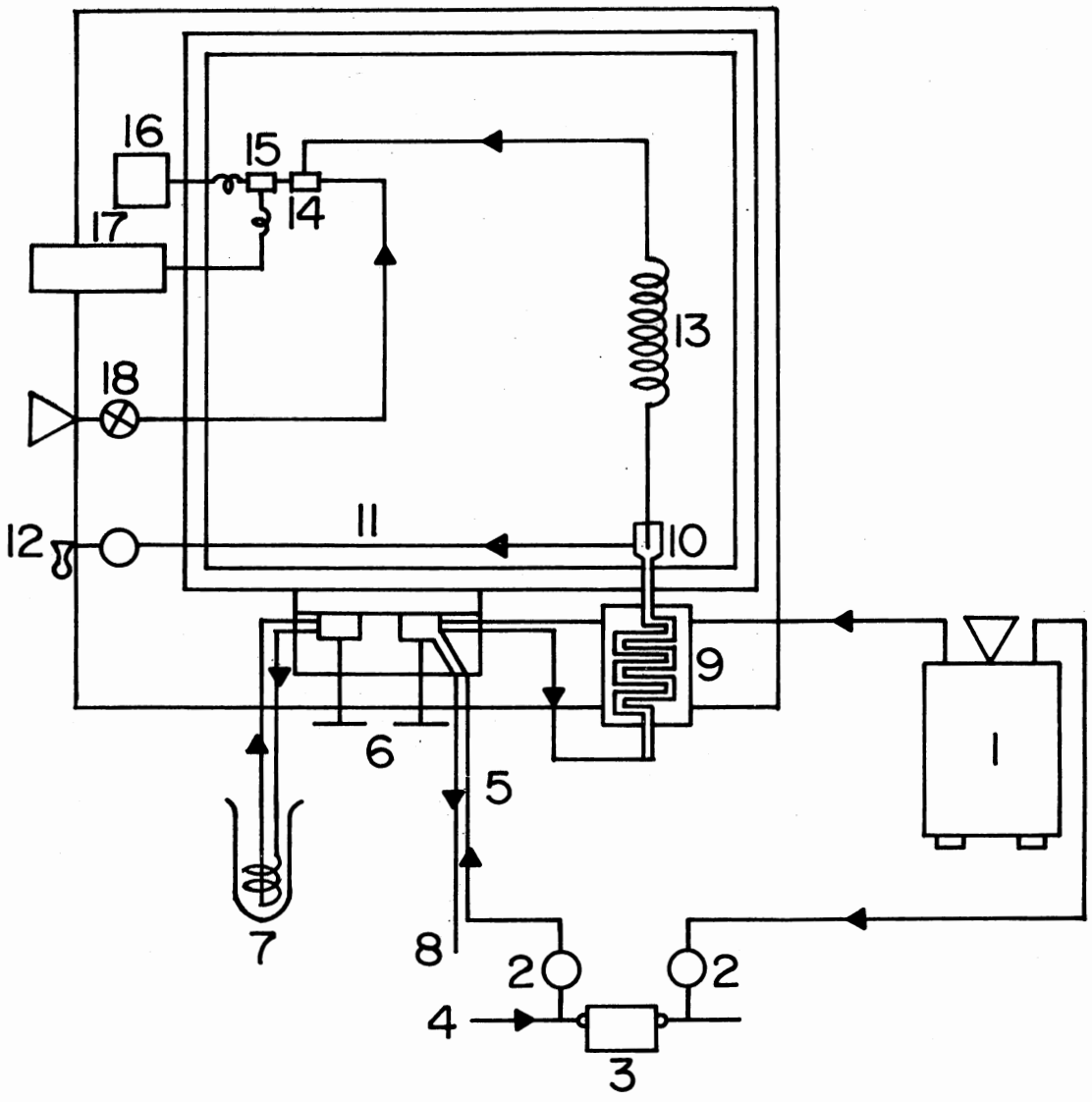


Figure 7. Functions of Valve Injection System: Position (1) Sample Collection in Liquid Nitrogen Trap, Position (2) Sample Isolation and Heating, and Position (3) Sample Injection onto Column

Figure 8. Modified Gas Chromatograph and Injection System: 1. Flow Controller, 2. Shut-Off Valve, 3. Tenax Column in an Aluminum Heating Block, 4. Breath or Urine Sample Inlet, 5. Sample Transfer, 6. Injection Valves in Heating Block, 7. Liquid Nitrogen Trap, 8. Injection Valves Outlet, 9. Expander in Heating Block, 10. Inlet Splitter, 11. Restrictor Tube for Splitter, 12. Shut-Off Valve With Bleed Line, 13. SCOT or WCOT Column, 14. Modified Low Dead Volume Union, 15. Effluent Splitter, 16. FID, 17. FPD, 18. Make Up Gas Stream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GC Modifications and Procedures

A HP Model 7620A Research Chromatograph equipped with flame ionization detectors was used in this study. Figure 8 shows the modified GC and the injection system.

Two 50 ft x 0.02 in inner diameter SCOT columns coated with Carbowax 20M were used. These columns were commercially obtained from the Perkin Elmer Company. The two columns were connected with a low dead volume union. A 500 ft x 0.03 in inner diameter WCOT column coated with SF 96 was also used. The column was coiled (U.S.D.A. Laboratory, Albany, California) in a basket weave design for better heat transfer. The cleaning and coating procedure of Teranishi et al. (1971) was used for the WCOT column with the exception that the flow rate during the coating procedure was kept constant at 0.5 ml/min using a Brooks Model 5840 Dual GC Mass Flow Controller. The coating apparatus consisted of a tee fitting with one arm of the tee used for filling and then capped, a 500 ml sample cylinder (Whitey, Model 304-HDF4-500) used for a reservoir, and a bellows sealed valve (Nupro, Model SS-4TW). The bellows sealed valve was necessary due to the use of concentrated nitric acid and concentrated ammonia during the cleaning process.

When using open tubular columns small sample sizes must be used. For this reason a HP Model 19118A inlet splitter was used for the urine samples. To insure complete mixing of the sample before splitting a relatively large heated volume, the expander, was placed before the splitter. A relatively high flow rate was established through the expander to prevent peak broadening due to diffusion. The split ratio was established by the length of 0.01 in inner diameter tubing off the

splitter to the room relative to the column length. With the SCOT column a split ratio of approximately 22:1 was used and with the WCOT column a ratio of approximately 9:1 was used. For the breath samples, the sample sizes were maximized by removing the splitter and expander. The carrier flow rate was regulated by either a Brooks Model 8744 Flow Controller or a Brooks Model 5840 Dual GC Mass Flow Controller.

The flow rate through the WCOT column was set at 5.78 ml/min as determined by a soap bubble meter. The resulting flow rate through the expander was 58 ml/min. With the SCOT column for both the breath and urine samples the flow rate was set at 1.73 ml/min. This flow rate was determined by measuring the retention time of the relatively unretained methane peak at room temperature. Equation 5 gives the calculation for finding the flow rate.

$$F = V_M/t_R \tag{5}$$

where V_M is the dead volume of the column and t_R is the retention time of the methane peak. When the splitter and expander were used the flow rate to the atmosphere was 37 to 38 ml/min. The flow rate through the expander was determined for both the WCOT and SCOT columns with a soap bubble meter.

The temperature control chassis contained two Model 19120A temperature controllers and one Model 7660A Multilevel Programmer. The temperature controllers gave the system the capability of heating and controlling the following equipment: (1) the Tenax adsorption column, (2) the injection valves, (3) the FID, (4) the FPD, (5) the expander, and (6) a helium ionization detector located on another GC. The Multilevel programmer provided precise temperature programming at 1 to 3

different rates as well as isothermal operation. For the SCOT column the temperature program used was isothermal at 60°C for 10 min, 2°C/min to 120°C, 1°C/min to 165°C, and isothermal at 165°C for 20 min. Due to the high susceptibility of Carbowax 20M to oxidation, the flow rate through the column was turned off at night. This necessitated a cleaning program before injection. The following was used: 60°C to 170°C at 6°C/min and isothermal at 170°C for 20 to 30 min. For the WCOT column the temperature program used was isothermal at 30°C for 10 min, 1°C/min to 65°C, isothermal at 65°C for 2 min, 0.5°C/min to 100°C, 2°C/min to 175°C, and isothermal at 175°C and hold.

The HP GC was designed for use with the relatively high flow rates used with packed columns and therefore had a large dead volume between the end of the column and the detector. To compensate for this dead volume and to insure the best flame conditions, a make up gas stream was installed at the end of the column. This entailed modifying an angle-pattern, low dead volume 1/4 in to 1/16 in union (O.S.U. Chemistry Instrument Shop). Capillary tubing was silver soldered in a hole drilled into the end of the fitting. This inlet was used for the make up gas stream. The column effluent was introduced into the side of the fitting and swept along at the higher flow rate of the make up gas. The make up gas flow was controlled with a Brooks Model 8744 Flow Controller.

The instrument was equipped with two plug-in signal modules, Model 7650A electrometers, which allowed the simultaneous monitoring of dual detectors. A HP flame photometric detector was installed to monitor sulfur compounds. An additional HP recorder Model 7127A was used to record the FPD trace. A HP Model 19034A Effluent Splitter was installed

so that the FID and FPD could be used simultaneously. The split ratio between the two detectors was determined by the length of 0.01 in inner diameter tubing between the splitter and the respective detectors. In this case a 1:1 split was used.

The operating conditions of the other parts of the GC are given in Table I. When peaks would have gone off scale with the attenuations given in Table I, the attenuation and/or range were changed until the top of the peak was on scale.

Subjects

The subjects chosen are designated as Subjects #1, #2, and #3. Their respective ages were 24, 33, and 22 years and were all female. Subject #1 was a diabetic under insulin treatment and a nonsmoker. Subject #2 was taking no drugs and was a nonsmoker. Subject #3 was taking birth control pills (Ovulen 21) and was also a nonsmoker. All the subjects fasted for 12 hr before samples were collected.

Urine samples from Subjects #1, #2, and #3 were analyzed to examine variations between individuals. Breath and urine samples from subject #3 were used for analysis on consecutive days as well as for the asparagus study. For the asparagus experiment approximately 15 oz was eaten before retiring.

Treatment of Data

The retention time of a compound is a characteristic of that particular compound and can be used for tentative identification. The method of McConnell and Novotny (1975) was used for the calculation of relative retention times: one peak was chosen as a reference and all

TABLE I

TYPICAL OPERATING CONDITIONS OF THE GC

Electrometer Settings:	FID	FPD
Function	A	A
Supression	Low	High
Range	SCOT-1, WCOT-10	10^3
Attenuation	SCOT-4, WCOT-1	8

Temperatures:

FID	250°C
FPD	200°C
Expander	115°C

Chart Speed: SCOT-0.5 in/min, WCOT-0.25 in/min

Input Module:	FID Detector 1	FPD Detector 1	
Gases:	Tank Settings	Approximate Flow Rate	Rotameter Settings
H_2	15 psi	40 ml/min	FID(A) 3.0
		50 ml/min	FPD 0.5
Air	26 psi	300 ml/min	FID(A) 3.0
		50 ml/min	FPD 50
O_2	50 psi	10 ml/min	FPD 10
He(make up gas)	42 psi	60 ml/min	
He(carrier gas)	40 psi		

the other peaks had their relative retention times calculated relative to that one peak. In this case the peak chosen was a sulfur peak.

Peak area reflects the amount of a compound present. Peak height measurement is the most widely used method of estimating peak area and was the method used here.

CHAPTER III

RESULTS AND DISCUSSION

The developmental aspects of the breath sampling system and the gas chromatograph modifications are enumerated here together with several illustrations of pertinent analytical problems encountered.

It was desirable to operate the initial program slightly above ambient temperature due to the appearance of several extremely volatile components early in the chromatograms. Since the oven was not thermally stable below about 50° C, a water cooled coil was installed in the oven and used with the WCOT column.

The WCOT column was expected to provide the best overall separation. It did, however, produce severe tailing for some of the early peaks. The coating procedure used may have caused the unsatisfactory performance. The curing steps during the coating procedure may have been insufficient to insure proper layering of the liquid phase. This was not investigated further due to the excellent performance of the SCOT column.

The SCOT column reduced the analysis time by one half with improved resolution and sensitivity. The oven cooling coil was not necessary with the SCOT column since the Carbowax coating liquified at 60° C. The WCOT column produced a lower column bleed than the SCOT column. This may have been due to the thermal instability of Carbowax 20M relative to SF 96 or more probably to a low SF 96 load in the WCOT column.

The lower column bleed may be significant for later MS work.

The purpose of the breath sampling system was to provide a system which minimized contamination. A system which was closed to the atmosphere was built in order to minimize room air contamination. The original design of the breath sampling system utilized exhalation and inhalation valves which had orifice sizes of 0.172 in and shut-off valves to isolate the Tenax which had orifice sizes of 0.172 in. The entire exhalation was directed through the Tenax column. A closed system was found not possible with this design, because the back pressure on the exhalation side was too high. At this point, ball valves with an orifice size of 0.281 in were installed both as the exhalation and isolation valves. The air actuator for the exhalation ball valve had a 1 sec response time. This allowed some of the supply air to flow through the Tenax without being used by the subject. Assuming the supply air was sufficiently pure, the above flow pattern would have had a negligible effect. The system was redesigned so that only part of the breath sample went through the Tenax. This resulted in a decrease in the rate of obtaining a sample of given volume. The pressure drop across the Tenax column was decreased by using a wire mesh in one end rather than glass wool to contain the Tenax. There was a tendency for water to build up in the system which caused a contamination problem. Several steps were taken to minimize this: a water aspirator upstream from the mouthpiece was removed, the 500 ml ballast tank was heated, the sampling system was partially disassembled and placed in an oven between samples, and the rest of the system was flushed with nitrogen. Data presented later illustrates the effectiveness of the closed system and the cleaning procedure.

The fluidics control circuit was initially designed with the length of the exhale cycle as well as the length of the inhale cycle being preset. This feature was subsequently improved by replacing the preset exhalation cycle with the pulse button. The length of exhalation was then goverened on each breath by the subject. The fluidics control circuit had several advantages: (1) it proved to be extremely reliable in continuous use due to the lack of moving parts, (2) the apparatus was convenient in a gas chromatography laboratory due to the availability of flow and pressure control equipment, and (3) the system was programmable to meet the needs of an individual. The limiting feature of the apparatus was the time limit imposed by fatigue of the subject. This could be alleviated by using a Schmidt trigger in the fluidics control apparatus which would make the circuit a true demand regulator. The control circuit would then be open to the breathing system and the plastic tubing used on the fluidic devices would have to be replaced with metal tubing.

A typical human urine chromatogram after ingestion of asparagus is shown in Figure 9. The figure is an artist's representation of the actual profile. The baseline was rezeroed as shown between pages 55 and 56. About 130 measurable peaks (not counting apparent composite peaks) were obtained in a total analysis time of 4 hr.

A comparison of urine profiles from Subject #3 taken on three consecutive days is shown in Table II. The mean relative retention time and the mean peak height are reported as well as the average deviations. Little interference was observed from the blank. Only two peaks were common to the blank and urine chromatograms and are designated with a "B". Approximately 34% of the peaks measured did not appear in all

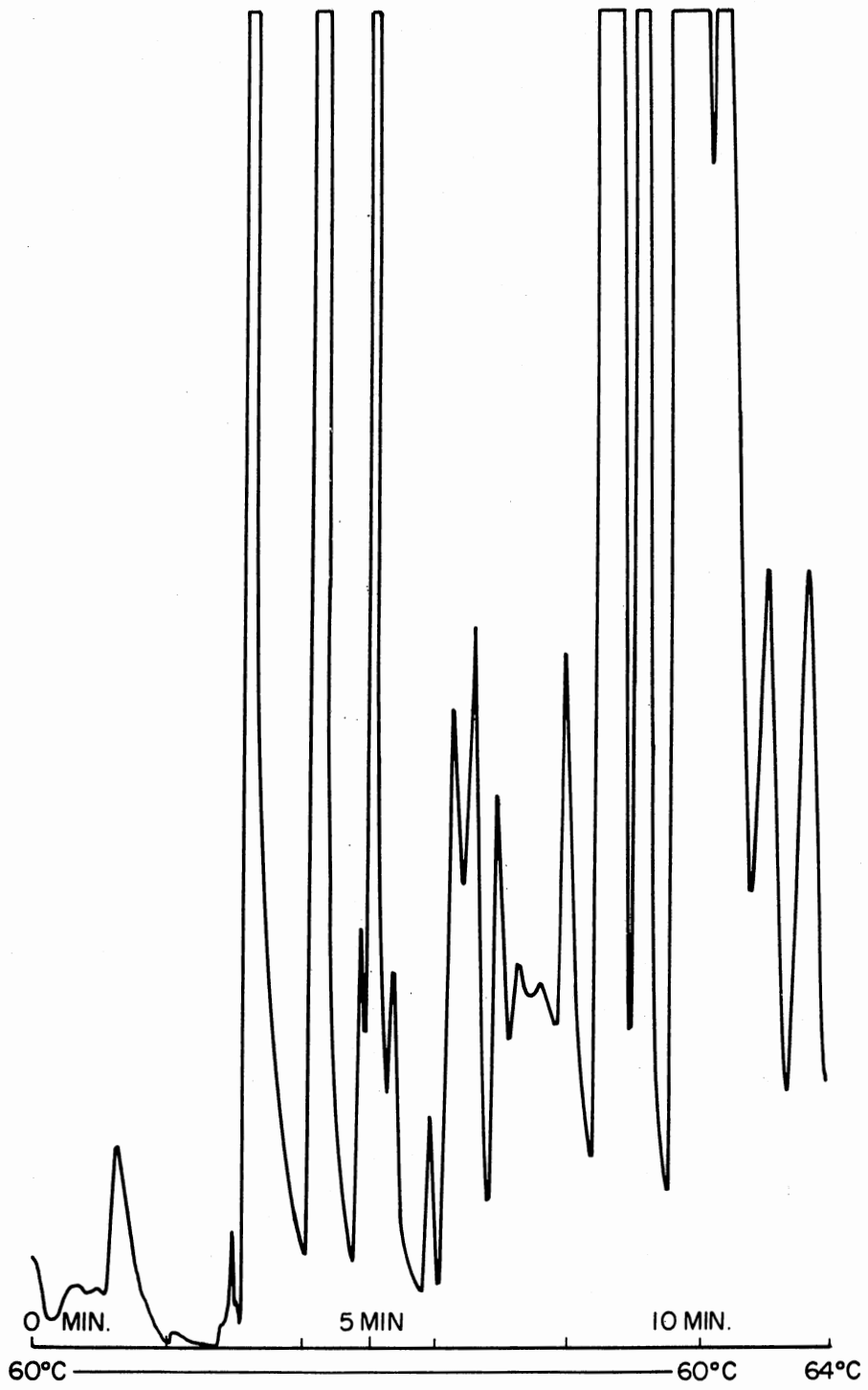

Figure 9. Artist's Representation of an Urine Chromatogram After Ingestion of Asparagus

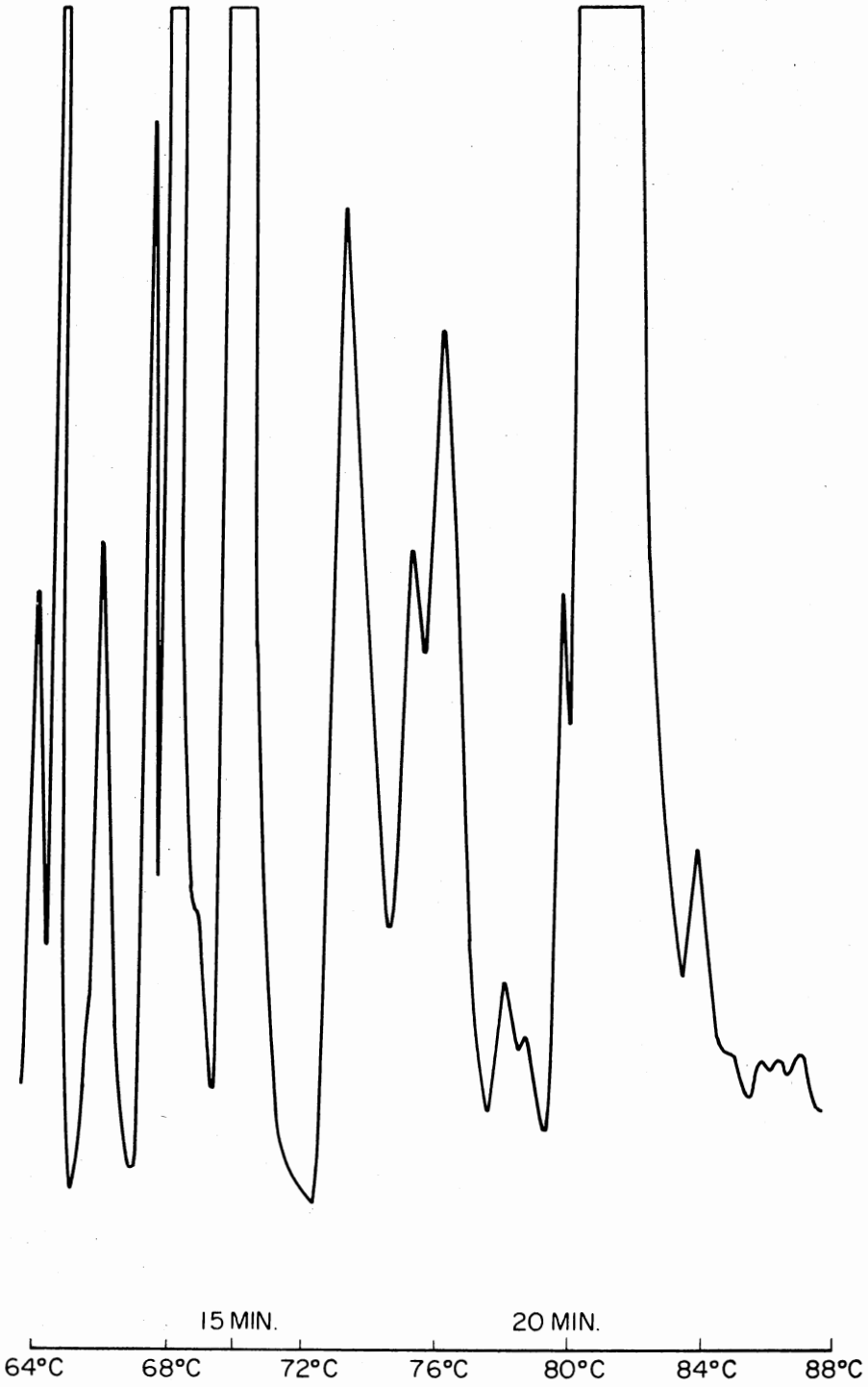

Figure 9. (Continued)

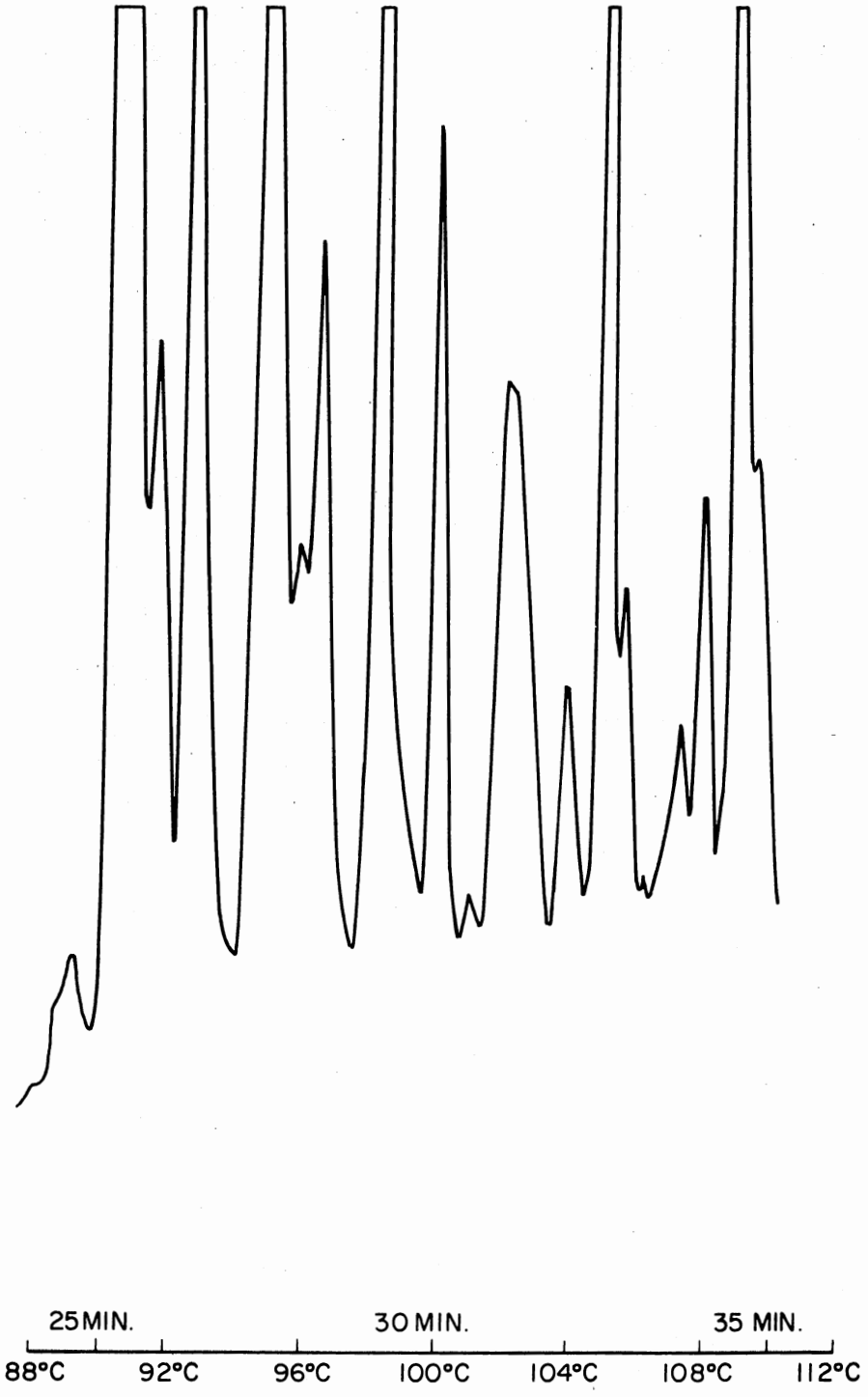

Figure 9. (Continued)

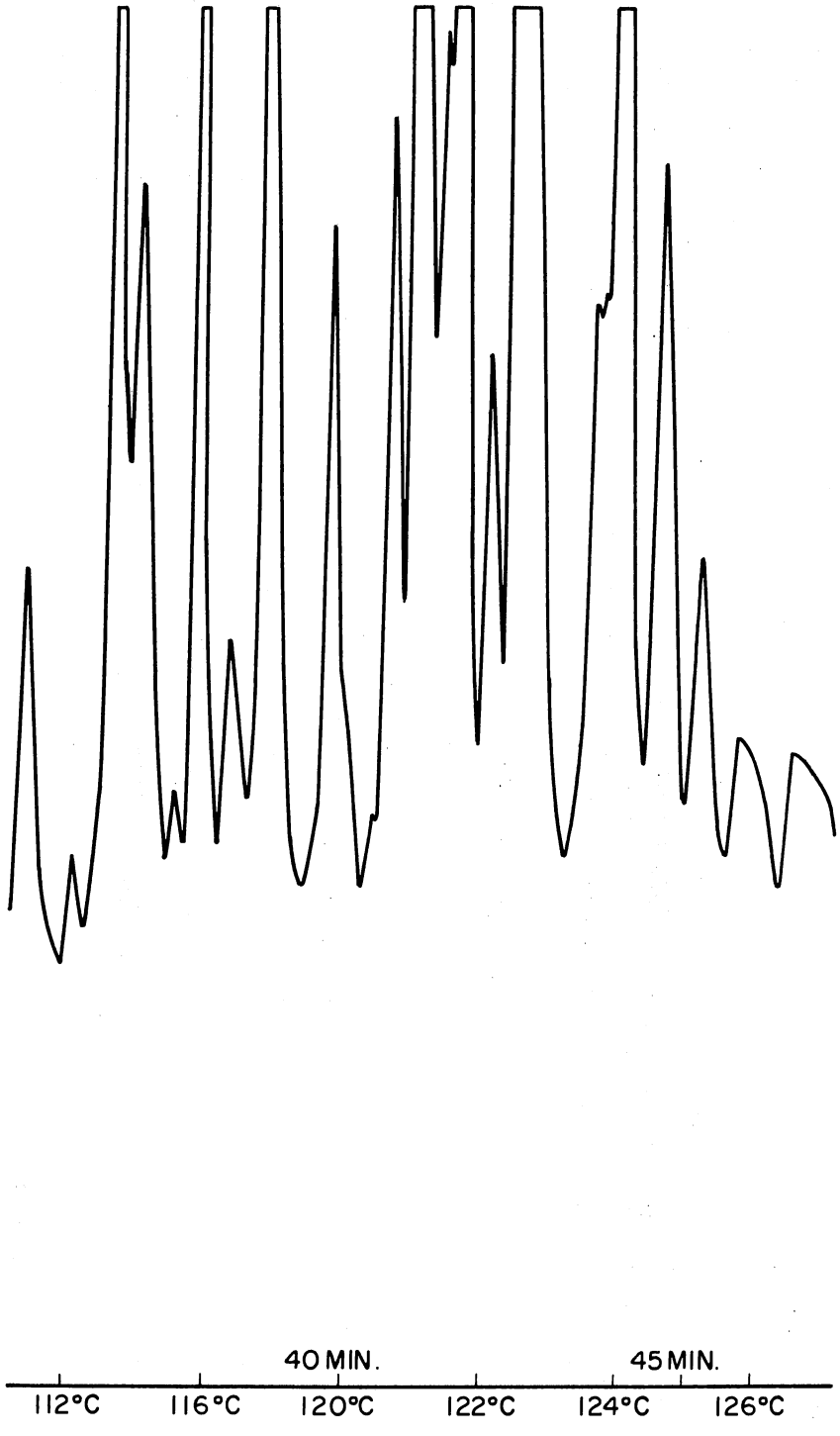

Figure 9. (Continued)

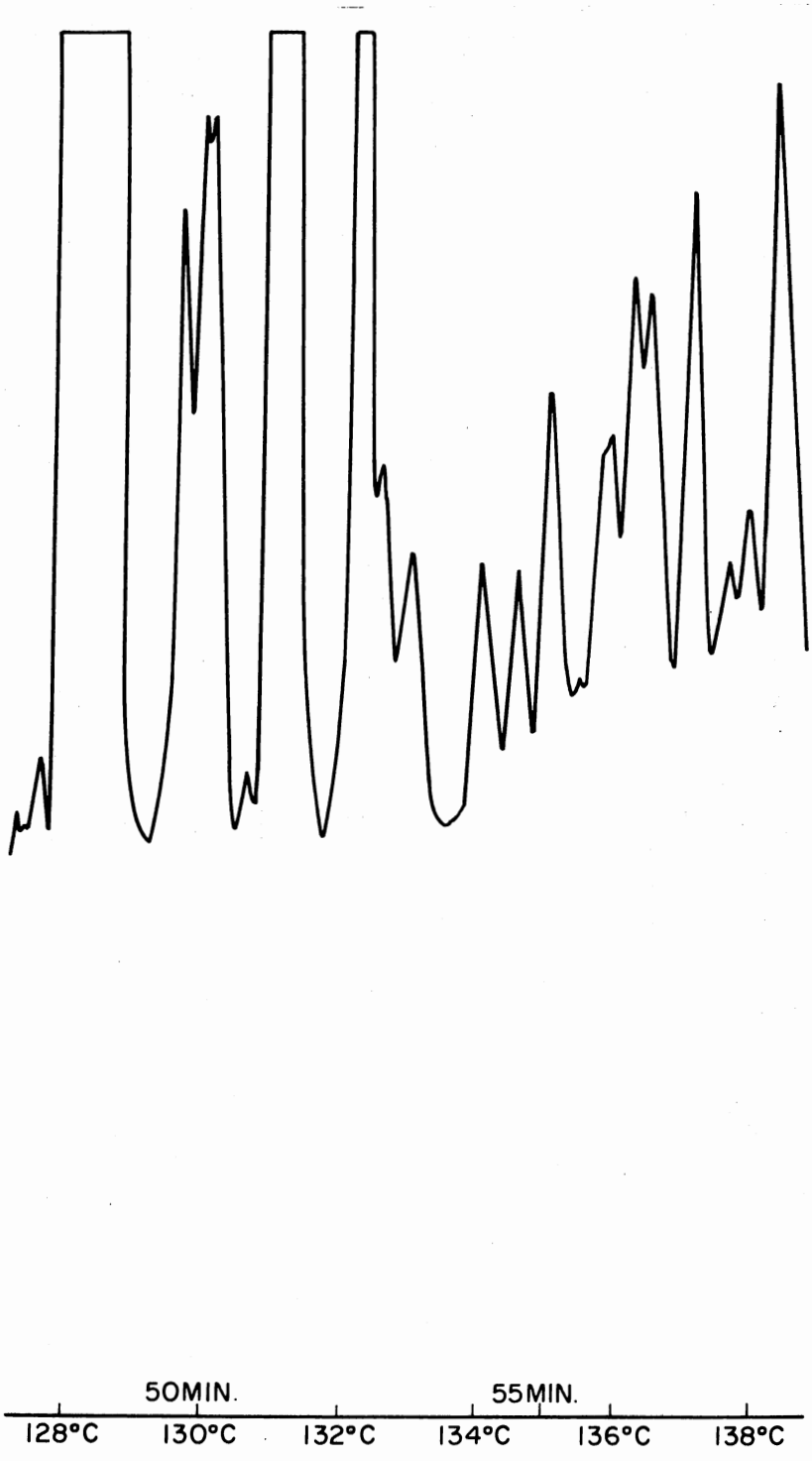

Figure 9. (Continued)

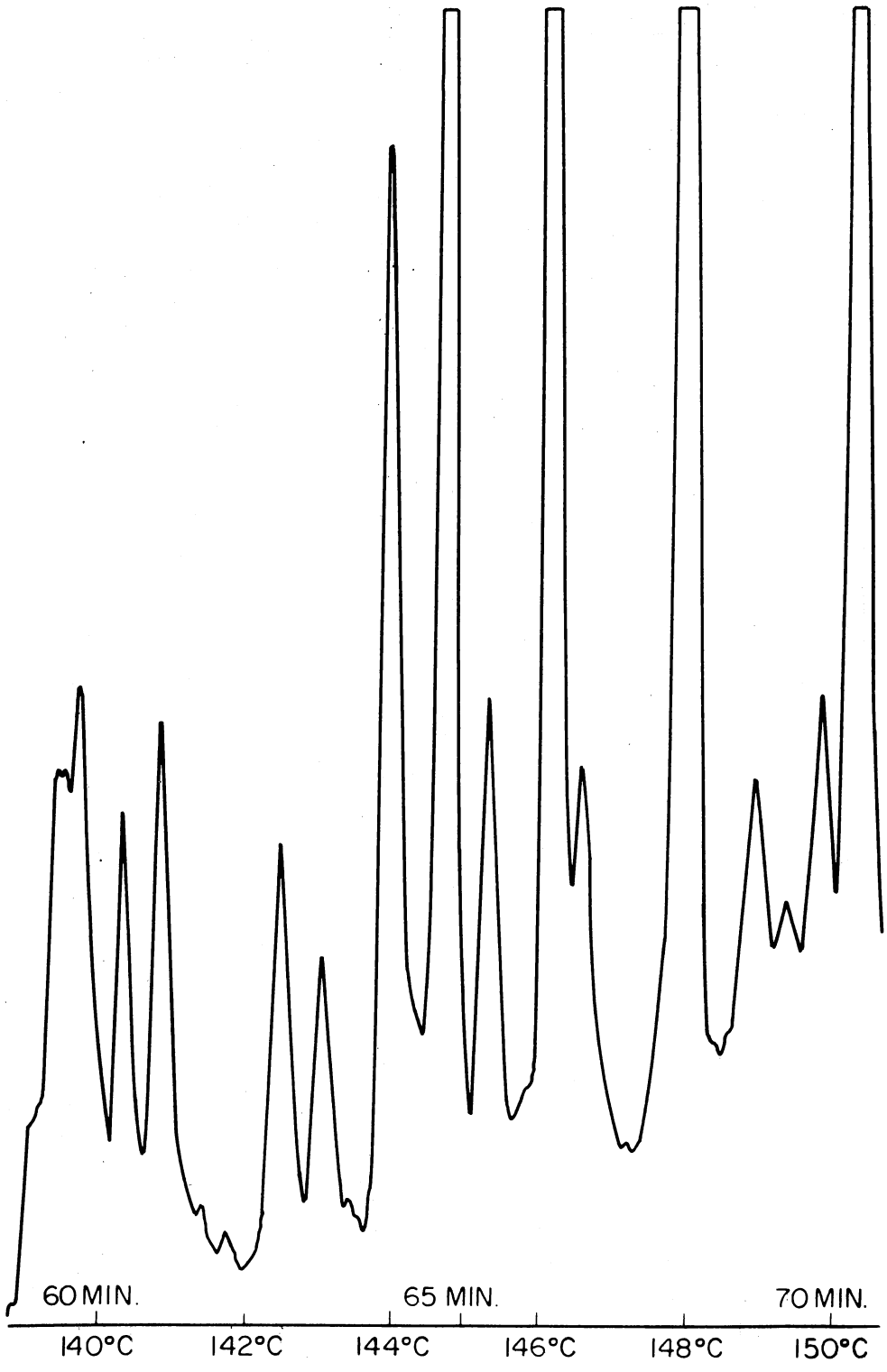

Figure 9. (Continued)

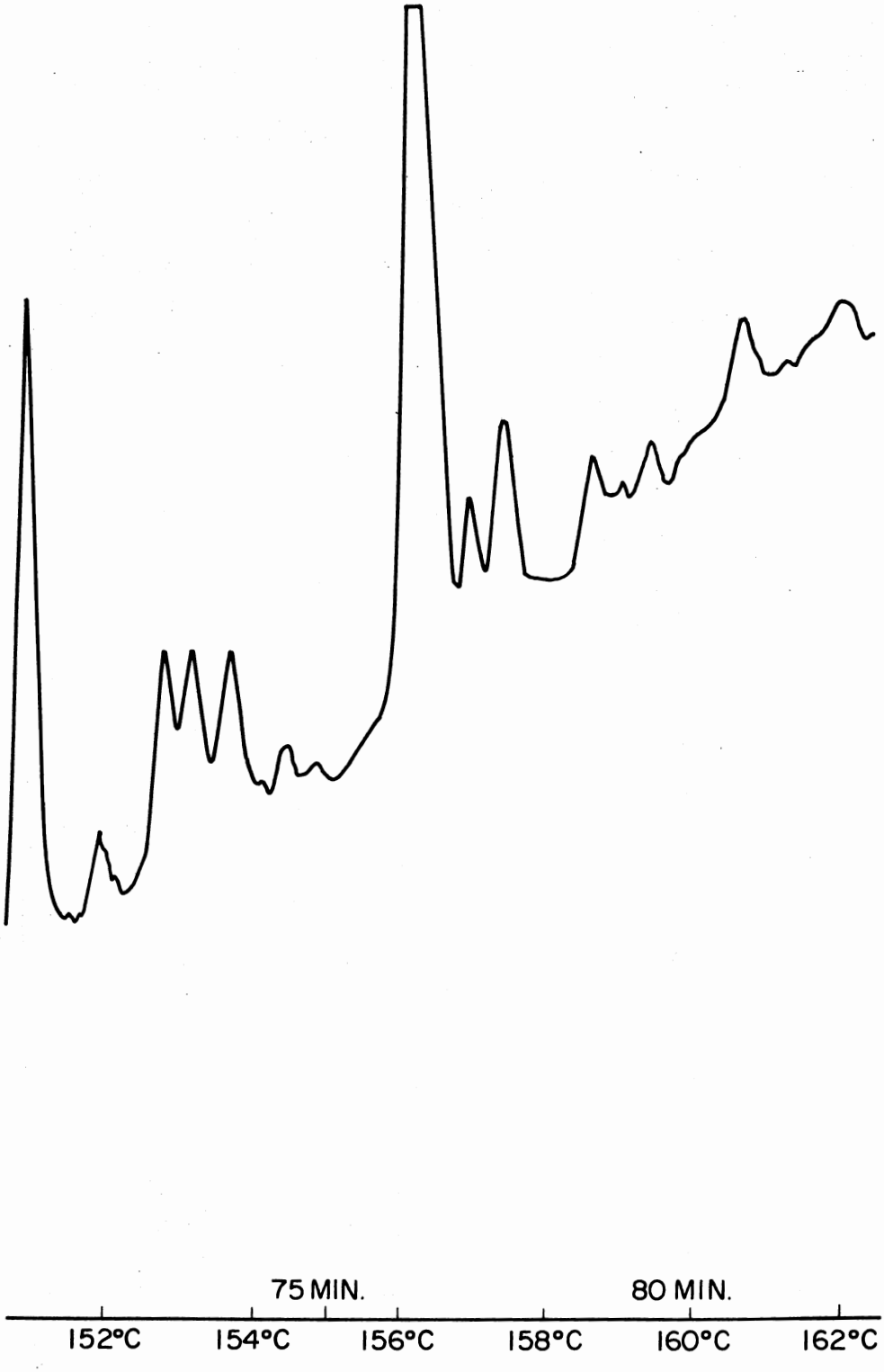

Figure 9. (Continued)

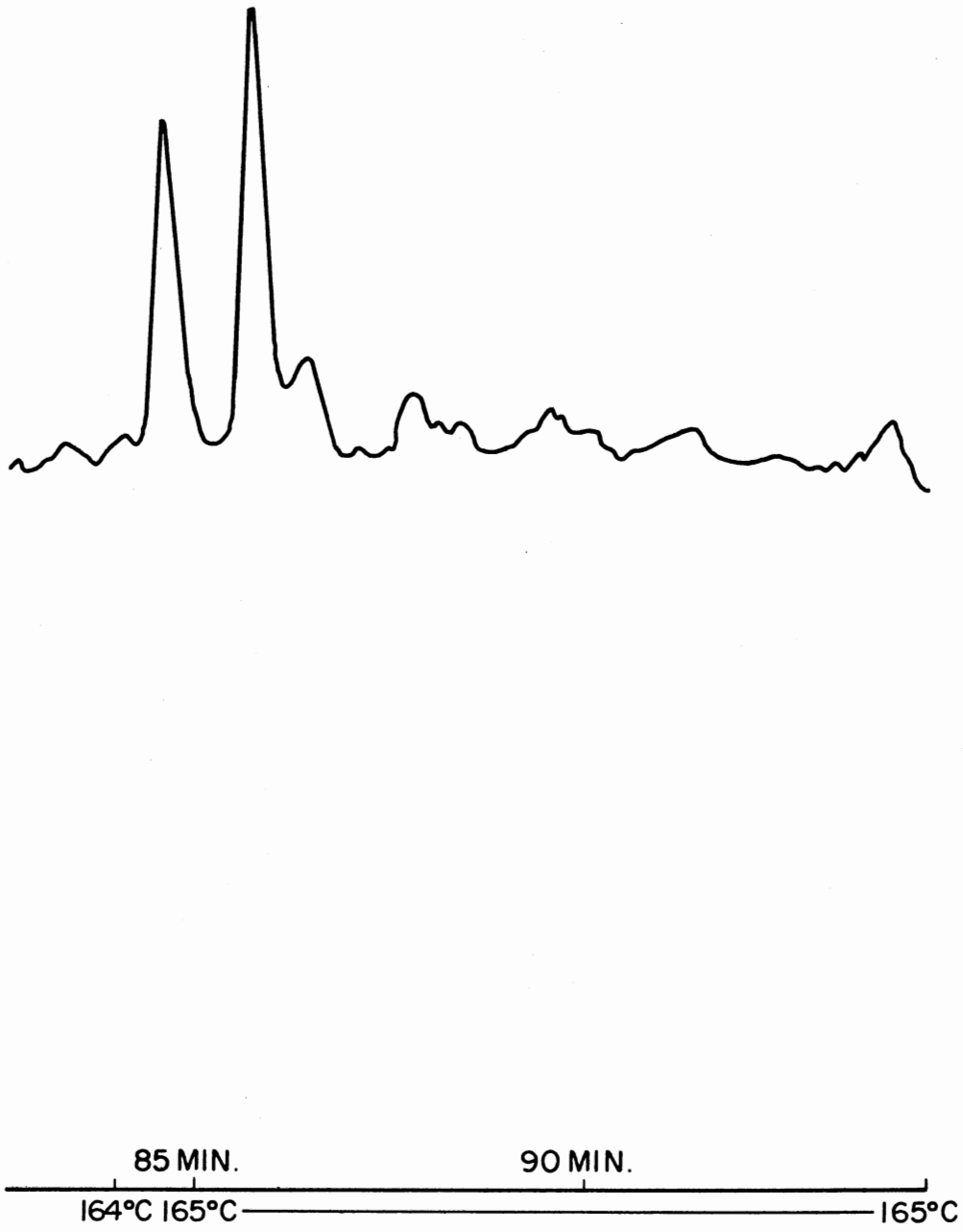

Figure 9. (Continued)

TABLE II

REPRODUCIBILITY OF URINE SUBSTITUENTS IN ONE SUBJECT
FOR THREE CONSECUTIVE DAYS

Mean Relative Retention Time	% Average Deviation	Mean Peak Height (cm)	% Average Deviation
0.0721[a]	----	1.40	-----
0.0754(B)	1.86	1.17	29.06
0.0777	2.12	1.93	22.80
0.0788[a]	----	119.2	-----
0.0813	1.83	87.40	62.32
0.1128[b] (S,)(B)	1.02	165.20	62.71
0.1265[c]	0.91	6.48	87.64
0.1322	0.74	2.92	84.25
0.1365	0.42	31.08	59.01
0.1471	0.32	5.32	66.92
0.1629	0.29	5.65	39.47
0.1723[a]	----	1.65	-----
0.1808	0.53	6.57	42.92
0.1917	0.88	26.53	59.63
0.2044[a]	1.06	10.83	56.51
0.2194[a]	----	2.85	-----
0.2217	0.75	16.88	105.5
0.2303[a]	----	2.65	-----
0.2386	0.70	57.67	51.71
0.2479(S)	0.48	5.20	70.58
0.2571[b]	0.66	112.10	54.22
0.2722[d,a]	1.22	188.4	7.86
0.3008	----	2.30	-----
0.3142[c]	1.29	7.05	56.31
0.3250	1.34	10.70	44.86
0.3334	1.35	6.78	41.45
0.3465	1.51	19.52	58.01
0.3590	1.26	23.43	19.16
0.4019	0.09	13.08	2.49
0.4079[a]	0.49	51.60	34.11
0.4458	----	2.90	-----
0.4360(S)	2.84	740.4	120.71
0.4623[d]	0.55	29.0	21.38
0.4958[d]	0.57	4.38	22.29
0.5418	0.14	1.60	56.25
0.5627[d,a]	0.17	16.05	95.47
0.5946[d,a]	----	1.50	-----
0.6089[d]	----	1.20	-----
0.6336[d,a]	0.01	1.90	31.58
0.6461[d]	----	1.15	-----
0.6654	0.14	1.95	33.33
0.6885[d]	0.03	60.27	29.06
0.7177	0.07	12.43	52.92

TABLE II (Continued)

Mean Relative Retention Time	% Average Deviation	Mean Peak Height (cm)	% Average Deviation
0.7343[d]	0.24	3.08	67.48
0.7433[d]	0.02	1.28	29.41
0.7500[d,a]	0.41	48.53	44.28
0.7659[d]	----	23.40	-----
0.7866[d,a]	0.08	20.25	39.26
0.7946[d]	----	1.20	-----
0.8127[d]	0.10	4.55	7.69
0.8364	0.27	13.35	37.83
0.8522 (S)	0.15	3.85	55.84
0.8678[d,a] (S)	----	1.30	-----
0.8780[d] (S)	0.18	9.58	6.53
0.9170[d]	0.17	6.13	15.10
0.9249[d]	0.01	4.68	79.68
0.9346[d,a]	0.14	11.27	41.22
0.9553[d,a] (S)	----	2.80	-----
0.9657[c]	0.21	1.00	10.00
0.9779[c] (S)	0.36	2.30	32.61
0.9905	0.14	32.03	56.54
1.000 (S)	----	3.03	4.13
1.023 (S)	0.15	79.00	131.14
1.038	0.11	1.57	48.23
1.049	0.11	14.23	29.66
1.075 (S)	0.17	4.48	34.70
1.079[a]	----	4.02	-----
1.100	0.16	593.00	26.59
1.109	0.18	37.28	37.28
1.116[a]	----	16.65	-----
1.124	0.14	19.68	15.98
1.138	0.18	6.48	29.01
1.148	0.25	48.73	32.74
1.175[c] (S)	0.30	7.23	19.03
1.184[a]	----	10.90	-----
1.189	0.19	23.27	65.14
1.204[c] (S)	0.17	7.40	9.46
1.211[c]	0.21	6.90	77.54
1.221	0.16	3.07	18.84
1.237	0.09	2.43	42.92
1.248[c]	0.28	1.43	19.30
1.259	0.12	3.40	25.49
1.273[c]	0.20	3.95	29.11
1.289	0.18	1.93	71.26
1.299	0.22	518.9	18.57
1.324[a]	----	1.60	-----
1.340[c]	0.07	8.70	11.49
1.349	0.16	11.47	31.01

TABLE II (Continued)

Mean Relative Retention Time	% Average Deviation	Mean Peak Height (cm)	% Average Deviation
1.368[c]	0.07	1.43	40.35
1.384	0.10	56.87	8.34
1.413	0.33	14.47	59.14
1.422	0.23	3.00	34.44
1.432	0.21	5.77	59.35
1.462	0.21	6.87	59.55
1.473	0.15	14.47	125.5
1.487	0.21	6.33	30.18
1.509	0.10	5.28	32.39
1.527	0.20	9.08	69.85
1.543	0.20	5.32	29.68
1.564[a]	----	1.50	-----
1.567	0.21	13.60	97.06
1.583[c]	0.13	4.10	12.20
1.597[c]	0.06	2.33	37.63
1.609[c]	0.25	14.90	94.63
1.615[c]	0.06	5.55	27.03
1.633	0.19	12.07	55.99
1.647	0.22	6.47	41.41
1.664[c]	0.21	11.40	64.91
1.691[b]	0.21	21.75	11.72
1.708	0.21	6.70	67.66
1.732	0.19	12.68	80.51
1.752	0.18	9.53	33.07
1.769	0.21	15.30	67.54
1.792	0.21	22.23	62.87
1.841	0.21	12.53	25.09
1.868	0.24	7.28	40.58
1.891[c]	0.13	1.30	30.77
1.909[c]	0.24	1.00	0.00
1.925	0.25	9.03	100.1
1.949[a]	----	2.10	-----
1.982[a]	----	1.85	-----
1.976[a]	----	32.4	-----
2.076	0.31	4.08	20.68
2.086	0.00	5.15	55.34
2.198[c]	0.27	4.60	69.57
2.322[a]	----	2.60	-----
2.784[a]	----	3.60	-----

(B) denotes a component occurring in the blank also.
(S) denotes a sulfur component.

[a]Peak appeared in only one chromatogram.

[b]Peak appeared in all three chromatograms, however, one was not attenuated in time to catch the top of the peak.

[c]Peak appeared in only two chromatograms.

[d]Peak appeared in only two chromatograms due to a wrong attenuator setting.

three chromatograms (not including those due to attenuator error). The differences were mainly quantitative in nature. Half of the peaks not common to all three chromatograms were small (3 cm or less). Slight differences in sample size could easily account for these peaks not appearing consistently. There were, however, some drastic differences observed for some peaks. Diet variations may have caused these differences.

The reliability of the GC system is reflected in the excellent reproducibility of the relative retention times; most average deviations were below 1%. This indicates the reproducibility of the oven temperature as well as the ability of the valve injection system to inject all of the components as a slug. The average deviation of the peak heights indicate a wide variation in the relative quantities of substituents from day to day. The effect of concentration variations whether due to the sampling system or the individual was examined. When each peak was compared from one day to the next, a pattern did not emerge which would have indicated that one sample was consistently larger than another. Thus it was deduced that the variations were due primarily to the individual's metabolism.

A typical human breath chromatogram is shown in Figure 10. The figure is an artist's representation of the actual profile. The baseline was rezeroed between pages 64-65. Approximately 45 measurable peaks were obtained in 2.5 hr.

A comparison of breath samples taken on two consecutive days from Subject #3 is shown in Table III. Only 15% of the peaks failed to appear in both chromatograms. The data in Table III as well as the data from the urine experiments in Table II was used for a correlation study

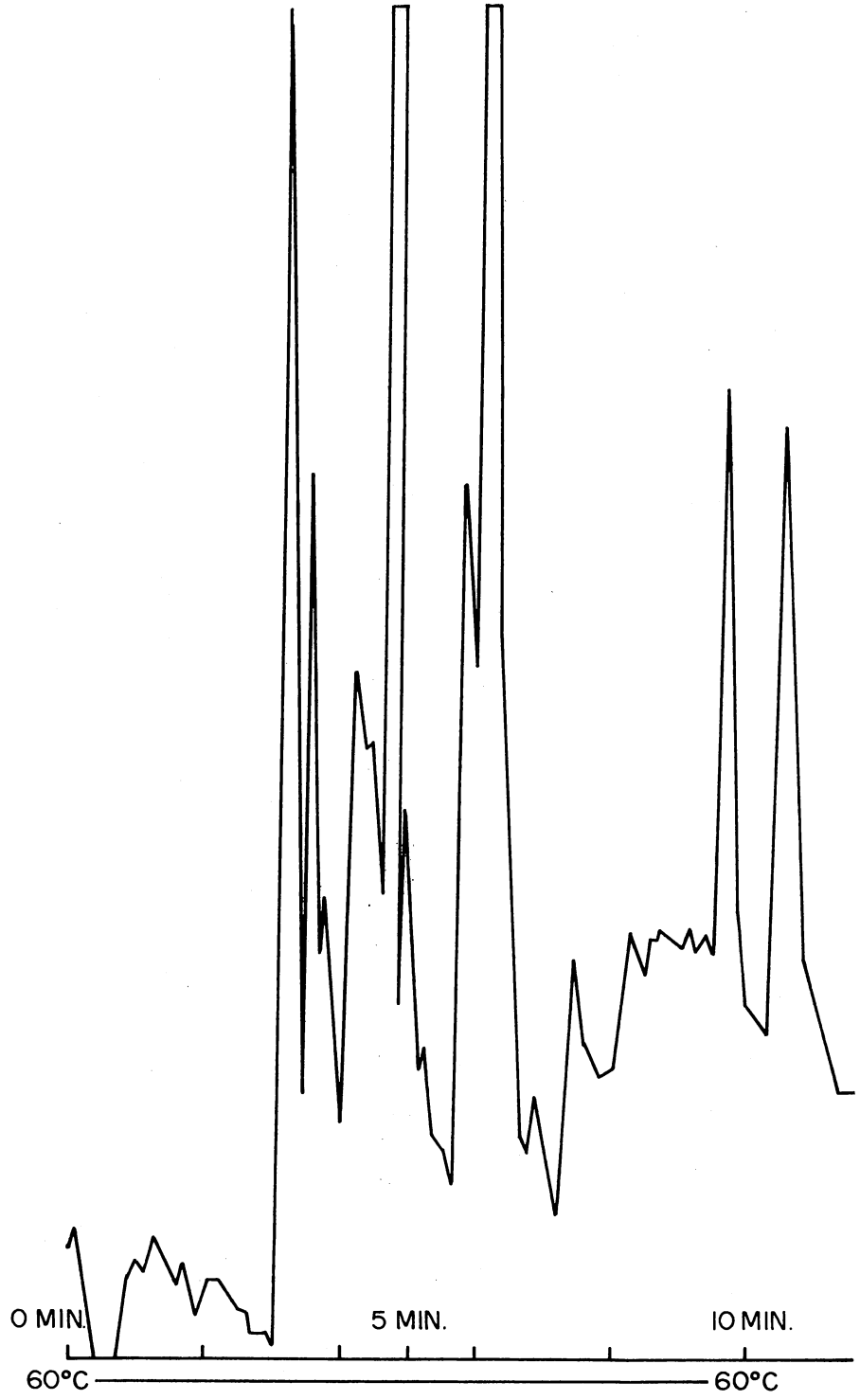

Figure 10. Artist's Representation of a Typical Breath Chromatogram

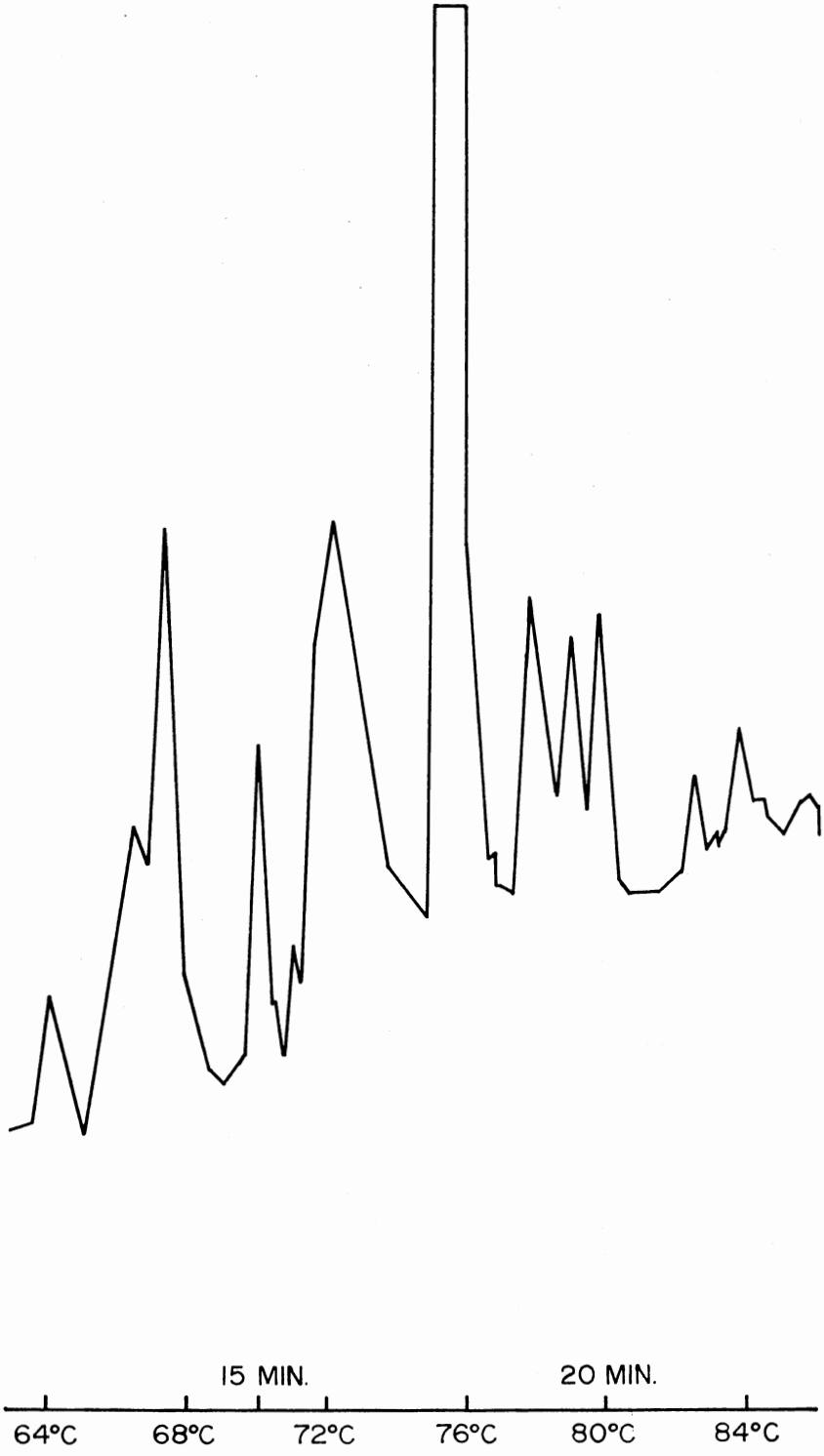

Figure 10. (Continued)

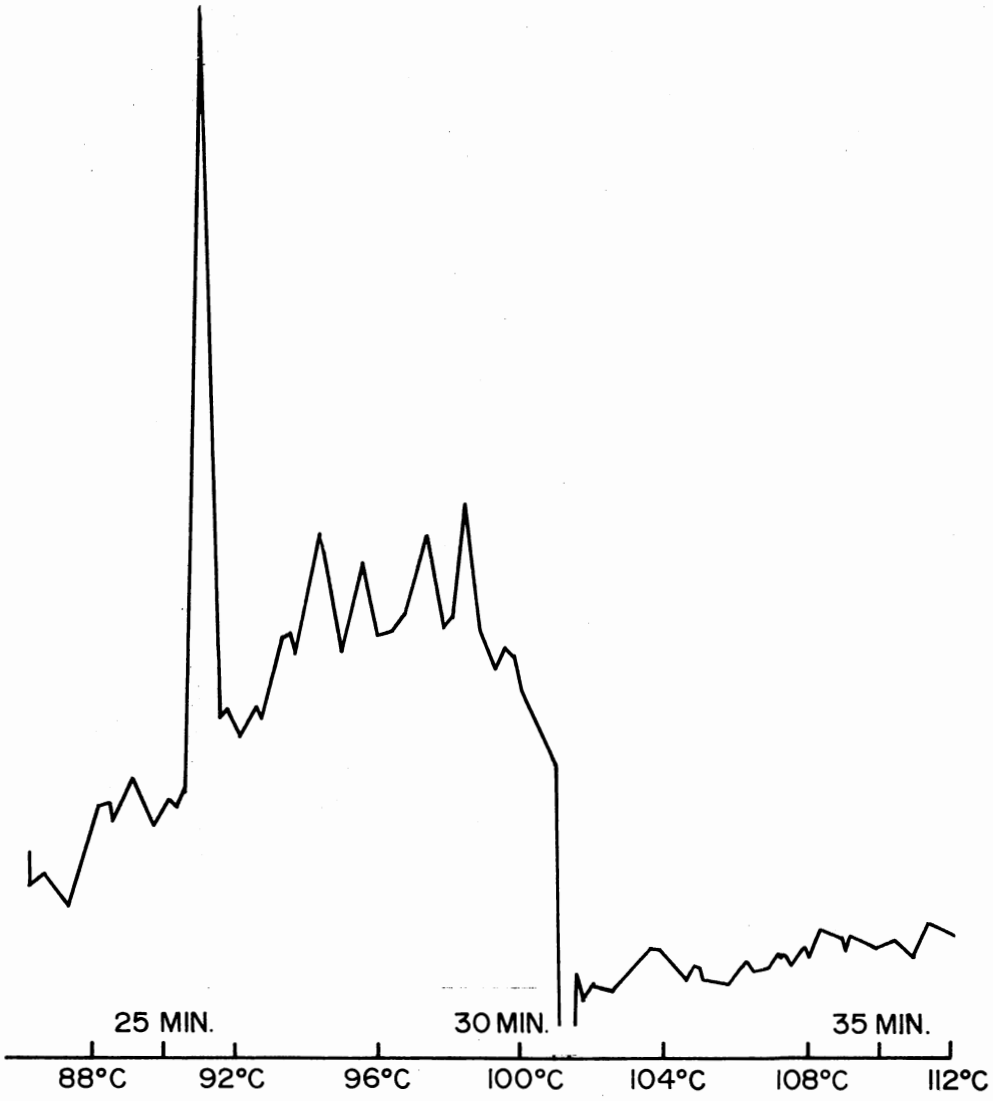

Figure 10. (Continued)

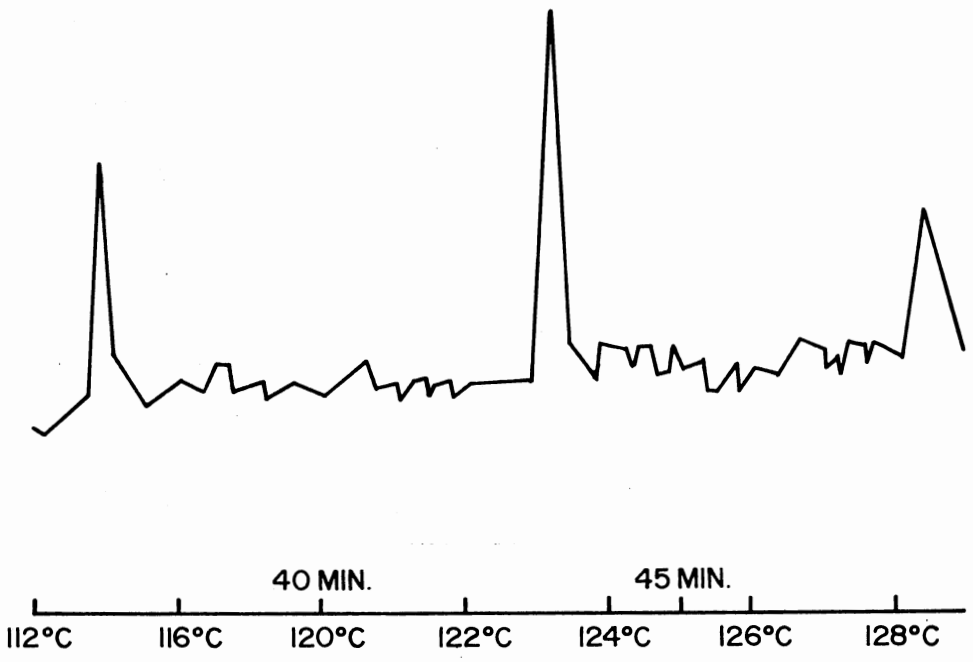

Figure 10. (Continued)

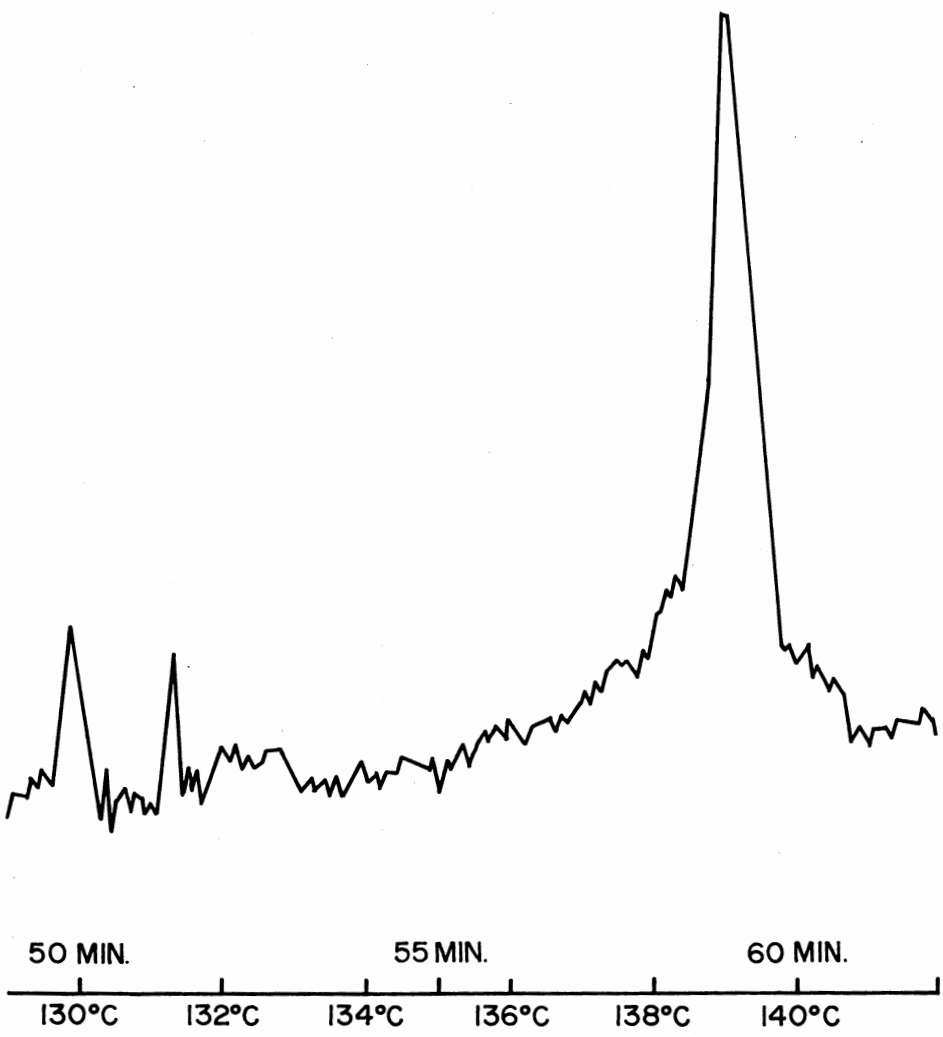

Figure 10. (Continued)

TABLE III

REPRODUCIBILITY OF BREATH SUBSTITUENTS IN ONE SUBJECT
FOR TWO CONSECUTIVE DAYS

Mean Relative Retention Time	% Average Deviation	Mean Peak Height (cm)	% Average Deviation
0.079	----*	13.85	-----
0.080	----	15.40	-----
0.086 (S) (B) (A)	4.65	23.70	5.91
0.087 (R) (B)	----	11.60	-----
0.093 (R) (B) (A)	3.23	16.10	.62
0.098 (R) (B) (A)	4.08	7.48	7.75
0.103	.97	7.65	-----
0.113 (R)	1.77	18.00	33.33
0.121	.83	0.55	-----
0.127 (S) (R) (B)	1.57	27.10	15.13
0.139 (R) (A)	1.44	1.03	2.91
0.159 (R) (B)	----	14.50	-----
0.163 (B)	3.68	74.6	.54
0.181	3.31	1.00	40.00
0.197 (B) (A)	3.05	3.03	51.82
0.220 (A)	2.73	3.40	35.29
0.230	1.74	3.80	18.42
0.233	----	2.80	-----
0.256 (B) (A)	2.73	8.28	32.97
0.278	3.60	9.35	38.50
0.318	2.83	1.60	56.25
0.348	3.16	3.35	52.24
0.358	3.07	7.40	4.73
0.396 (R) (B) (A)	2.53	7.68	23.18
0.410	2.44	2.10	11.90
0.405 (R)	----	2.55	-----
0.429 (A)	2.56	5.73	53.75
0.468 (S) (R) (B)	1.92	138.6	74.31
0.502 (B)	1.59	4.73	13.11
0.521 (R) (B)	2.30	3.50	34.29
0.530 (R) (B)	1.51	3.60	41.67
0.566	1.41	1.70	5.88
0.585	1.37	1.60	25.00
0.679	1.18	13.90	-----
0.726 (A)	.96	1.98	11.62
0.744	.81	1.20	16.67
0.768	.78	1.30	26.92
0.783	.64	1.80	30.56
1.000 (S)(R) (B)	----	7.18	40.11
1.169	.09	8.75	26.29
1.306	.46	3.23	2.48

TABLE III (Continued)

Mean Relative Retention Time	% Average Deviation	Mean Peak Height (cm)	% Average Deviation
1.346	.37	3.25	4.62
1.385	.36	2.65	1.89
1.595	.19	11.80	10.17

(S) denotes a sulfur component.

(R) denotes a room air component.

(B) denotes a blank component.

(A) denotes a supply air component.

of the organic volatiles and will be discussed again in connection with Table VII.

The reproducibility of the retention times for the breath chromatograms is not as good as for the urine chromatograms. For the breath profiles the splitter was removed to maximize the sample size. This required setting the flow controller at 1.73 ml/min which is below its optimum operating range. Thus small differences in flow rate could cause the increased average deviations.

The breath sampling system was designed to keep contamination at a minimum. The occurrence of impurities in a blank run is designated in Table III by a "B". These blank components comprised about 33% of the observed breath components and in most cases they were in much smaller amounts. These impurities reflect contamination from the supply air as well as inherent contamination. Those components in Table III which also occurred in a sample of the supply air are marked with an "A". There were some inconsistencies in the minor peaks between those components occurring in the blank and supply air samples. Those breath sample peaks in Table III which appeared also in a room air sample are designated with an "R". Table IV is a tabulation of the relative retention times of all the room air components. Since only 33% of the room air components appeared in the breath chromatogram, it was deduced that room air contamination was not significant.

The breath sampling system represented an improvement over previous systems (Conkle, 1973). The apparatus was reliable and relatively simple. The system of Conkle involved a complicated pattern of bellows, etc., and had a significant contribution from impurities in his system.

Table V shows a comparison of urine chromatograms from three sub-

TABLE IV

ROOM AIR COMPONENTS

Relative Retention Time			
0.083	0.239	0.507	0.913
0.091	0.247	0.520	0.925
0.097	0.262	0.531	0.953
0.109	0.312	0.599	1.000 (S)
0.114	0.323	0.622	1.121
0.122 (S)	0.341	0.640	1.146 (S)
0.128	0.394	0.756	1.332
0.136	0.404	0.778	1.361
0.156	0.435	0.821	1.515
0.192	0.466 (S)	0.867	

TABLE V

COMPARISON OF THE OCCURRENCE OF URINE SUBSTITUENTS IN THREE SUBJECTS

Relative Retention Time		
Subject #1	Subject #2	Subject #3
0.311	0.316	0.302
0.319	---	0.316
0.424	0.421	0.422
0.433	---	---
0.439	0.438	0.438
0.448	0.447	0.448
0.472	0.468	0.470
0.519	0.516	0.520
---	0.522	---
0.536	---	0.533
0.583	0.581	0.583
0.613	0.608	0.611
---	---	0.648
0.663	0.660	0.662
0.695	0.697	0.692
---	---	0.707
---	---	0.714
0.735	0.730	---
---	---	0.753
---	---	0.759
---	---	0.769
0.789	---	---
---	---	0.799
0.802	0.798	0.799
---	---	0.834
0.844	---	---
0.853	---	0.853
0.856	0.855	---
0.881	---	---
---	0.876	0.877
0.889	0.884	0.887
0.897	0.895	0.893
0.904	---	---
0.926	0.923	0.924
---	---	0.936
0.973	0.972	---
1.000	1.000	1.000
1.025	---	---
1.032	1.032	1.033
---	1.035	---
1.049	1.046	---
---	---	1.057

TABLE V (Continued)

Relative Retention Time		
Subject #1	Subject #2	Subject #3
1.068	1.067	1.069
1.078	1.077	1.078
1.095	1.093	1.094
---	---	1.101
1.131	1.133	1.135
1.168	1.165	1.166
1.186	1.183	1.182
1.190	1.187	1.194
1.237	1.232	1.240
1.255	1.251	1.255
---	1.257	---
1.265	1.263	---
1.326	1.326	1.328
---	---	1.344
1.363	1.361	1.363
1.391	1.393	1.393
---	---	1.400
1.409	1.412	1.408
1.432	1.430	1.431
1.457	1.453	1.455
1.506	1.506	1.508
---	1.526	1.527
1.542	1.547	1.539
1.563	1.563	1.561
1.585	1.585	1.582
1.607	1.605	1.603
1.609	1.611	1.614
1.631	---	1.628
---	---	1.652
---	---	1.665
---	---	1.674
1.754	1.758	1.752
1.792	---	1.791
---	1.799	1.804
1.813	1.819	1.811
1.821	1.828	1.819
1.833	1.839	1.831
1.844	1.849	1.843
---	---	1.846
1.858	1.865	1.856
1.862	1.869	1.859
---	---	1.873
---	---	1.903
1.913	---	1.911
1.932	---	1.929

TABLE V (Continued)

Relative Retention Time		
Subject #1	Subject #2	Subject #3
1.956	1.972	1.955
---	---	1.983
---	---	1.999
---	---	2.030
2.027	2.038	---
2.076	2.082	
2.085	2.097	
2.126	2.139	

jects, one of which was diabetic. The WCOT column was used for this experiment only. About half of the components occurred in all three chromatograms. On comparing Table II and V it appears that variations from person-to-person are greater than those of an individual from day-to-day. The interesting part of this experiment was the fact that the diabetic subject was not the one who accounted for most of the differences. Subject #3 accounted for about half of the differences.

Table VI shows an example of diet effects, in particular, the effect of ingestion of asparagus on Subject #3. The experiment was duplicated, however the time of the ingestion of asparagus was different. In Experiment #1 the asparagus was eaten directly before retiring and in Experiment #2 the asparagus was eaten for the evening meal. Only those peaks which were significantly different between the control and asparagus runs are shown. The control is data from Table II. From this experiment it is apparent that diet can affect urine constituents rather drastically. This result is in contrast to that of Zlatkis and Liebich (1971) who concluded that diet effects were minimal. The degree to which diet effects account for the differences seen in day-to-day analyses and in analyses of different individuals is an open question and one which needs more study.

The promise of breath analysis is the possibility that breath analysis can give information about some bodily processes which urine cannot. The initial step in studying this hypothesis is to see how well urine and breath components correlate. The data shown in Tables II and III has been retabulated in Table VII to show the correlation. The peaks were considered to have the same retention time if the range of the retention times, as determined by the mean plus and minus the aver-

TABLE VI

COMPARISON OF URINE SUBSTITUENTS BEFORE AND AFTER INGESTION OF ASPARAGUS

Mean Relative Retention Time for Control ± Average Deviation	Mean Peak Height for Control (cm) ± Average Deviation	Relative Retention Time for Asparagus	Peak Height for Asparagus (cm) Experiment #1	Peak Height for Asparagus (cm) Experiment #2
---	---	0.4546 ± 0.022	2.05	4.05
1.075 ± 0.001	4.48 ± 1.56	1.075 ± 0.000(S)	35.90	12.40
---	---	1.317 ± 0.005(S)	61.60	16.80
1.909 ± 0.005	1.00 ± 0.00	1.914 ± 0.015(S)	135.2	28.00

TABLE VII

CORRELATION OF BREATH AND URINE COMPONENTS

Mean Relative Retention Time for Urine ± Average Deviation	Mean Relative Retention Time for Breath ± Average Deviation
0.0777 ± 0.0016, 0.0788	0.079
0.0813 ± 0.0015	0.080
----	0.086 ± 0.004 (S)(B)(A)
----	0.087 (R)(B)
----	0.093 ± 0.003 (R)(B)(A)
----	0.098 ± 0.004 (R)(B)(A)
----	0.103 ± 0.001
----	0.113 ± 0.002 (R)
----	0.121 ± 0.001
----	0.127 ± 0.002 (S)(R)(B)
0.1365 ± 0.0006	0.139 ± 0.002 (R)(A)
----	0.159 (R)(B)
0.1629 ± 0.0005	0.163 ± 0.006 (B)
0.1808 ± 0.0010	0.181 ± 0.006
0.1917 ± 0.0017	0.197 ± 0.006 (B)(A)
0.2194	0.220 ± 0.006 (A)
0.2303	0.230 ± 0.004
----	0.233
0.2571 ± 0.0017	0.256 ± 0.007 (B)(A)
----	0.278 ± 0.010
0.3142 ± 0.0040, 0.3250 ± 0.0044	0.318 ± 0.009
0.3465 ± 0.0052	0.348 ± 0.011
0.3590 ± 0.0045	0.358 ± 0.011
0.4019 ± 0.0004	0.396 ± 0.010 (R)(B)(A)
0.4019 ± 0.0004	0.410 ± 0.010
0.4019 ± 0.0004	0.405 (R)
----	0.429 ± 0.011 (A)
----	0.468 ± 0.009 (S)(R)(B)
0.4958 ± 0.0029	0.502 ± 0.008 (B)
----	0.521 ± 0.012 (R)(B)
----	0.530 ± 0.008 (R)(B)
0.5627 ± 0.0010	0.566 ± 0.008
----	0.585 ± 0.008
0.6782 ± 0.002	0.679 ± 0.008
0.7177 ± 0.0005, 0.7343 ± 0.0018	0.726 ± 0.007 (A)
0.7433 ± 0.0002, 0.7500 ± 0.0030	0.744 ± 0.006
0.7659	0.768 ± 0.006
0.7866 ± 0.0007	0.783 ± 0.006
1.000 (S)	1.000 (S)(R)(B)
----	1.169 ± 0.001

TABLE VII (Continued)

Mean Relative Retention Time for Urine ± Average Deviation	Mean Relative Retention Time for Breath ± Average Deviation
1.299 ± 0.0029	1.306 ± 0.006
1.340 ± 0.0010, 1.349 ± 0.0022	1.346 ± 0.005
1.384 ± 0.0013	1.385 ± 0.005
1.597 ± 0.0001	1.595 ± 0.003

(S) denotes a sulfur component.

(R) denotes a room air component.

(B) denotes a blank component.

(A) denotes a supply air component.

age deviation, overlapped. In some cases more than one possibility existed for overlap; these are indicated in the table. Around half of the breath substituents appeared to be in urine also. This result is significant enough to merit further research into the question of correlation.

The FPD was invaluable in attempting to correlate the breath and urine components. Choosing a reference peak with which to calculate the relative retention times was greatly simplified. The breath and urine chromatograms were sufficiently different to make difficult the initial determination of a peak common to both chromatograms. Without the FPD it would have been necessary to use an internal standard.

CHAPTER IV

SUMMARY AND FUTURE WORK

Urine and breath sampling systems were designed to collect the trace organic volatiles present in breath and urine. The breath sampling system was designed to minimize contamination from room air and from the system itself. A unique fluidics control circuit was used to control the air supply. A low dead volume injection system was designed which was compatible with the concentration technique and type of samples used. A HP Model 7620A GC was modified so that it accommodated open tubular columns.

The analysis system as a whole provided for efficient collection, maximum concentration, and excellent resolution of the volatiles. The low average deviations of the retention times demonstrated the reproducibility of the GC conditions.

Variations from an individual on a day-to-day basis, variations between three individuals, and asparagus induced variations were studied. Differences between the three individuals were greater than the differences in an individual from day-to-day. The ingestion of asparagus had a definite effect on the urine volatiles.

The relative retention times for breath and urine components were correlated. Approximately half of the breath components occurred in urine also. This result is significant enough to merit further research.

There are several areas in which modifications to the existing apparatus would give improved analyses. The supply air contained several impurities which did appear in the breath chromatograms. A cleaning procedure would be desirable to eliminate this contamination.

The fluidics control circuit could be converted to a true demand regulator with the use of a Schmidt trigger. There was a wide range of concentrations of volatiles which were recorded. In order to prevent the GC operator from having to attenuate these peaks, a logarithmic recorder could be used.

The interface of the GC system with a MS would produce the identities of the components in a profile. A physiologist then would be able to study the history of the substituents.

The factors affecting profiles are undoubtedly numerous and complex; they may be due to metabolic processes or to variables such as diet, etc. Long range future work will involve studying the variations in profiles and their causes. Diet effects are probably a major factor. Studies of the composition of foods before ingestion and the resulting profiles after ingestion could provide a great deal of information on nutrition.

In conclusion, the metabolic profile type of analysis for breath and urine shows great promise in the study of human metabolism. The complex processes within the body require analyses which will follow the changes in several components simultaneously.

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