ANALYSIS OF VOLATILE METABOLITES IN BIOLOGICAL FLUIDS BY HIGH RESOLUTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY

A Dissertation

Presented to

the Faculty of the Department of Chemistry

College of Arts and Sciences

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Farid Shunbo

May 1974

To Fadia and Sherine

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ABSTRACT

The work described here includes the development of a new method for concentrating and analysing organic volatiles in body fluids (urine and blood). The concentrating was accomplished by using a suitable solid adsorbent, Tenax GC, to adsorb the volatiles which are present in very small amounts. The analysis was done using high resolution gas-chromatography.

The purpose behind the development of such a method is to provide early indications of trace metabolic disorders resulting from pathological conditions. However, certain guidelines had to be established before the analysis and interpretation of profiles from pathological cases could be made. Such guidelines were established by analysing body fluids, urine in particular, of three healthy individuals before, during and after an 80 day period of controlled diet, during which, the different physiological activities of the three individuals were carefully monitored.. Results indicated that there were no changes in the profiles of each individual for the period in question. Such"normal" profiles were used for comparison with profiles obtained from patholo-gical samples.

Comparison of the "normal" profiles with profiles obtained from the analysis of over fifty samples from diabetic patients gave a clear indication that differences do exist. The complexity of the profiles obtained from analysing urine prompted the analysis of

organic volatiles in blood (serum and plasma) which, as was expected had less volatlies and presented a more simplified picture. Simple profiles were also obtained from urine samples by using specific detectors.

TABLE OF CONTENTS

Chapter		Page
	Dedication	i
	Acknowledgement	ii
	Abstract	iii
I.	INTRODUCTION	1
II.	EXPERIMENTAL	7
	A. Introduction	7
	B. Adsorbents for Sampling	9
	1. Porapak P	10
	2. Carbon Molecular Sieve (Carbosieve)	10
	3. Tenax GC	12
	C. Sampling System	13
	1. Trap-Insert	13
	2. Modified Injector Port	15
	3. Sample Preparation	15
	4. Storage of Samples	19
	D. Sample Transfer and Analysis	20
	1. Precolumn-Trap	20
	2. Four-Port Valve	22
	3. Analytical Columns.	23
	a. Preparation	25
	b. Cleaning and Etching	25

TABLE OF CONTENTS (CONTINUED)

Chapter	Page
c. Coating	27
4. Chromatographic Conditions	29
5. GC-MS Analysis	30
III. RESULTS AND DISCUSSION	32
A. Sampling System	32
B. Organic Volatiles in Urine	36
C. Organic Volatiles in Blood	64
IV. CONCLUSION	72
BIBLIOGRAPHY	73

LIST OF TABLES

Table		Page
I.	Volatile organic compounds in normal human urine . . .	38
II.	Volatile organic compounds in urine of subjects with Diabetes Mellitus	48
III.	Volatile organic compounds in normal human serum . . .	71

LIST OF FIGURES

Figure		Page
1.	Poly-p-2,6-diphenylene oxide Tenax GC	11
2.	TENAX modified injector port	14
3.	Sampling system	16
4.	Manifolds for simultaneous preparation of more than one Trap-Insert	18
5.	Sample transfer system	21
6.	Four -port valve	24
7.	Modification of ends of needle stock column	26
8a,b.	Component distribution and capacity of Tenax GC for a gas sample	33
9.	Reproducibility of FIDprofiles from same urine sample	34
10.	Effect of different storage conditions on compounds trapped on Tenax GC	35
11.	Comparison of volatiles in urine of three healthy individuals	42
12.	Effect of diet on volatiles in urine of healthy individual	43
13.	Comparison of sulfur profiles of three healthy individuals	44
14.	Comparison of volatiles in urine of diabetic and normal individuals	46

LIST OF FIGURES (CONTINUED)

Figure		Page
15.	Comparison of urinary volatile organic sulfur compounds from normal and diabetic individuals	47
16.	Profile of a diabetic with high blood sugar and on no insuline	53
17.	Profile of an adult diabetic on insuline	54
18.	Profile of a juvenile diabetic on insuline	55
19.	Components in diabetic urine	56
20.	Profile of diabetics on therapy different than insuline	57
21.	Comparison of profiles of four different diabetics.	58
22.	Comparison of profiles of two diabetics on insuline	59
23.	Comparison of profiles of two diabetics, one treated and the other not	60
24.	Comparison of profiles of two diabetics of the same family	61
25.	Comparison of profiles of two diabetics of the same family	62
26.	Sulfur profiles in diabetics	63
27.	Volatiles in serum of healthy individuals	66
28.	Volatiles in serum of three healthy individuals	67
29.	Comparison of plasma and serum volatiles	68
30.	Volatiles in serum of two diabetics.	69
31.	Comparison of volatiles in serum of diabetic and healthy	70

CHAPTER I

INTRODUCTION

INTRODUCTION

In the past few years, there has been a substantial growth of interest with respect to the role of biochemical screening and profile techniques in the diagnosis of disease states and the problems of identifying the cause of abnormal clinical chemistry findings. The interest in this facet of clinical chemistry was greatly enhanced by the advancement of analytical technology.

A precedent to screening and profile techniques is expansion of knowledge about the nature and control of metabolic processes which has progressed to such a degree that, it is possible to consider all diseases to be either a result of a primary metabolic abnormality or a metabolic derangement resulting from some extrinsic cause. For example, the inborn errors of metabolism would fall in the first category and the catabolic response to a broken leg into the second.

The concept of "inborn errors of metabolism" was first postulated in 1908 by Sir Archibald Garrod,[1] who commented about the pathogenesis of the metabolic disorders and predicted their biochemical mechanism. He believed that each successive step in the metabolism of sugars, proteins, and fats was governed by a particular enzyme. The intermediate products that are formed have only momentary existence and are subject to further changes as soon as they are formed. Therefore, metabolism should be pictured as a

series of successive, discrete steps. According to this view, when any one step fails, the intermediate product will not be converted to the succeeding metabolite, and will be excreted rather than being allowed to accumulate or to be metabolized in some abnormal manner.

A more updated version of Sir Garrods' concept is one which takes into account not only the multitude of biochemical reactions taking place in the body but also the concentration of different substances present in it or produced by it. The biochemical reactions taking place in the body do so with a high degree of precision and speed, almost all of them are catalyzed by enzymes and, are subjected to carefully controlled regulatory mechanisms inside the cells. Accordingly, the different metabolites produced under normal and healthy conditions do so in a fairly constant concentration. In the case of "inborn errors", one or more of the biochemical processes may have been defective from birth, on the other hand, the onset of a certain disease at a later stage in life may cause one or more of the biochemical processes to become defective either temporarily or permanently. In either case, the concentration of substances normally present in the body and required for maintaining a normal healthy state is changed. This in turn manifests itself in a change of concentration of normal metabolites, the change occuring in either direction and, in many cases new abnormal metabolites may also occur.

It therefore seems reasonable to make the assumption that

if one were able to identify and determine the concentration of all compounds present in the human body, whether they be high molecular weight or low molecular weight in nature, one would probably find that almost every known disease would result in characteristic changes in the biochemical composition of the cell and body fluids. It is of interest to mention at this point that the preceding assumption is the basis of the hypothesis put forth by Pauling, which he calls "Orthomolecular Medicine" and is defined as "the preservation of good health and the treatment of disease by variation of the concentrations in the human body of substances normally present in the body and are required for health." [2,3]

An important problem, therefore, concerns the detection and analysis of the various compounds present in body fluids, such as, blood, urine, saliva, sweat, and breath. A problem which has proven to be rather challenging, especially, if one takes into account the fact that the number of metabolites present in such body fluids could exceed several hundred or even several thousand.

The interest in this facet of clinical chemistry coupled with, the advent of high resolution analytical methods have offered a promising start to this problem of multicomponent analysis. High speed liquid chromatography and, the synergism of gas liquid chromatography, mass spectrometry, and computer methods are two types of high resolution analytical techniques that have been invaluable in making headway in the analysis of body fluids.

Three areas in which plausible developments have taken place are:

1. The determination of specific enzyme defects or metabolites associated with known detectable disorders.[4-8]

2. The testing of selected members of the general population with high resolution analytical techniques for physiologically related or unrelated compounds, in order to establish metabolic profiles in health and disease states.[9-15]

3. The determination of a limited group of body constituents, known to be indicators of disease states, in large numbers of the general population.[16-18]

Although, the work done in these areas and in this domain of biochemical research, in general,[19-31] has yielded very informative results concerning health and disease states and in some instances has led to the discovery of new inborn errors of metabolism,[32] it has until now been concerned mainly with nonvolatile constituents of biological fluids, that is, steroids, carbohydrates, amino acids, fatty acids, urinary acids, etc., and very little attention has been paid to the more volatile fraction in biological fluids. This lack of interest could in part be attributed to the fact that the analysis of nonvolatile constituents in body fluids requires sampling methods which are conventional and, employ wet chemistry procedures that are well known to the chemist.

It must be realized, however, that the analysis and identification of the volatile constituents in biological fluids could result in profiles furnishing a substantial amount of information

on health and disease states in humans, or any living organism for that matter. That the idea is far fetched could be ruled out if the attention is turned to the analysis of food; in general, food in itself is made up of many complex high molecular weight compounds, however, it is the volatiles in food which, sometimes are present in trace amounts that are responsible for the odor and flavor and, it is the analysis and identification of such volatiles that has paved the way for the so called "artificial flavor" industry today.[33] In an analogous manner, analysis and identification of the volatile constituents in body fluids could prove to be of enormous diagnostic value.

Contrary to the few attempts that have been made in analyzing volatiles in body fluids such as, saliva,[34] breath and urine,[35-38] the work described here has been devoted to the development of a novel, inexpensive, and rapid procedure for sampling and analyzing volatiles in urine and blood from healthy and pathological cases with particular emphasis on Diabetes Mellitus. The main purpose behind the developments of such a procedure is fourfold:

1. To establish profiles of normal individuals uder strict controlled dietary conditions and/or otherwise.

2. To compare these profiles with those from patients with Diabetes Mellitus in its different forms, and note any differences.

3. Based on the results from (1) & (2) to further develope and refine the procedure

so that it could be used as a diagnostic tool in clinical laboratories for the early detection of diabetes.

4. Assuming that differences are found, and certain compounds are found in patients with Diabetes Mellitus which are absent in healthy individuals, or vice versa, it would then be possible to investigate the biochemical processes taking place in the body that could be the reason behind the presence or absence of such compounds. If this is ever accomplished in the case of Diabetes Mellitus and extended to other serious diseases, it would result in a major breakthrough in the early diagnosis and treatment of fatal diseases.

CHAPTER II

EXPERIMENTAL

EXPERIMENTAL

A. INTRODUCTION:

Since, the work described here was primarily concerned with the development of a novel procedure for sampling, concentrating, and analyzing organic volatiles in body fluids, it is, therefore, only appropriate to define first the term:

> "The volatile fraction may be defined either in terms of the method of isolation (e.g., headspace analysis, liquid-liquid extraction, liquid-solid extraction, and vacuum distillation), or the fact that such molecules can be chromatographed in the gas phase without the pretreatment necessarily used for the nonvolatile organics."[39]

The idea of analyzing organic volatiles in itself is not new and, there are numerous reports in the literature during the past decade that relate to the analysis of volatiles such as those responsible for odor and flavor in fruits, dairy products, and foodstuff in general. The direct sampling of volatiles contained in such material, however, presented numerous problems. In almost all the sampling procedures reported, a prior scheme of isolation and/or collection of the volatiles is often used. Some examples of the sampling procedures used are solvent extraction, steam distillation, vacuum distillation, and sampling of headspace volatiles. Of all these methods the simplest and most practical, by far, is the headspace method. Although the other methods are also simple and

often quite useful, they do possess certain limitations. Solvent extractions, for example, not only are they time consuming, but they also introduce problems due to solvent impurities and solvent removal (or concentration of sample) prior to chromatographic analysis. Steam and vacuum distillations, on the other hand, involve additional manipulation of the collected volatiles and often subsequent solvent extraction.

It was the absence of such limitations in the headspace methods that has made it the one most preferred for sampling organic volatiles in air, and body fluids. While the high efficiency and exceptional resolving ability of high-resolution gas chromatography have made it a preferred method for analyzing complex mixtures of volatile material, the introduction of headspace samples directly onto a chromatographic column is seldom possible. An alternative would, therefore, have to take into consideration the concentration of organic volatiles or trace volatiles whether they be in body fluids or in air prior to gas-chromatographic separation. This prompted several workers to explore several methods for concentrating organic volatiles prior to high-resolution gas chromatography.[35, 40-50] For the analysis of the headspace organic volatiles in urine and blood as described in this work, the approach used for the concentration of volatiles is, in essence, one which utilizes the properties inherent in certain solid porous polymers to adsorb and concentrate the headspace organic volatiles with subsequent thermal desorption of the

trapped compounds onto a chromatographic column for analysis. The grounds on which the choice of adsorbent was made is discussed in the following section.

B. ADSORBENTS FOR SAMPLING:

In evaluating the various kinds of adsorptive materials that could be used for trapping and concentrating volatiles in biological fluids, careful consideration should be given to the nature of the compounds to be analyzed and particularly to the nature and properties of the sorptive material itself, which include selectivity, thermal stability, and extent of surface activity.

Studies on the selective retention of various solid adsorbents used as column packing material for gas chromatography could be easily traced back to the early years of gas chromatography. Such studies and more recent ones,[51,52] have provided a significant amount of information concerning the selective retention and surface activity of the various materials.

In order to make a proper choice of sorptive material for concentrating the volatiles in body fluids extreme care was excersized in avoiding some of the most common adsorbents, such as, silica gel, alumina, and charcoal which, exhibit excessive surface activity towards many classes of compounds and, makes their use fairly limited because of irreversible adsorption and decomposition

problems.

Of the three chromatographic packing materials studied in this work, only 2,6-diphenyl-p-phenylene oxide porous polymer, Figure 1, (known under the commercial name Tenax-GC, and distributed by Applied Science Laboratories, Inc., State College, Pa) has proven to be the most suitable adsorbent in terms of both relative inertness and sufficient thermal stability.

1. Porapak P

Porapak P,(Waters Associates, Framingham, Mass), a styrene-divinylbenzene porous polymer with a surface area 300 m^2/g and an upper temperature limit of 250°C was first used as an adsorbent for trapping and concentrating the headspace volatiles over urine. As an adsorbent, it first appeared to be very promising, however, due to its low thermal stability Porapak P is not sufficiently stable at temperatures above 200°C and, bleeding during thermal desorption and the production of artifacts during subsequent analysis made it less desirable for use as an adsorbent.

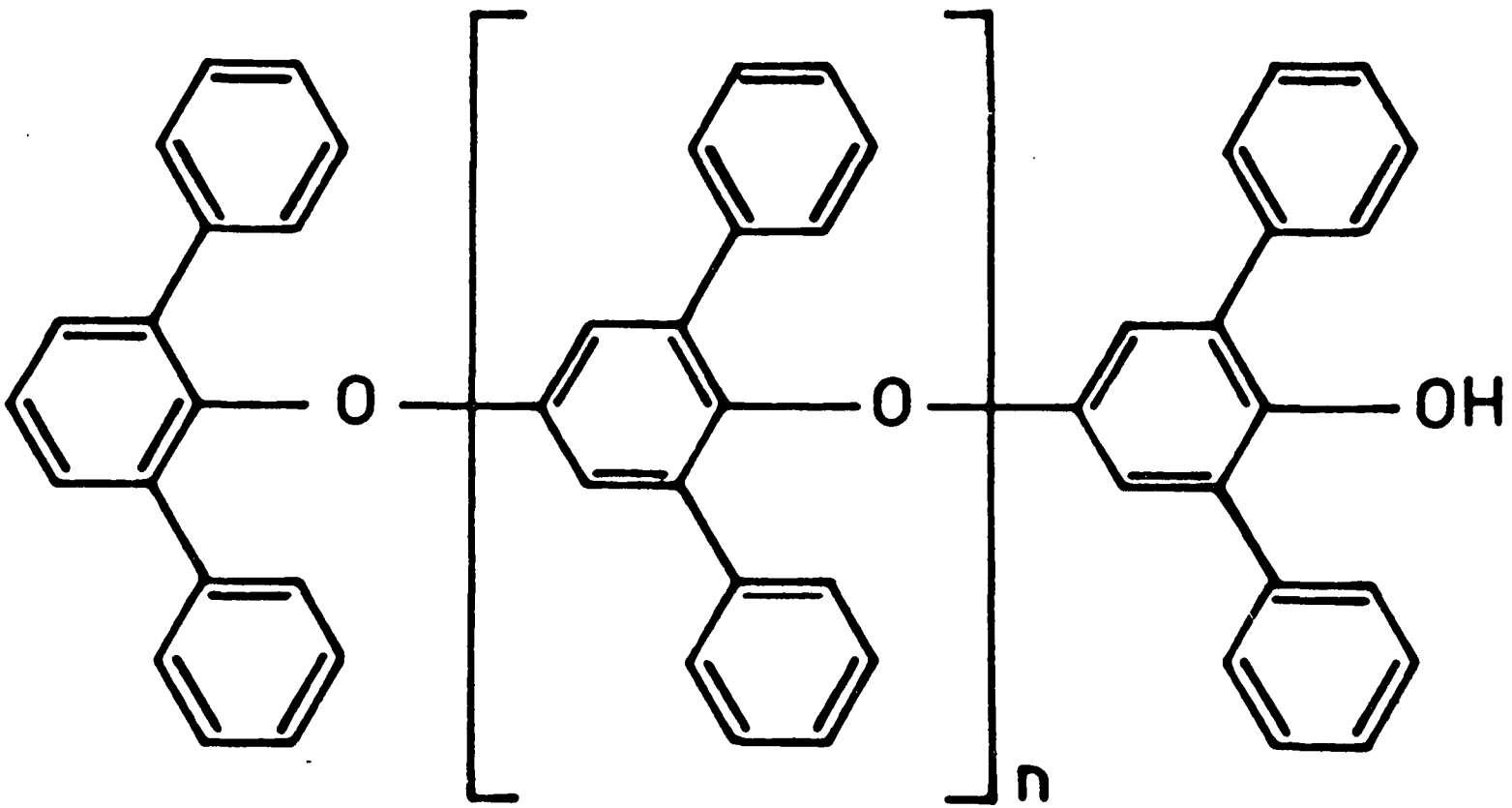
2. Carbon Molecular Sieve (Carbosieve).

Carbosieve, prepared by thermally cracking polyvinylidene chloride (Supelco, Inc, Bellefonte, Pa). The enormous surface area of this material (1000 m^2/g) and its high temperature stability made it almost impossible to ignore the possibility of using it as an adsorbent for trapping and concentrating volatiles in body fluids.

FIGURE 1

POLY-p-2,6-DIPHENYL-PHENYLENE OXIDE

TENAX GC



TENAX Poly-p-2.6 Diphenyl-Phenylene Oxide

As was expected, it showed great promise as an adsorbent, however, there were two limitations to the use of Carbosieve that had to be considered. First, its high reactivity with ambient volatiles demanded extreme care in handling, regeneration, and storage. The second, and maybe, the most important limitation is, the fact that temperatures well above 400°C are needed in order to desorb the organic volatiles which, would result in pyrolytic effects leading to erroneous results. Therefore,"for qualitative analysis of unchanged molecules, the convenience of desorption with heat cannot be employed using Carbosieve. On the other hand, Carbosieve showed great promise as a gas purifier and/or trapping system, if desorption by solvents is preferred or required."[39]

3. Tenax GC

From the investigation of the previous two adsorbents it was concluded that neither would be adequate. While the low temperature stability of Porapak P prevented efficient desorption of higher molecular weight compound, the high temperature stability of Carbosieve required desorptive temperatures which would ultimately destroy the sample.

The third packing material investigated was a porous polymer of 2,6-diphenyl-p-phenylene oxide, Figure 1, known commercially as Tenax GC (Applied Science Laboratories, Inc., State College, Pa). This adsorbent which has been characterized as having unusual and

desirable chromatographic properties,[53] appeared to fulfil both requirements of efficient adsorptivity and desorptivity. The temperature stability of Tenax GC is relatively quite high, which allows it to sustain temperatures as high as 400°C without its destruction which makes it very useful for a variety of sample types, ranging from the gas phase analysis of tobacco smoke[54] to aroma studies[55] and air pollution.[56] The properties of Tenax GC have recently been explored more fully.[57]

C. SAMPLING SYSTEM:

1. Trap-Insert

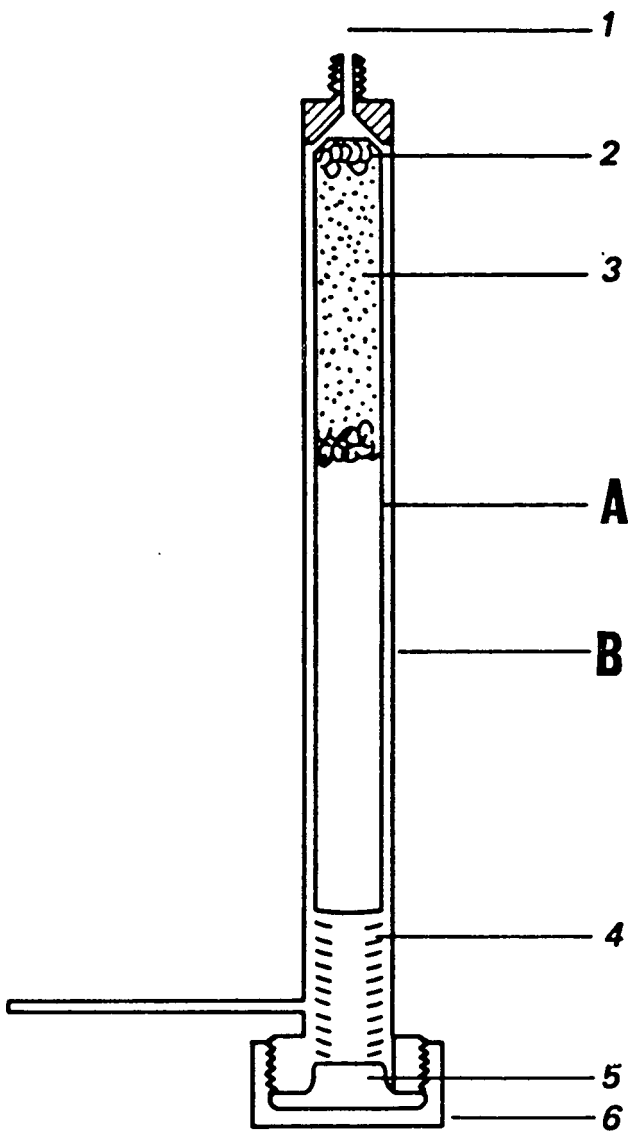
Once the choice of adsorbent had been made it was then necessary to develope the sampling system into one with which optimum reproducibility of results could be achieved, and also one that would provide a convenient method of sampling, storing, transfer, and analysis of sample or samples under investigation. In order for the system to meet all the previously mentioned requirements, a prototype glass trapping chamber was designed, which ultimately becomes an insert for a modified injector port (described in the next section).

The trap is a glass tube 11cm x 10mm OD, 8mm ID. The tube is filled with 2ml (0.35g) Tenax GC (35/60 or 60/80 mesh) with a plug of silanized glass wool at both ends to prevent spilling of the solid adsorbent , Figure 2. One end of the glass tube is tappered, so as

FIGURE 2

A. TRAP-INSERT

B. MODIFIED INJECTOR PORT



- **A** Tenax-Trap Insert, **B** Modified Injector Port

1 Pre-column inlet, **A** Trap-insert, **B** Modified injector port, 2 Silanized glass wool, 3 Tenax-GC, 4 Spring, 5 Teflon plug, 6 Steel cap

to provide an efficient seal inside the injector port.

2. Modified Injector Port

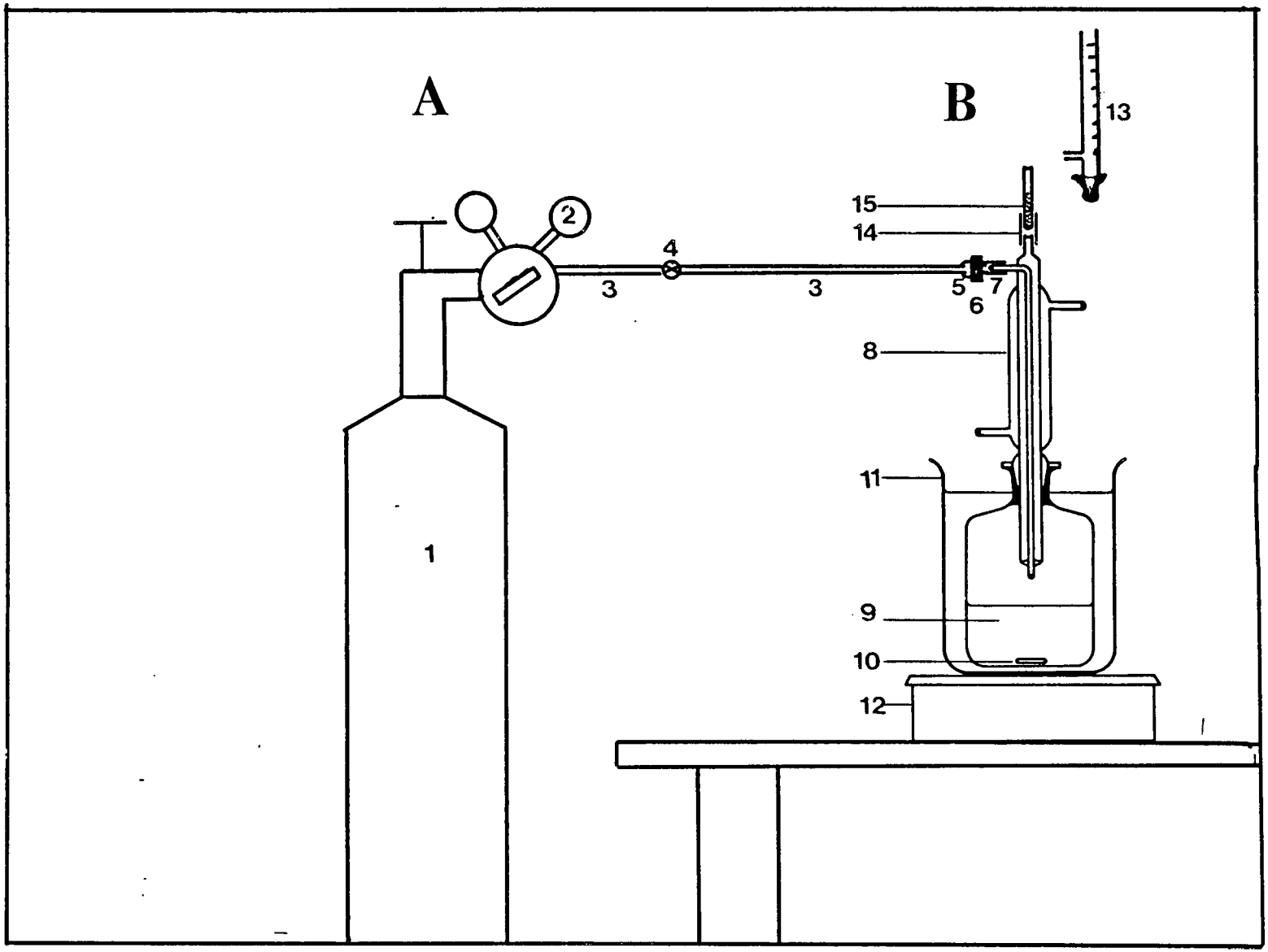
An injector port made from either stainless steel or brass was constructed in such a manner so as to replace one of the two existing injection ports of a Perkin-Elmer 900 gas chromatograph, Figure 2. The modified injector port was also constructed in a manner that would allow it to accomodate the Trap Insert mentioned in the previous section, after the Trap-Insert is placed inside the port for sample transfer, a spring is used to maintain a tight seal between the tapered end of the Trap-Insert and the outlet of the port.

3. Sample Preparation

Figure 3 illustrates the design of the apparatus used for the collection and concentration of urine or blood (serum or plasma) headspace volatiles. For reasons of clarity the apparatus is divided into two parts. Part A, the gas or carrier source and, part B the aqueous volatile sampler. In part A zero grade nitrogen (1) flows through a pressure regulator (2) which is usually adjusted within the range 20-30 psi in order to give the desired flow rates through the entire system. The nitrogen is carried through the system by means of a 1/8 in. Teflon line (3) and through a needle valve (4) which is used to further control the flow of nitrogen and restrict it to

FIGURE 3

SAMPLING SYSTEM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20 ml per min. Part B, the "aqueous volatile sampler" consists of a 500 ml glass bottle (9) in which is placed a 25 ml sample of urine, 7.5g of ammonium sulphate, and a stirring bar (10). In case of blood analysis, a 5 ml sample of serum or plasma is placed in the bottle, and no ammonium sulphate is added. In either case, the bottle containing the sample is in turn placed in a 2 liter pyrex beaker (11) containing water, this serves as a boiling water bath, which implies that during the entire sampling period the sample is at 100°C. A hot-stirrer-plate (12) not only keeps the water bath temperature at 100°C during the entire sampling period but, also allows the urine, serum, or plasma being sampled to be stirred.

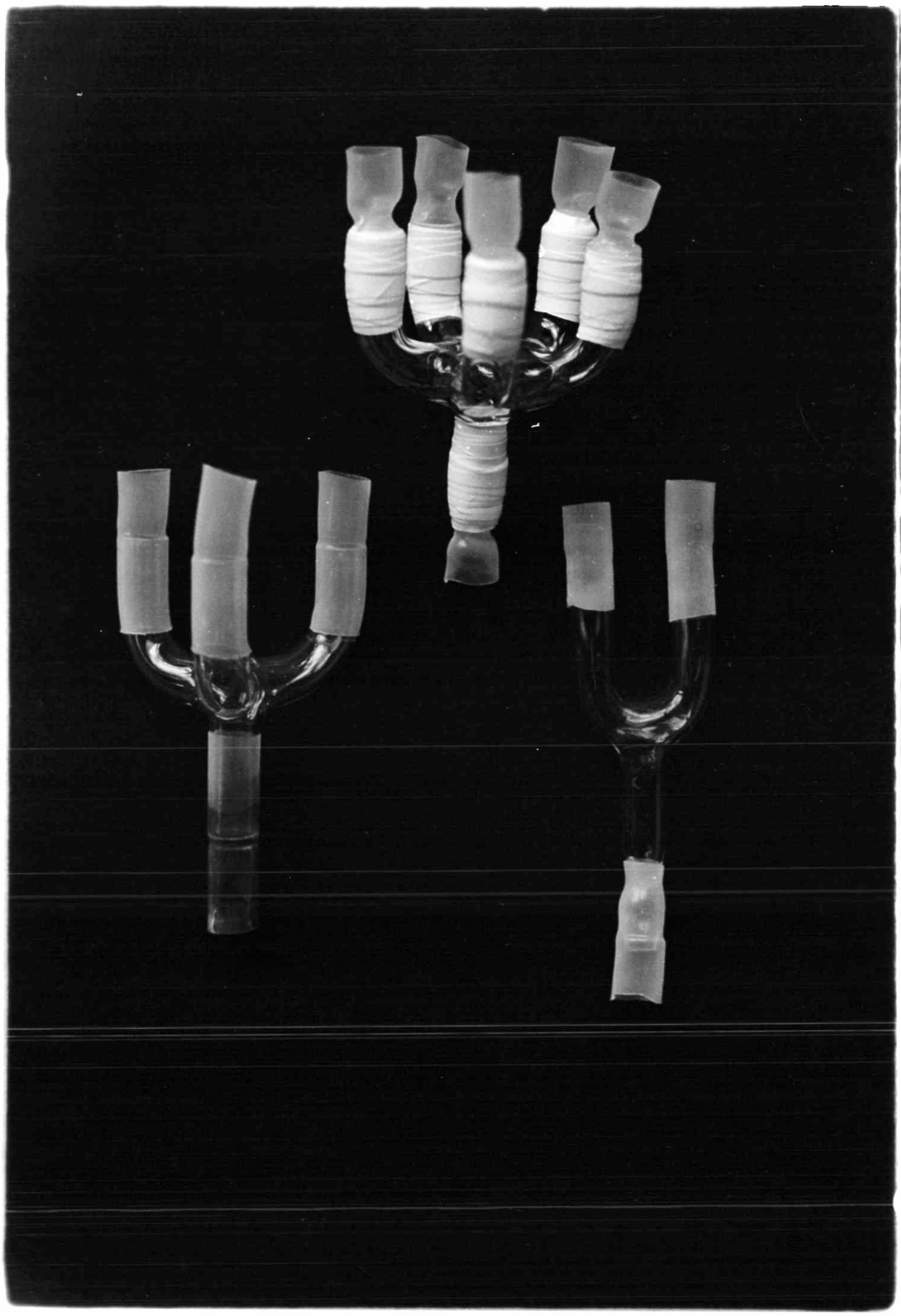
In order to remove the water from the urine vapors without removing the volatile organic compounds, the bottle containing the sample is fitted with a chilled water 4°C condenser (8). In such a case it is possible to reflux the water without preventing any of the important compounds from continuing on to the Tenax tube. The water that does pass through the condenser will also pass through the hydrophobic Tenax GC unadsorbed. The fact that the organic compounds in the urine vapor do not condense in the chilled water condenser is due to their presence in small amounts and their possession of very low thermodynamic activity.

To start sampling, parts (A) and (B) in Figure 6 are connected, this is done by connecting the 1/8 in. Teflon line (3) of part (A) to the nitrogen inlet of part (B) by means of a

FIGURE 4

MANIFOLDS FOR SIMULTANEOUS PREPARATION

OF MORE THAN ONE TRAP-INSERT



1/8 - 1/4 in. reducer union (6); the 1/8 in. end of the reducer is connected to the Teflon line (3) with a 1/8 in. nut and, the 1/4 in. end is attached to the nitrogen inlet by means of a short piece of shrinkable Teflon tubing (7).

Once the connection between parts (A) and (B) was completed a "Trap-Insert" is attached to the outlet of the condenser with a Teflon sleeve and the flow is monitored with a bubble-flow meter (13) and controlled to give a flow of 20ml/min by means of the needle valve (4). Consequently, the sample is purged for half an hour in case of urine samples, or one hour in case of serum and plasma samples. At the end of the sampling period the "Trap-Insert" is stored in an appropriate vial (described in the next section) and saved until it is ready for analysis.

A very interesting additional feature that we have added to this sampling system is that, several "Trap-Inserts" may be used simultaneously, especially, when it is desired to have more than one sample for different types of analysis. The different manifolds used to accomplish the trapping and concentration of the organic volatiles on more than one "Trap-Insert" are shown in Figure 4.

4. Storage of Samples

After the headspace volatiles over urine or serum samples are trapped and concentrated by purging them through the Trap-Inserts containing the porous polymer adsorbent Tenax GC, they were either

analyzed immediately after sampling or stored for analysis at some future date. Effective storing was accomplished by placing the loaded Trap-Inserts in 20 x 125mm culture tubes (KIMAX; Kimber Products, Owens, Illinois) which are firmly secured by plastic screw caps lined with Teflon. At first the culture tubes containing the loaded Trap-Inserts were stored in freezers at temperatures of -10°C. However, after several experiments at different storage temperatures and conditions it was determined that storage at room temperatures was quite adequate. To demonstrate further the effectiveness of such a simple storing system two Trap-Inserts were prepared from the same urine sample and, one was mailed from Houston, Texas to Tuebingen, Germany and back while, the other Trap-Insert was stored in our laboratory in Houston. On the analysis of both Trap-Inserts the results were found to be identical.

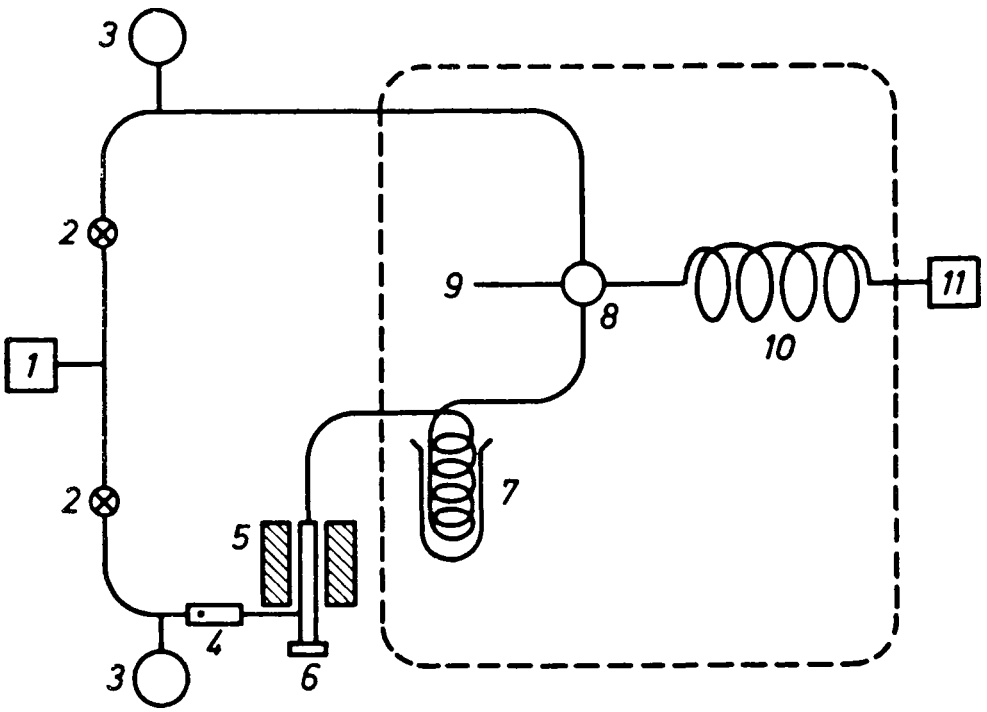
D. SAMPLE TRANSFER AND ANALYSIS

1. Precolumn-Trap

The question of transferring the organic volatiles adsorbed on the Tenax GC to the chromatographic column to be analyzed may at first seem a simple matter, however, it was discovered that the use of highly efficient open tubular columns imposed certain requirements as to how the headspace volatiles from urine or serum should be desorbed and injected into the chromatographic column. In order to

FIGURE 5

SAMPLE TRANSFER SYSTEM



- Sample Analysis and Transfer Flow System

1 Carrier (He)	6 Modified injector port
2 Toggle switch	7 Pre-Column in dry ice
3 Pressure regulator	8 4-port valve
4 Rotameter	9 Vent
5 Injector block	10 Separating column

make full use of the efficiency characteristic of open tubular columns, the sample injected by means of a syringe should enter the column in form of a "plug", in practice, this is made possible by the use of splitting devices. Another factor that should be taken into consideration is the amount of sample injected, large samples usually result in overloading the column, therefore, making it less efficient.

In order to simulate conditions as those just mentioned for the injection of headspace volatiles into chromatographic columns, the Trap-Insert is placed in the modified injector port whose temperature is maintained at 280-300°C at all times. The desorbing sample is then transferred (purged) into a pre-column trap, usually, a 1m x 0.5mm ID open tubular column coated with the same phase as the analytical column which is attached to the outlet of the injector port. By cooling the pre-column in liquid nitrogen, and purging the desorbing sample for 15 minutes at a flow of 20ml/min, it is possible to condense the purged sample in the form of a plug within the pre-column prior to its introduction into the analytical column, a schematic of such a system for sample transfer and analysis is shown in Figure 5.

2. Four-Port Valve

Once the sample had been thermally desorbed, transferred, and condensed in the pre-column the next step was to transfer it, or more precisely inject it into the chromatographic column for analysis.

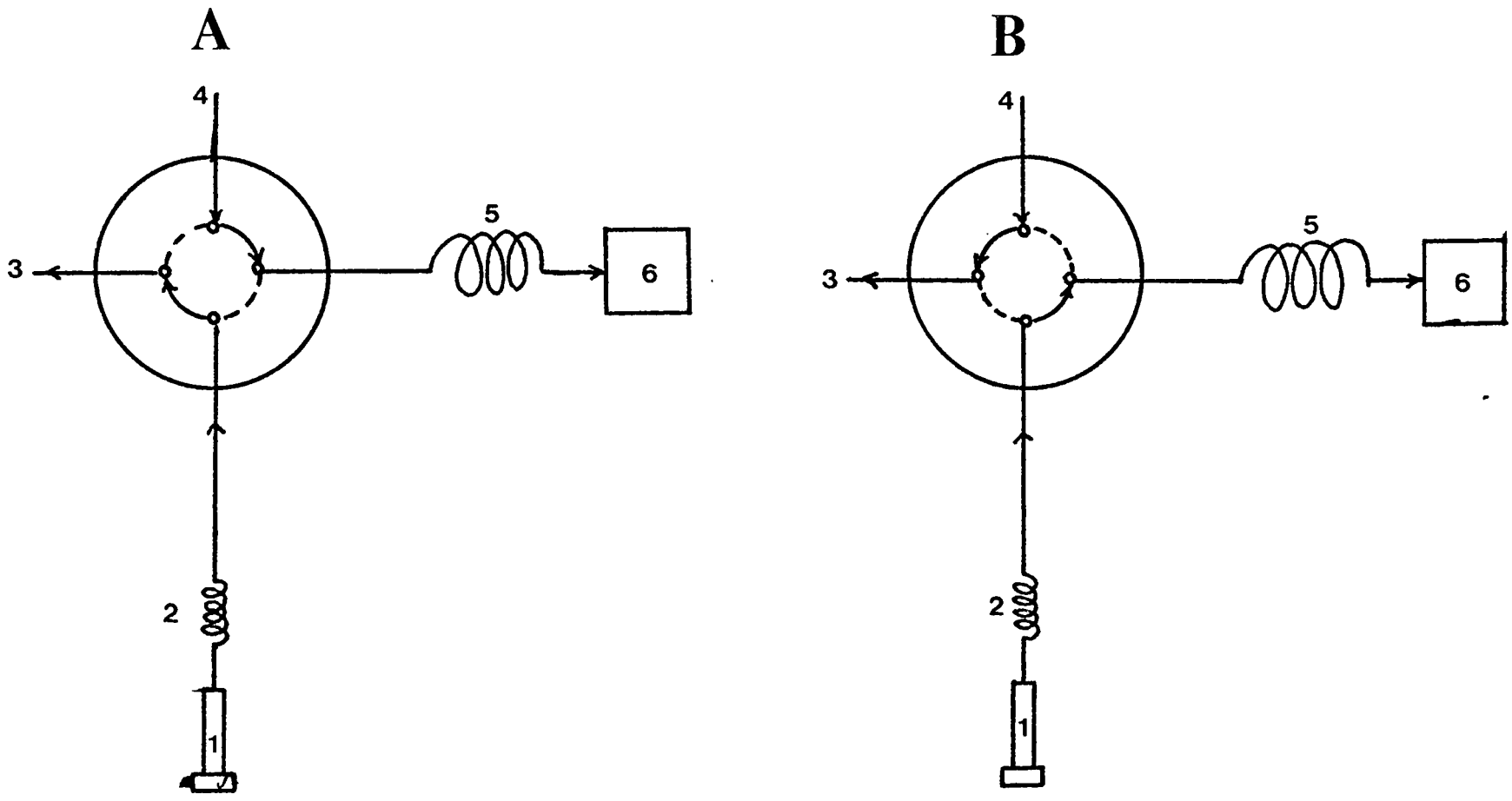
For this step, a four-port valve (Valco, Inc., Houston, Texas) Figure 5, was used as part of the system, a more detailed description of the functions carried out by the valve is shown in Figure 6. For the fifteen minutes during which the sample is being desorbed and purged into the precolumn immersed in liquid nitrogen the four-port valve is in the position indicated by the solid lines as shown in Figure 10a; at the end of the fifteen minute period, the valve is switched to the position indicated by the solid lines as in Figure 10b, the flow of carrier gas is then adjusted to a predetermined value to give a flow of 3.5ml/min measured at the end of the column, once this is done the liquid nitrogen bath is removed, the sample injected into the chromatographic column, and the analysis started.

3. Analytical Columns

During the course of this project, investigations dealing with the development of highly efficient capillary columns, best suited, for the analysis of organic volatiles in biological fluids or in the atmosphere were undertaken.[58-60] For the work described here, the analyses were all carried out on nickel capillary columns. The reason behind choosing nickel as the column material was the possibility of substantially increasing the surface area of the interior wall of the column by etching with acid prior to coating, as will be explained in a succeeding section.

FIGURE 6

FOUR-PORT VALVE


A
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1

a. Preparation:

The tubing purchased from Handy and Harman Tube Co., Norristown, Pa. was received in coils of 100m x 0.5mm ID and 0.8mm OD (needle stock). Newly acquired columns were first checked for leaks by closing off one end and applying high pressures (1000 psi) at the other. Once it was confirmed that the column was free from any defects, it was then necessary to modify the ends of the column in order to make appropriate connections to the instrument. This modification was achieved by slipping short pieces of stainless steel tubing, 5cm x 1.0mm ID and 1.6mm OD over the ends of the needle stock tubing and silver soldering them (Figure 7). The stainless steel sleeve was then fitted with 1/16 in. ferrules and nut needed for connecting the column to the instrument.

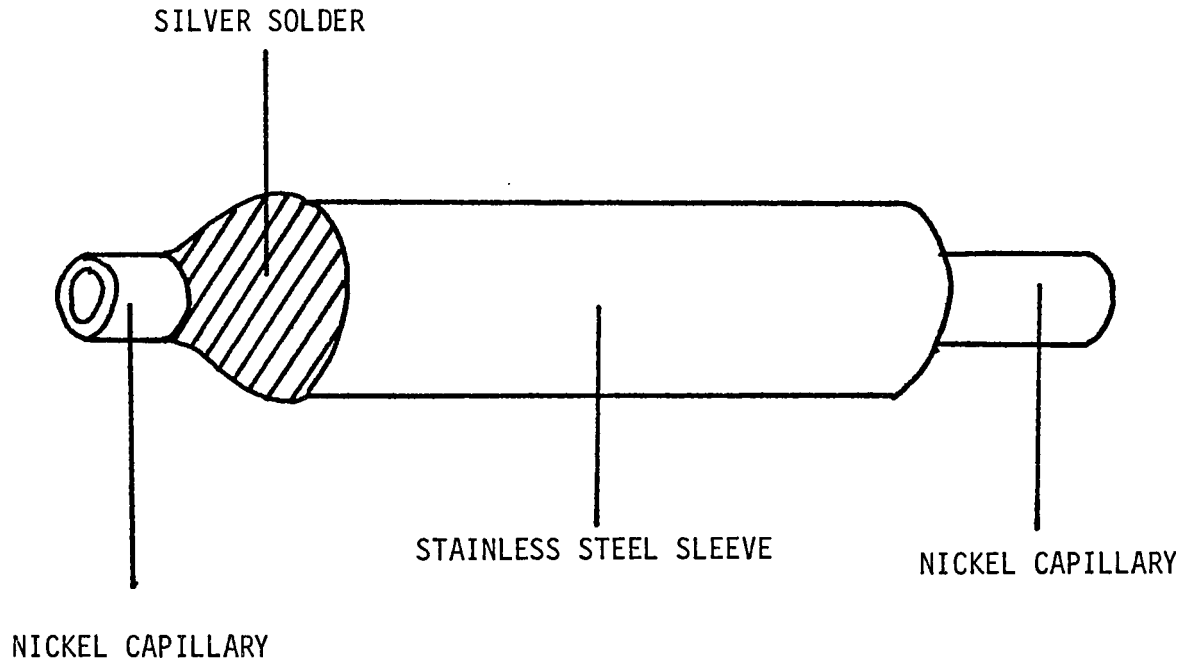
b. Cleaning and Etching:

For the cleaning of the new column it was first connected to a stainless steel pressure reservoir, with prepurified nitrogen (IWECO, Houston, Texas) used as the pressure source. Cleaning of the new column was accomplished by washing with several 50 ml portions of methylene chloride, methyl ethyl ketone, water, and acetone. The washings were done by pushing the solvents through the column by applying pressures as high as 1000 psi. All solvents were of spectro-grade quality. Finally, the column is dried by allowing prepurified nitrogen under high pressure to flow through for approximately on hour.

FIGURE 7

MODIFICATION OF ENDS OF

NEEDLE STOCK COLUMN


SILVER SOLDER
STAINLESS STEEL SLEEVE
NICKEL CAPILLARY
NICKEL CAPILLARY

for approximately one hour.

In order to etch the clean column, a stock solution of etching acid, consisting of concentrated nitric acid, glacial acetic acid, and distilled water in equal proportions was first prepared. A diluted portion of the stock solution (1 part stock solution to 6 parts distilled water) was then used to fill the entire column, both ends of the columns were then dipped into a beaker filled with water to prevent the acid from reacting with the soldering connections. The etching reaction withing the column was allowed to proceed for sixteen hours. At the end of the reaction, the etching solution is pushed out of the column and should be green in color. Collection of the etching solution in a graduated measuring cylinder made it possible to estimate the volume of the column.

Following etching, the column was washed with dilute ammonia (or a dilute solution of sodium bicarbonate) distilled water several times, and finally with acetone and allowed to dry as mentioned previously.

c. Coating:

The liquid phase used in this work was Emulphor-ON 870 a polyoxyethylated fatty alcohol (GAF Corporation, New York, N.Y.). A 10% w/w coating solution was prepared by dissolving 10g of the liquid phase in 67.3g of spectrograde quality chloroform. The clean, etched, and dried column was coated with the 10% coating solution

twice; for the first coating 5 ml of coating solution were placed into a small stainless steel reservoir, the end of the column was immersed in a beaker filled with water and pressure applied to the reservoir from a tank of pre-purified nitrogen. The pressure was adjusted, usually 5 - 10 psi, so that air being expelled out of the column would do so at a rate of about one bubble every 1 - 2 seconds. Coating required a period of about 6 hours, however, the flow of nitrogen through the column was allowed to continue uninterrupted for a period of approximately 20 hours to ensure drying. The second coating was carried out more carefully and with only 1 ml of the same coating solution. The vessel used for the second coating was equipped with a fine regulatory valve in order to ensure fine adjustment of the applied pressure, which in turn results in a thin uniform thickness (film) of liquid phase coated on the walls of the column. As in the case with the first coating , the flow of nitrogen was left uninterrupted for a period of 20 hours and then increased slightly to 10 psi for 24 hours to completely dry the column.

Before the column was ready for use it was necessary to condition it . This was done by placing the column into the oven cavity of the gas chromatographic instrument and connecting one end (direction opposite to that of coating) to the carrier gas inlet.

The pressure of carrier gas applied to the inlet is set at 10 psi, to give a flow of approximately 3.5 ml/min., measured at the end of the column. The temperature of the oven is then

raised from ambient temperature to 30°C, below the temperature limit of the phase at a rate of 0.5°C/min. In the case of Emulphor-ON 870 the temperature limit is 190°C, so the temperature is raised to 170°C and after two hours, the temperature is raised again at the same rate to the temperature limit of the phase and maintained for a minimum of two days.

4. Chromatographic Conditions

The conditioned column was then connected to the system as shown in Figure 5. As explained earlier, the volatiles previously trapped and concentrated on the adsorbent, are thermally desorbed in the modified injector port and purged at a gas flow rate of 20ml/min through a pre-column trap immersed in liquid nitrogen which allows the volatiles to condense in the form of plug. Consequently, by means of a four-port valve the pre-column trap is connected to the analytical column and the liquid nitrogen bath removed. To start the analysis, the oven door is closed and the temperature programmer operated isothermally at 80°C for 15 minutes followed by a rise of 2°C/min up to 180°C and, finally, isothermal operation at 180°C for 35 minutes.

The gas chromatographic instruments used for performing the analysis on the organic volatiles in urine and blood were a Perkin-Elmer 900 (Perkin-Elmer, Norwalk, Conn) equipped with a dual flame ionization detector and, a Shimadzu gas chromatograph,

Model GC-5 AP_5 (American Instrument Co., Silver Spring, Md),, equipped with a flame ionization detector and a sulfur specific flame photometric detector.

5. GC-MS Analysis

An LKB 9000 mass spectrometer (LKB-Prodkter AB,S-161, 25 Bromma 1, Sweden) modified by replacing its gas chromatographic unit with a Perkin-Elmer 900 gas chromatograph, was used for identification purposes. The ionization voltage was 70 ev and scan time for a mass range of 20 to 200 units was 4.5 seconds. Temperatures of the separator and ion source were set at 220°C and 250°C, respectively. Analytical procedures and chromatographic conditions were the same as described in the previous section. Mass spectral interpretation was accomplished by comparing the spectra obtained with the spectra of known compounds which is available in our laboratory.

Recently, analysis of organic volatiles in urine, serum, and plasma were carried out on a GC/MS system different than the one mentioned above and which consisted of a Model CH5 mass spectrometer connected to a spectrosystem 100 computer and mated to a Model 2701 gas chromatograph (all from Varian MAT, 28 Bremen 10, Germany). In this system the separator used was of the Biemann-Watson design. Make up gas was introduced at the end of the column to optimize the performance of the separator. Spectra were obtained using an ionization voltage of 70 ev with an exponential scan mode, requiring

approximately 4 seconds for a mass range from 10 to 300 mass units. Temperatures of the separator and ion source were 220°C and 250°C. Chromatographic conditions were identical to those mentioned earlier, in the latter system the Model 2701 gas chromatographed was equipped with the modified Tenax injection port .[61]

CHAPTER III

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

A. SAMPLING SYSTEM

Since the sampling method developed in our laboratories is in essence the backbone of the project described here, it was essential to evaluate it very closely, especially with regard to the adsorbent used and reproducibility of the results obtained.

The evaluation of the adsorbent, Tenax GC, was done in connection with research on the analysis of tobacco smoke. In analysis of the latter it was found that in a 35 ml puff , the organic compounds present in the smoke exceeded the capacity of the adsorbent.[55] To investigate this further five Trap-Inserts were connected in series and a sample of 500 ml of natural gas from a line in the laboratory was drawn through the five tubes. Figures 8a and 8b demonstrate the FID FPD (sulfur) profiles of such a sample. I t is evident from the sulfur profiles that very few sulfur containing compounds escape the first tube. The FID profiles, on the other hand, indicate that the higher molecular weight compounds tend to displace lighter ones which, is in accordance with the priciples of adsorbtion chromatography. The potential usefulness of using Tenax-GC as an adsrobent, therefore, lies in its ability to enrich selectively trace quantities of polar and high molecular weight compounds.

In order to test the system for reproducibility, a pooled urine sample from several members of our group was collected and

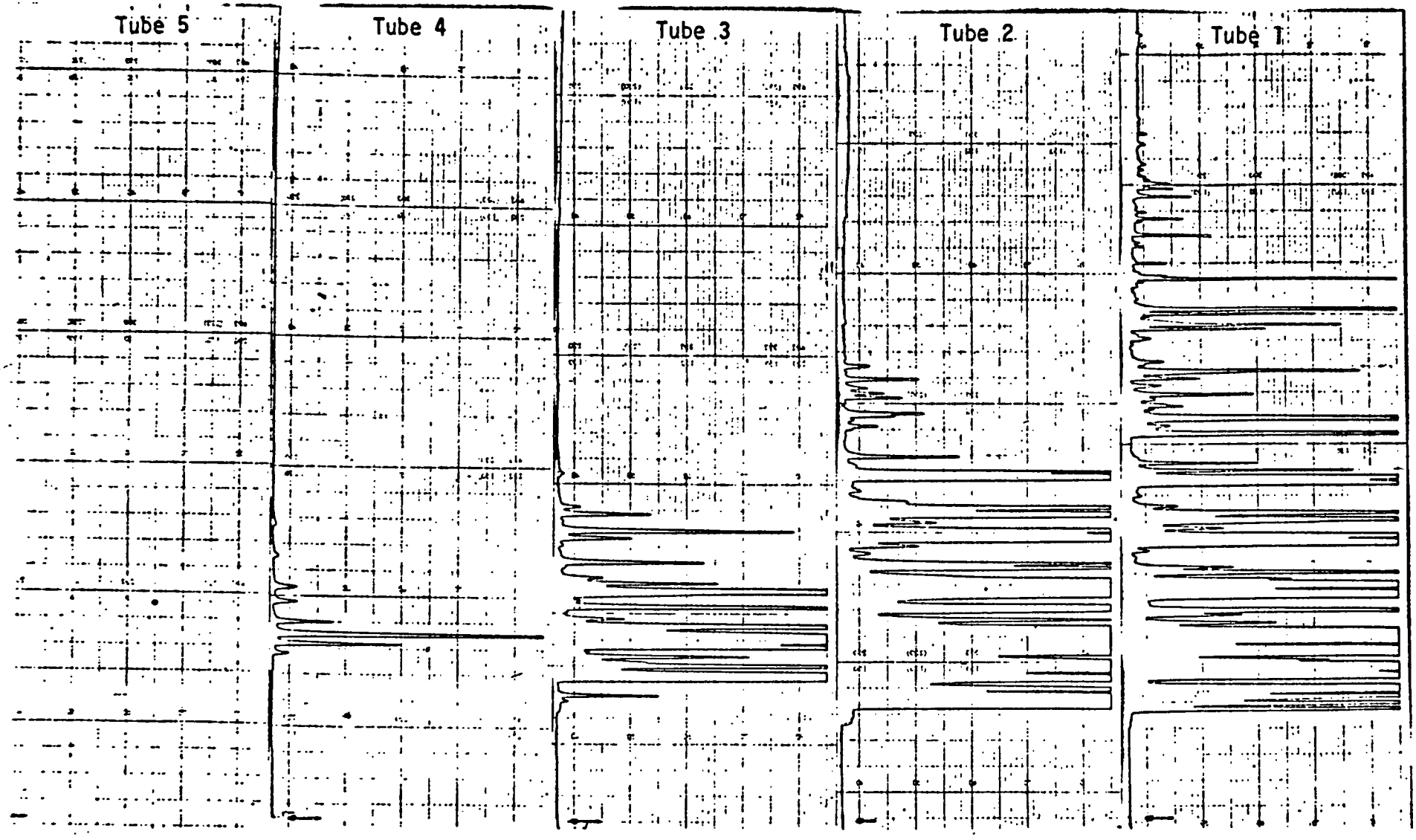
FIGURE 8

CAPACITY OF TENAX GC FOR A GAS SAMPLE
ADSORBED IN FIVE TRAP-INSERTS IN SERIES

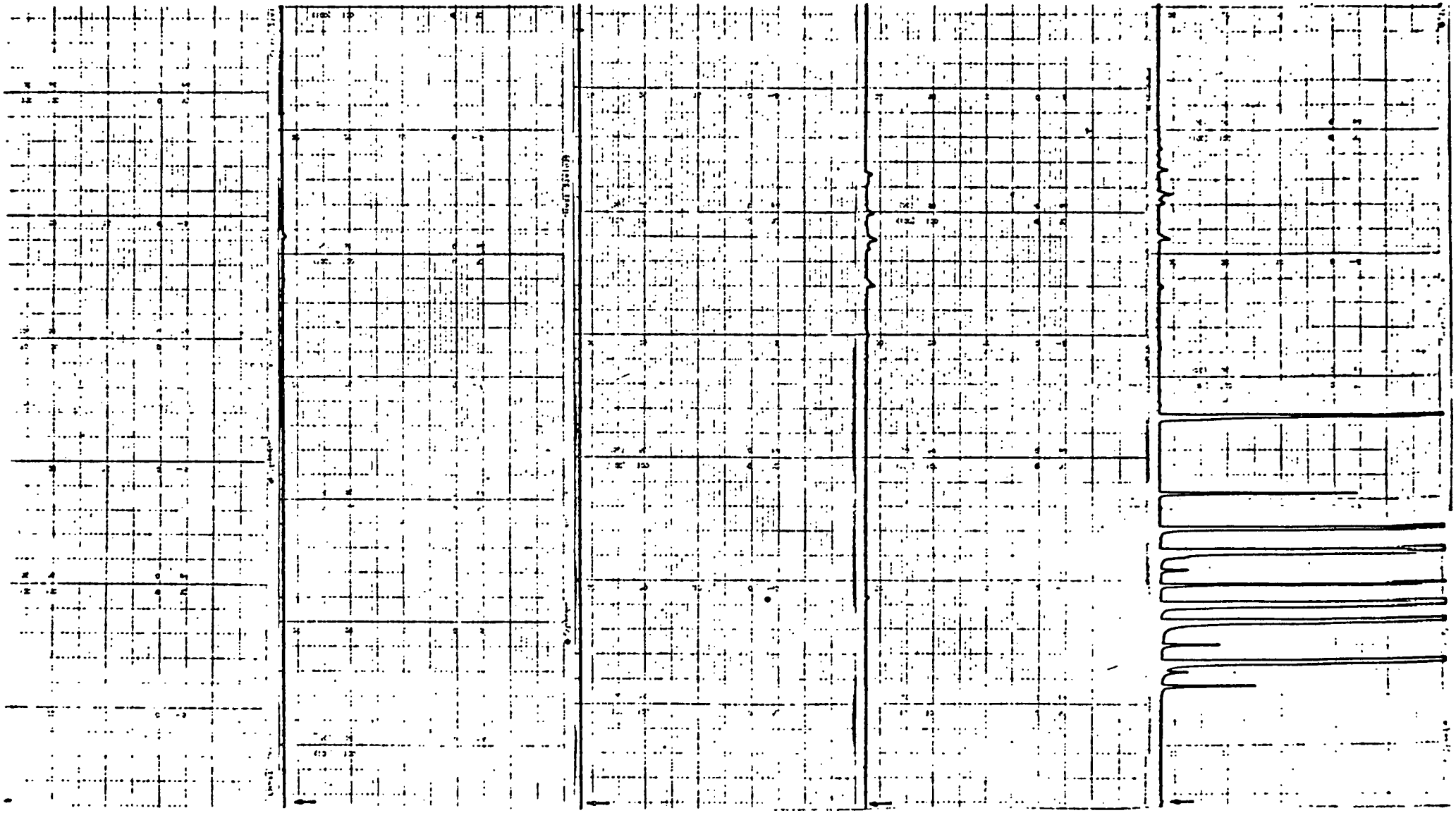
A. FLAME MONITOR FM

B. FLAME PHOTOMETRIC DETECTOR FPD

CAPACITY OF TENAX FOR A GAS SAMPLE
ADSORBED IN FIVE TUBES IN SERIES



FLAME MONITOR FM



FLAME PHOTOMETRIC DETECTOR FPD

FIGURE 9

REPRODUCIBILITY OF FID PROFILES

FROM SAME URINE SAMPLE

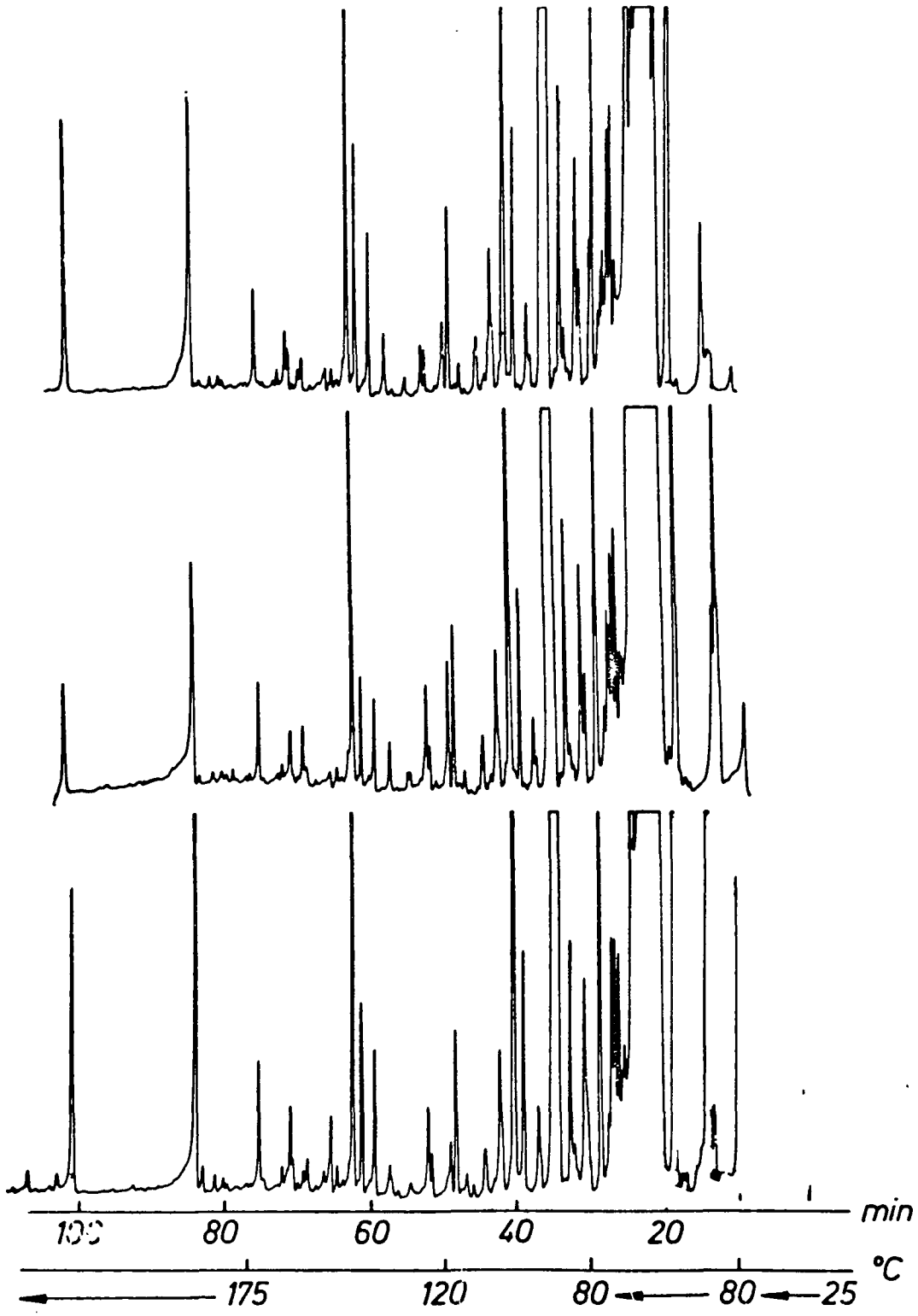
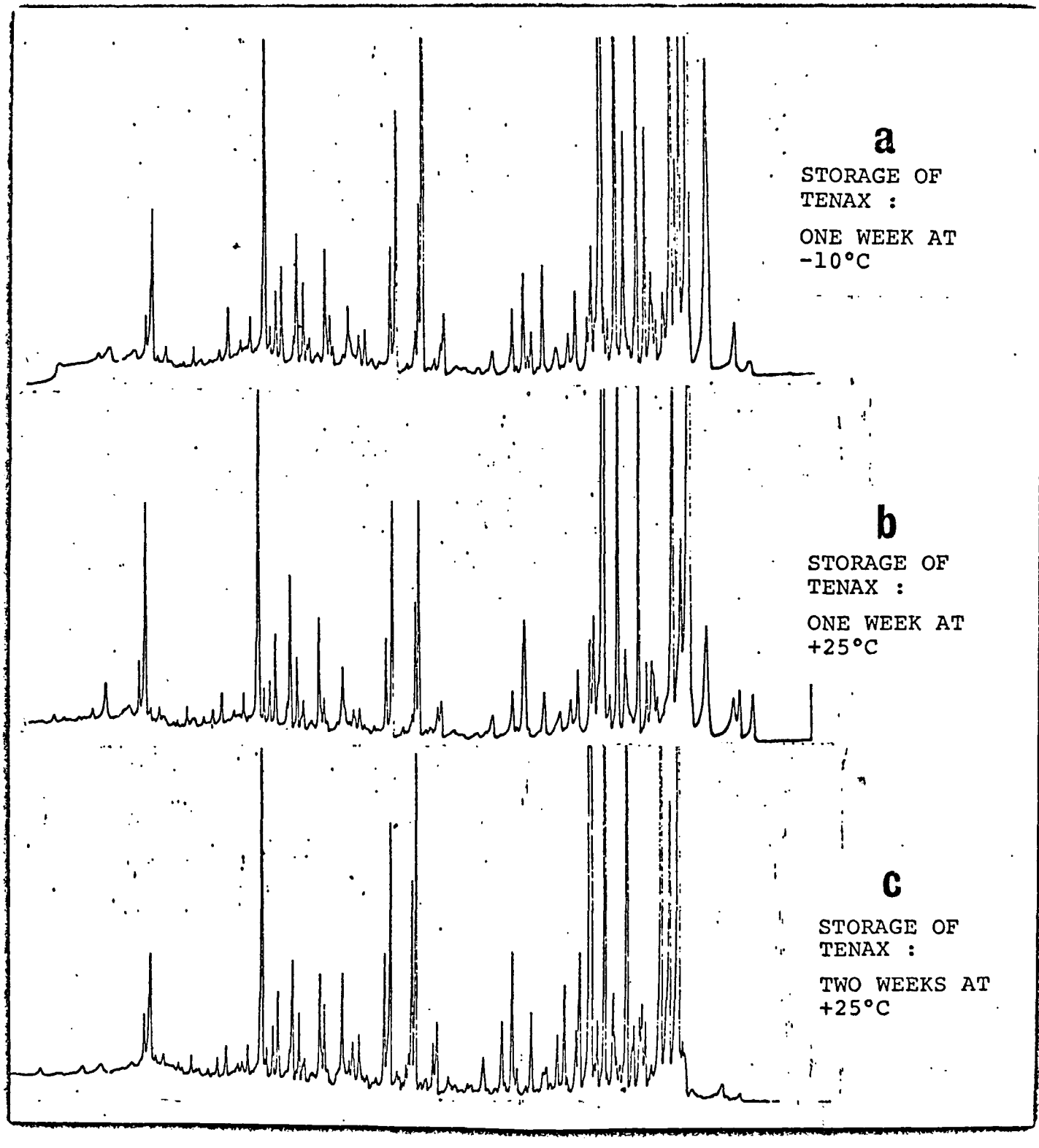

100
80
60
40
20
min
°C
175
120
80
80
25

FIGURE 10

EFFECT OF DIFFERENT

STORING CONDITIONS


a
STORAGE OF TENAX :
ONE WEEK AT -10°C
b
STORAGE OF TENAX :
ONE WEEK AT +25°C
c
STORAGE OF TENAX :
TWO WEEKS AT +25°C

divided into ten equal portions and stored in a refrigerator at 4°C. Each aliquot was sampled and analyzed as described earlier on different days. The results obtsined from three such samples that were sampled and analyzed thre days apart are shown in Figure 9. The reproducibility is quite apparent.

I n a similar manner, the method of storing the trapped samples was investigated. Volatile compounds from the same urine sample were simultaneously trapped on three Trap-Inserts. The Trap-Inserts were stored in the culture tubes mentioned earlier under different conditions and, the results are shown in Figure 10. The results not only demonstrate the effectiveness of the storage method but also its versatility since, this method will allow samples previously trapped to be shipped to another laboratory for analysis or stored indefinitely.

B. ORGANIC VOLATILES IN URINE:

A major difficulty that was encountered in the stepwise development of the procedure into one for the early detection of disease was, that of establishing the basic guidelines or profiles of normal individuals according to which distinction between healthy and pathological cases could be made.

To establish such a basis, several hundred urine samples from healthy individuals were acquired, these included daily samples taken from each individual while under a controlled diet for a

TABLE I

VOLATILE ORGANIC COMPOUNDS IN NORMAL HUMAN URINE

Peak Number*	Compound
	Acetone
1	2-Butanone
2	Ethanol
	Propionaldehyde
3	3-Methyl-2-butanone
	2,3-Butanedione
	2,3-Dimethylfuran
4	2-Pentanone
5	2,4-Dimethylfuran
	Chloroform
	4-Methyl-2-pentanone
	3-Methyl-2-pentanone
	2(?)-Methyl-5(?)-ethylfuran
	Toluene
6	Dimethyldisulfide
	3-Hexanone
7	2,3,5-Trimethylfuran
8	5-Methyl-3-hexanone
9	3-Penten-2-one

TABLE I (CONTD)

Peak Number*	Compound
	1-Methylpyrrole
10	4-Methyl-3-penten-2-one
11	1-Butanol
12	4-Heptanone
	C_4-Furan
	Thiolan-2-one (tent)
13	2-Heptanone
	3-Methylcyclopentanone
	6(?)-Methyl-3-heptanone
	3-Octanone
	Methylpyrazine
	2-n-Pentylfuran
	2,6- or 2,5-Dimethylpyrazine
	Allylisothiocyanate
	2,3-Dimethylpyrazine
	Furfural
	2,3,5-Trimethylpyrazine
	Vinylpyrazine
	2-Methyl-6-ethylpyrazine

TABLE I (CONTD.)

Peak Number*	Compound
14	Pyrrole
	2-Nonane
	Acetylfuran
	p-Methylpropenylbenzene
15	Benzaldehyde
	B-Pinene (tent.)
	2-Methyl-6(?)-vinylpyrazine
	2-Methylpyrrole
	Dimethylpyrrole
	1-Butylpyrrole (tent.)
16	Carvone
17	Piperitone
	p-Cresol

*Peak numbers refer to numbers in Figure 12. Identified compounds not numbered appear in the chromatograms between two numbered peaks in the order given in this Table. No numbers were assigned to these constituents because the experimental conditions for the GC-MS analyses were different from the conditions for GC analyses, with consequent slight changes in peak sizes and retention times.
"tent." = tentative.

period extending over eight weeks. The sampling and analysis of over one hundred of those samples established most of the guidelines needed to start the project.

The first and most interesting result was observed when a a urine sample from each of the healthy individuals collected on the same day was analyzed for headspace volatiles. Figure 11 shows an example of such an analysis which, inspite of certain similarities between the profiles of the three individuals does show basic differences and confirms what has always been referred to as "Biochemical Indiviuality."

The effect was investigated by analyzing urine samples from each individual before and after being put on the controlled diet. Numerous analyses showed that diet had little effect on the profiles obtained as shown in Figure 12. Most of the organic volatiles in healthy urine have been identified by mass spectrometry and are shown in Table I.

Due to the complexity of the profiles obtained using flame ionization detectors and, the need for simplifying the profiles as much as possible, simultaneous use of specific detectors such as the flame photometric detector (FPD) for sulfur compounds was undertaken. Profiles of the fraction of sulfur containing compounds in the headspace volatiles over urine for the three healthy individuals shown in Figure 13. It is quite obvious that the overall profiles have been immensely simplified, and in addition individuality seems to have been retained inspite of the use of specific detectors.

FIGURE 11

VOLATILES FROM URINE OF THREE
HEALTHY INDIVIDUALS TAKEN
ON THE SAME DAY

"BIOCHEMICAL INDIVIDUALITY"

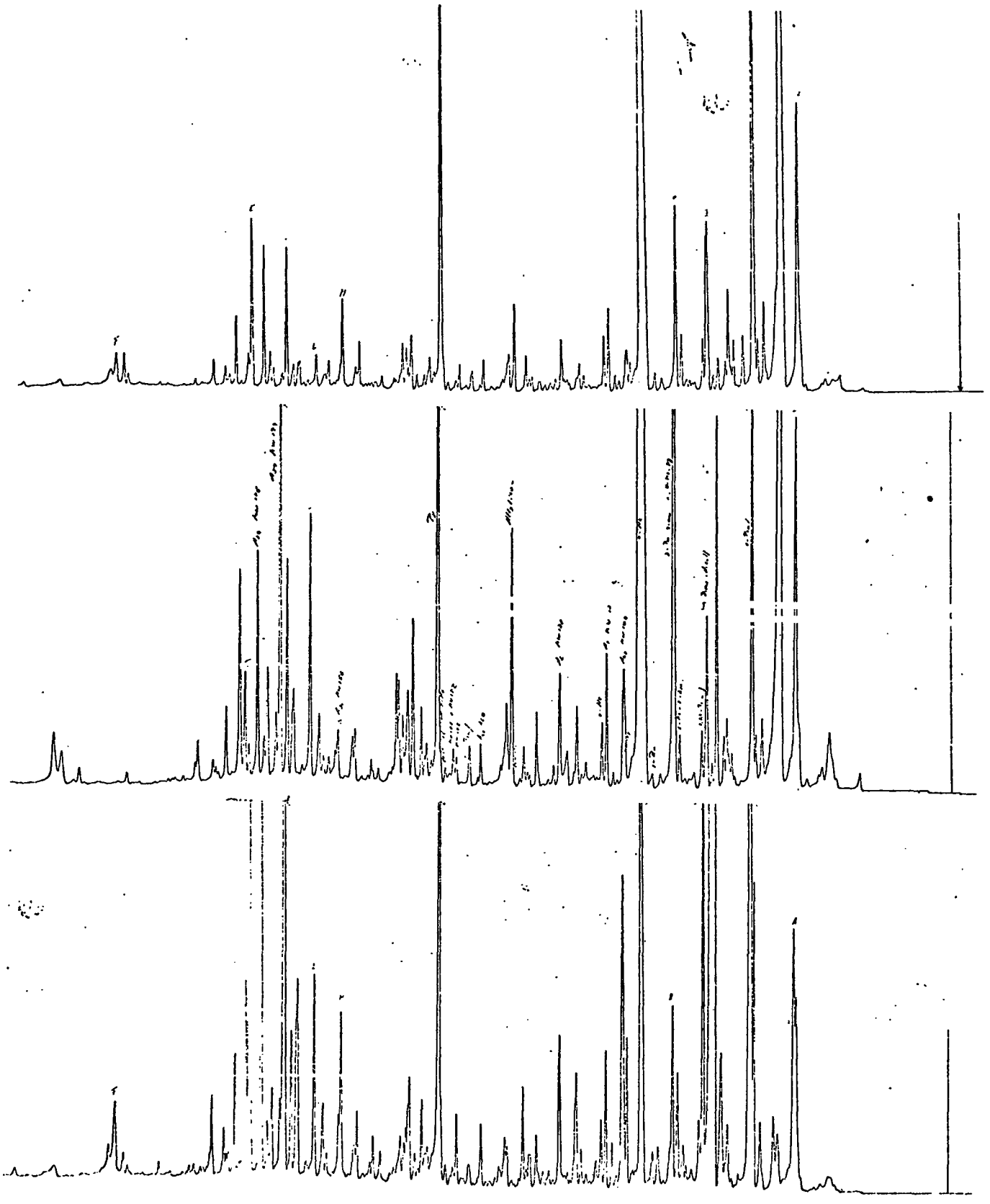
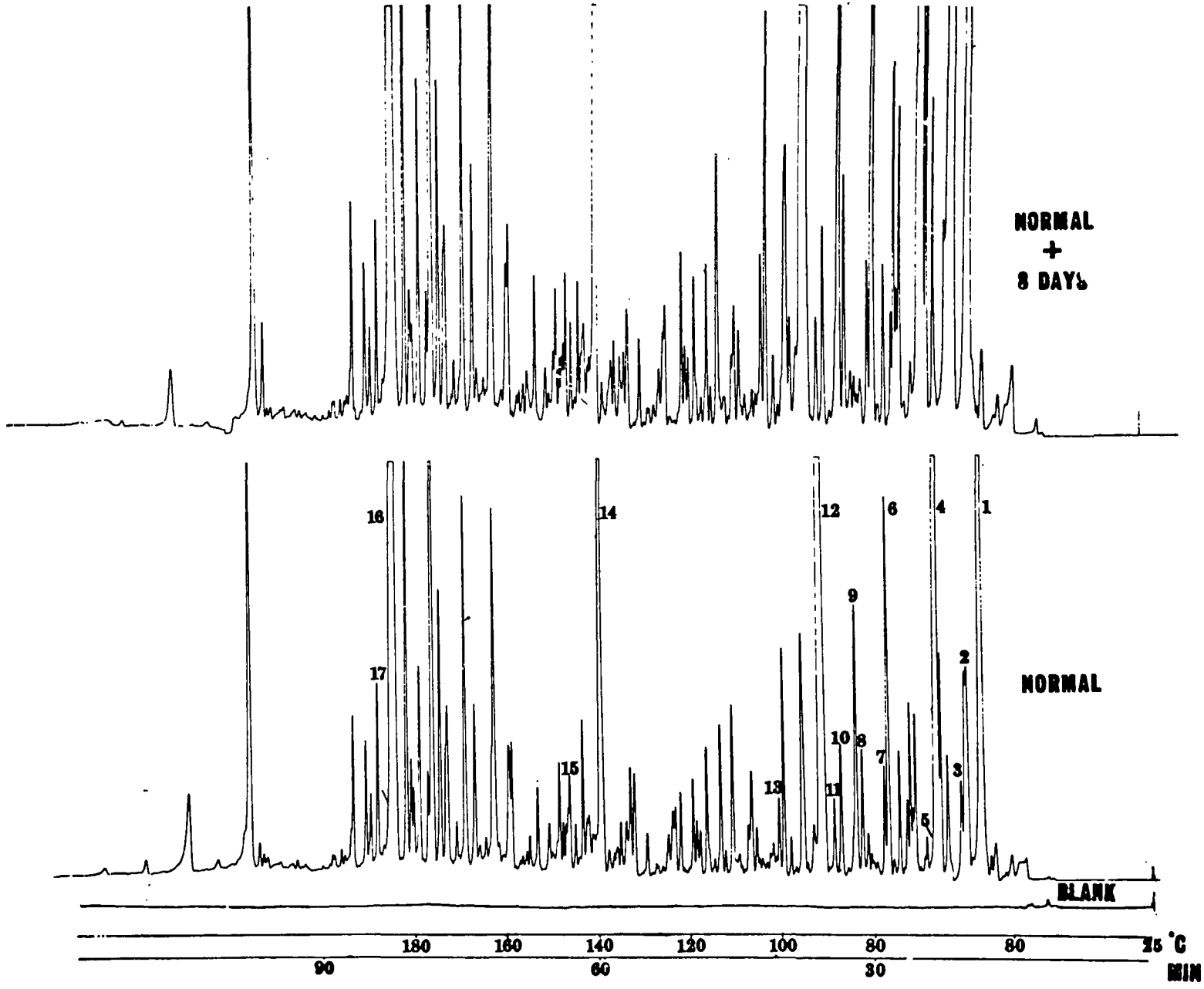


FIGURE 12

VOLATILES FROM URINE OF HEALTHY
INDIVIDUAL SAMPLED EIGHT
DAYS APART

"EFFECT OF DIET"

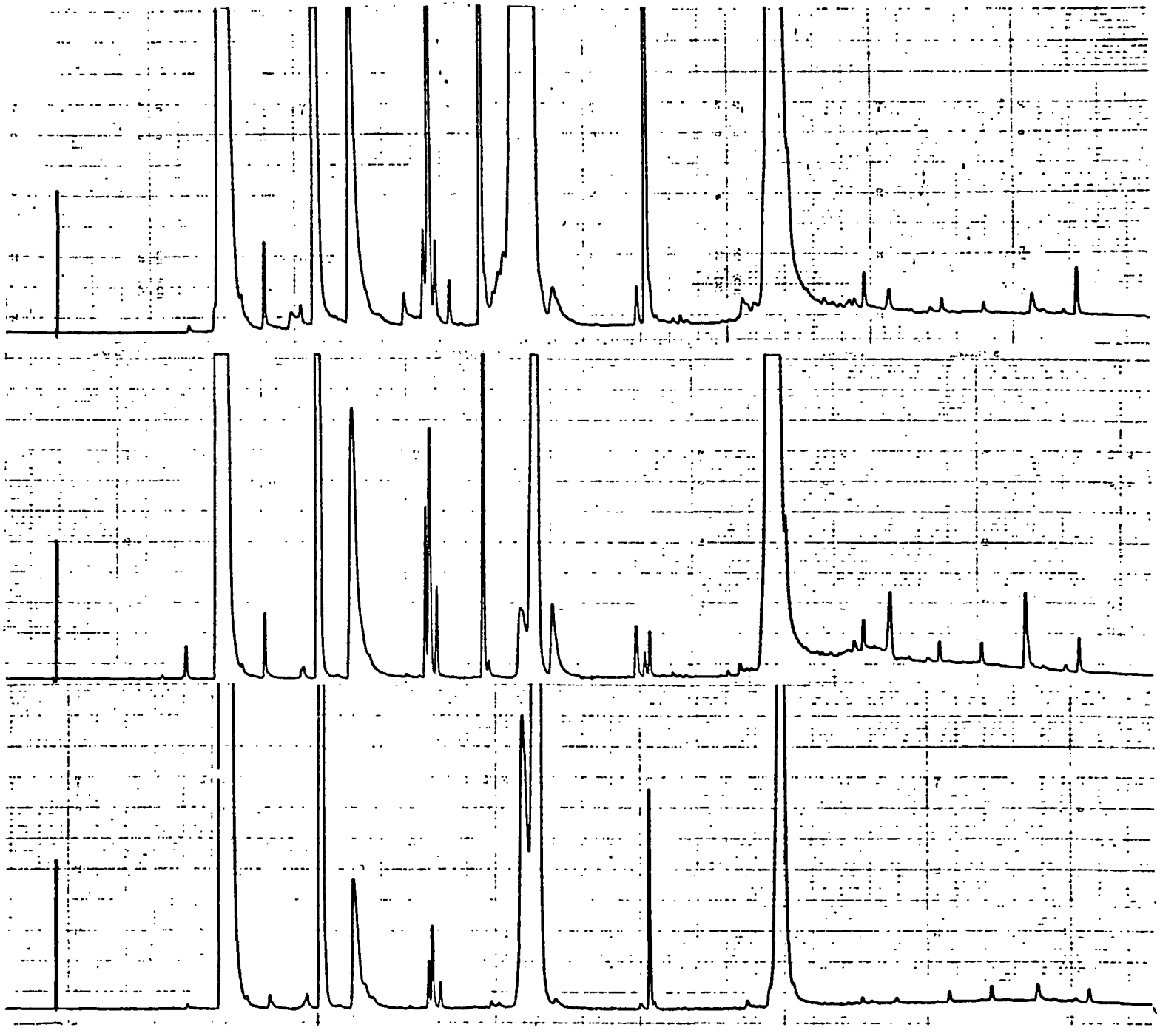


Urinary volatile organic compounds from the same individual sampled eight days apart (conditions in text).

FIGURE 13

COMPARISON OF SULFUR PROFILES

FOR THREE HEALTHY INDIVIDUALS



It was only after several hundred urine samples from the three healthy individuals were analyzed and normal profiles were established that the work on urine from patients with confirmed cases of Diabetes Mellitus was started. The diabetic samples (urine) analyzed ranged from different forms of the disease to those from diabetic patients on different therapies such as, insulin or oral agents.

Most significant about the profiles obtained from the diabetic urines was, the fact that they were all different than those obtained from any of the healthy individuals Figure 14, demonstrates the difference between healthy (normal) and diabetic profiles obtained on a flame ionization detector. Peak numbers on the normal profile refer to numbers in Table I, and peak numbers on the diabetic to those in Table II. Differences in sulfur profiles for normal and a diabetic urine is shown in Figure 15.

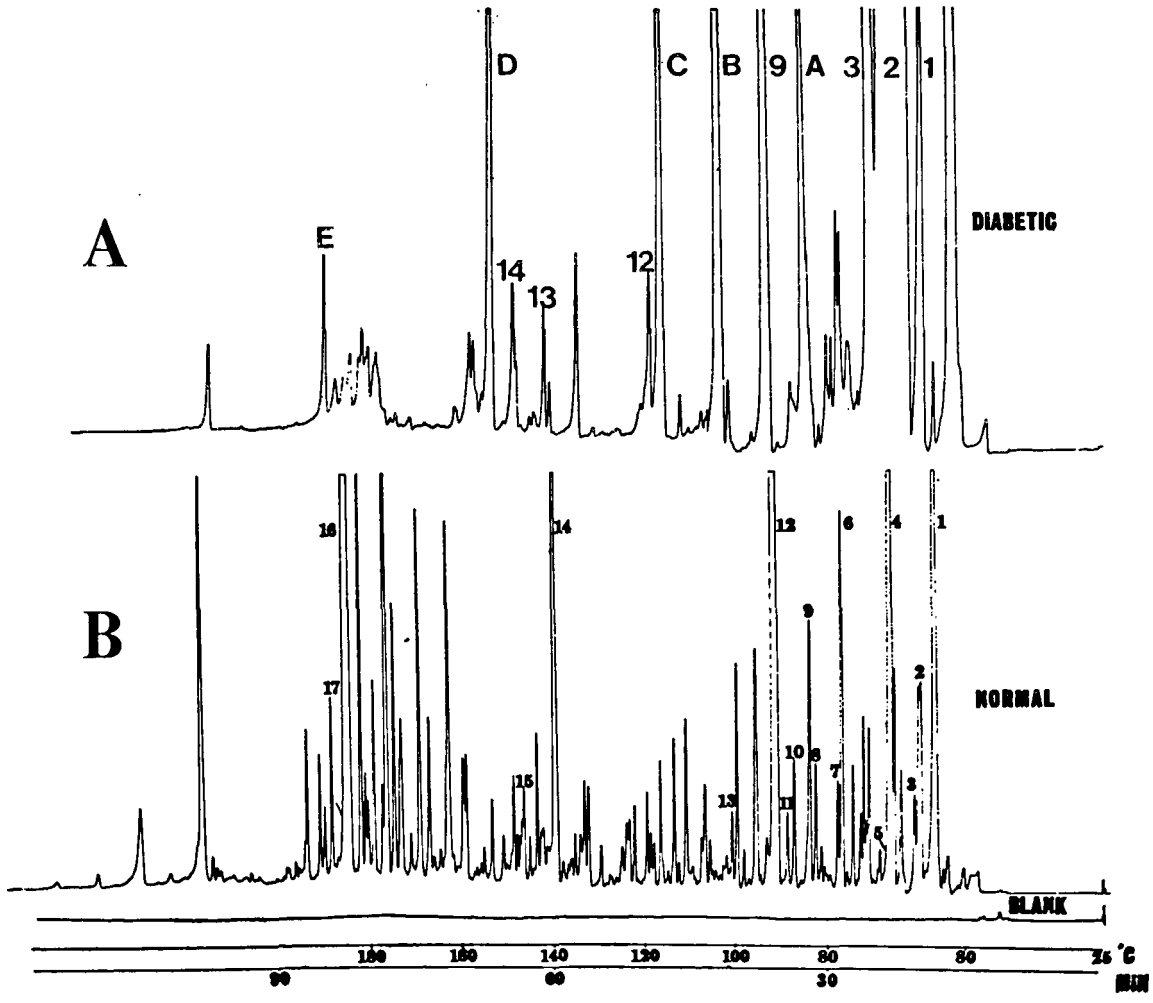
Insignificant as they may seem at first, the differences in the profiles were real and sufficient to expand the project to the study of as many diabetic urines as possible. Analysis of over 50 urine samples from subjects with Diabetes Mellitus were investigated. All the subjects had overt carbohydrate intolerance. As mentioned earlier some were on insulin others on sulfonylurea (oral agents) and the remainder were on no therapy. Some of the more interesting diabetic profiles are represented by Figures 16, 17 and 18. The first profile was that of a diabetic with high blood sugar who was not on

FIGURE 14

UNIRINARY PROFILES(FID)

A. VOLATILES IN DIABETIC URINE

B. VOLATILES IN NORMAL URINE



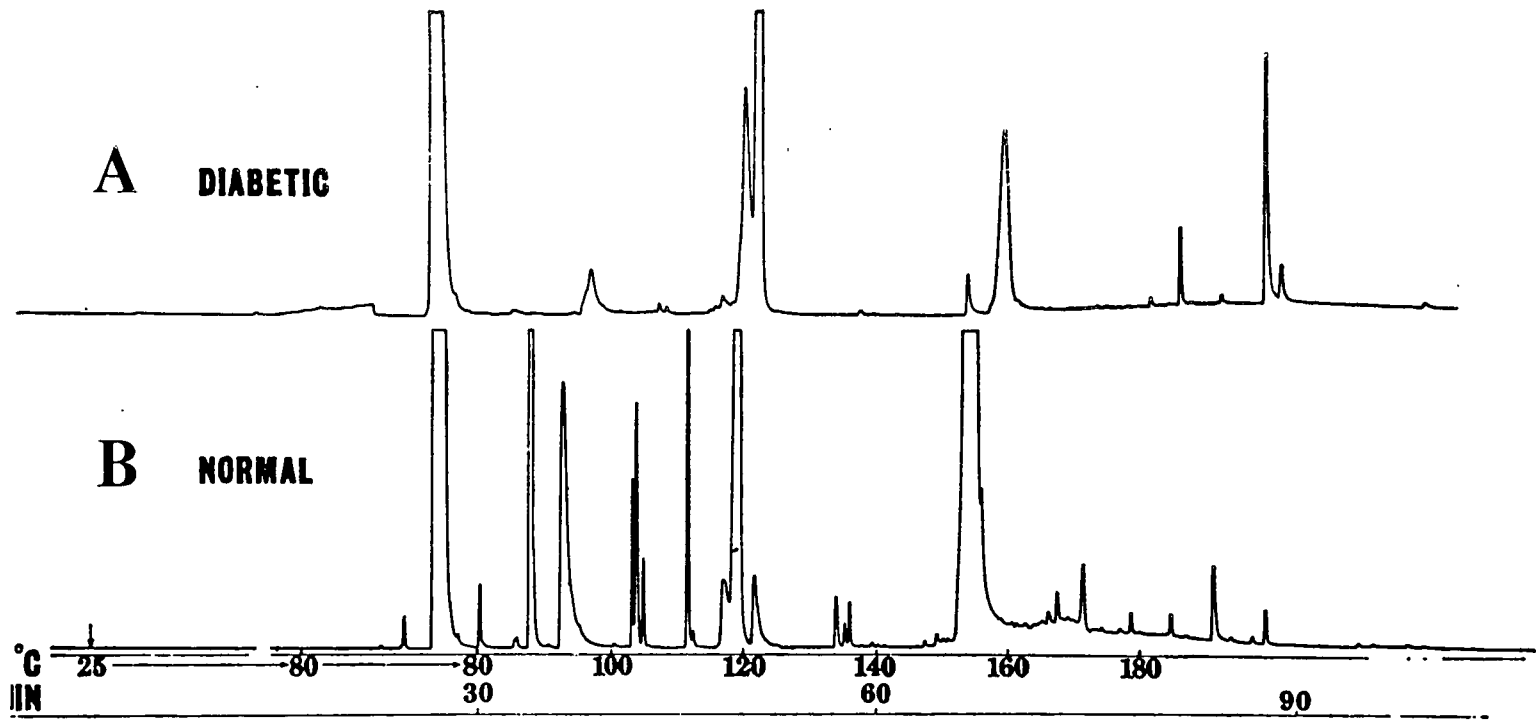
Comparison of urinary volatile organic compounds from normal and diabetic individuals

FIGURE 15

URINARY PROFILES (FPD)

A. VOLATILES IN DIABETIC URINE

B. VOLATILES IN NORMAL URINE



SULFUR VOLATILES

Comparison of urinary volatile organic sulfur compounds from normal and diabetic individuals

TABLE II

VOLATLE ORGANIC COMPOUNDS IN URINE OF SUBJECTS WITH DIABETES MELLITUS

Peak	Compound
	Acetaldehyde
	Acetone
1	2-Butanone
2	Ethanol
	2-Propanol
	Propionaldehyde
	3-Methyl-2-butanone
	2,3-Dimethylfuran
	2,3-Butanedione
3	2-Pentanone
	2,4-Dimethylfuran
	Chloroform
	4-Methyl-2-pentanone
	3-Methyl-2-pentanone
	Toluene
	2(?)-Methyl-5(?)-ethylfuran
K	*1-Propanol*
4	Dimethyldisulfide
	3-Hexanone

TABLE II (CONTD.)

Peak	Compound
5	2,3,5-Trimethylfuran
A	*2-Methyl-1-Propanol*
7	3-Penten-2-one
	1-Methylpyrrole
	1-Butanol
9	4-Heptanone
	3-Heptanone
	Pyridine
	Pyrazine
B	*2-Methyl-1-butanol*
B	*3-Methyl-1-butanol*
11	2-Heptanone
	3-Methylcyclopentanone
	Dimethylbenzene
	3-Octanone
	1-Pentanol
	6(?)-Methyl-3-Heptanone
L	*Methylpyrazine*
	3-Hydroxy-2-butanone
	2-n-Pentylfuran

TABLE II (CONTD.)

Peak	Compound
C	*Cyclohexanone*
	Picoline
	2,6- or 2,5,-Dimethylpyrazine
12	Allylisothiocyanate
M	*2,3-Dimethylpyrazine*
	Furfural
	2-Methyl-6-ethylpyrazine
N	*Vinylpyrazine*
N	*2,3,5-Trimethylpyrazine*
13	Pyrrole
	2-Nonanone
14	Benzaldehyde
D	*Several isomers with mol wt 130 and a characteristic fragment at 112, probably octanols*
	1-Octanol
	Dimethylethylpyrazine
	2-Methyl-6(?)-vinylpyrazine
O	*C_4-Pyrazine*
P	*C_4-Pyrazine*

TABLE II (CONTD.)

Peak	Compound
Q	*C_5-Pyrazine*
R	*Dimethylvinylpyrazine (tent.)*
	2-Methylpyrrole
	1-Butylpyrrole (tent.)
16	Carvone
	Piperitone
	Phenol
	p-Cresol

therapy, the second that of an adult diabetic on insulin and, the third represents a profile of a juvenile diabetic on insulin.

Mass spectrometric analysis of the samples of insulin treated diabetics whose profiles are shown in Figures 17 and 18 revealed several characteristic classes of compounds. All the compounds identified are shown in Table II. The italicized components could be considered characteristic of insulin-treated diabetics; these compounds are either not present in normal urines or their concentration in diabetics is higher by several orders of magnitude over that of normals. The characteristic components of diabetic urine are shown in Figure 19. The profile of the patient with juvenile diabetic shown in Figure 18 was characterized as having large amounts of pyrazines, the reasons for which have not been investigated. On the other hand, the profile represented by Figure 17, was characterized as having high concentrations of cyclohexanone and the octanols. Aliphatic alcohols were also identified in samples of diabetic patients who were not treated with insulin, however, in much smaller amounts (Figure 20).

To investigate the correlations between volatile metabolites produced in the different forms and stages of Diabetes Mellitus and in the different forms of treatment, a whole gamut of diabetic samples were analyzed and the profiles are shown in Figures 21-23. Another facet of Diabetes Mellitus that was also investigated is the possibility of the genetic inheritance of the disease. The results are shown in Figures 24 and 25, each Figure represents profiles of a diabetic female and

FIGURE 16

PROFILES OF A DIABETIC WITH HIGH BLOOD SUGAR AND ON NO INSULIN

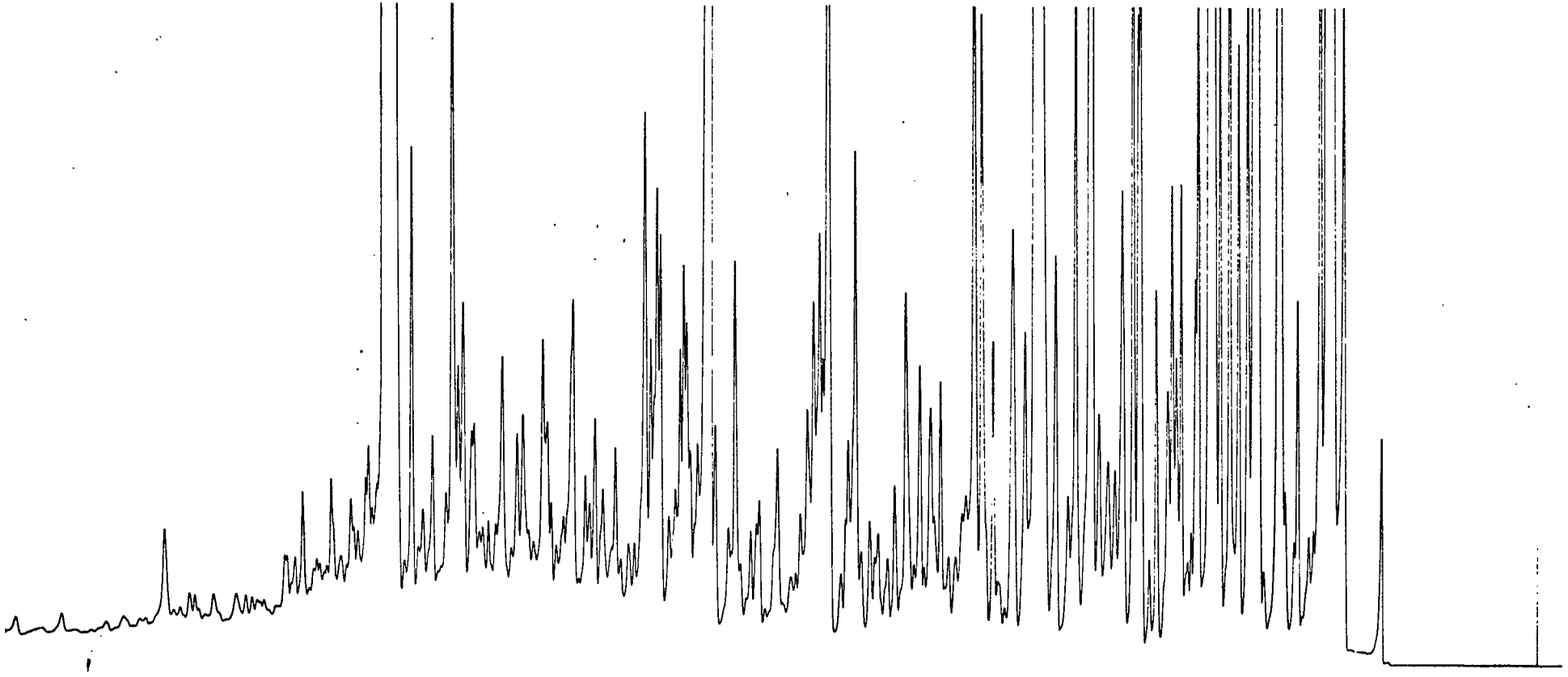


FIGURE 17

PROFILE OF AN ADULT DIABETIC

ON INSULIN

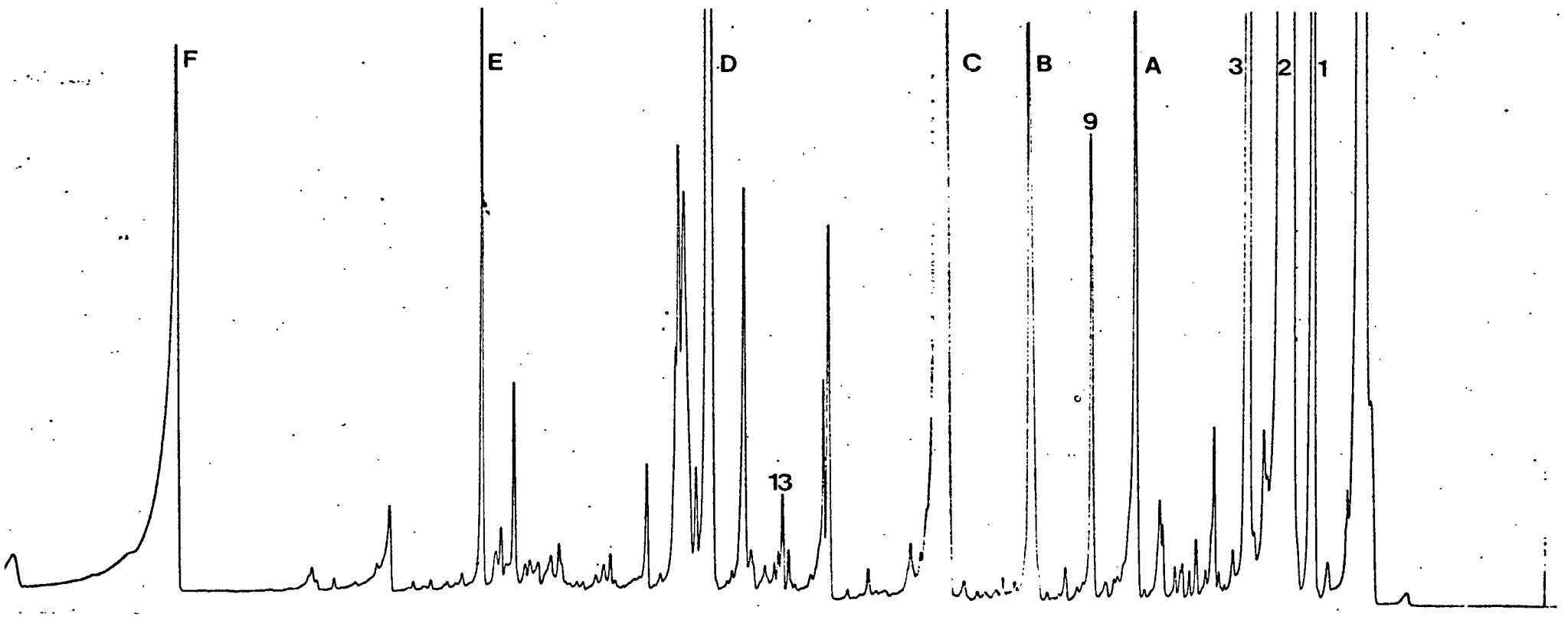

F
E
D
13
C
B
9
A
3
2
1

FIGURE 18

PROFILE OF A JUVENILE DIABETIC

ON INSULIN

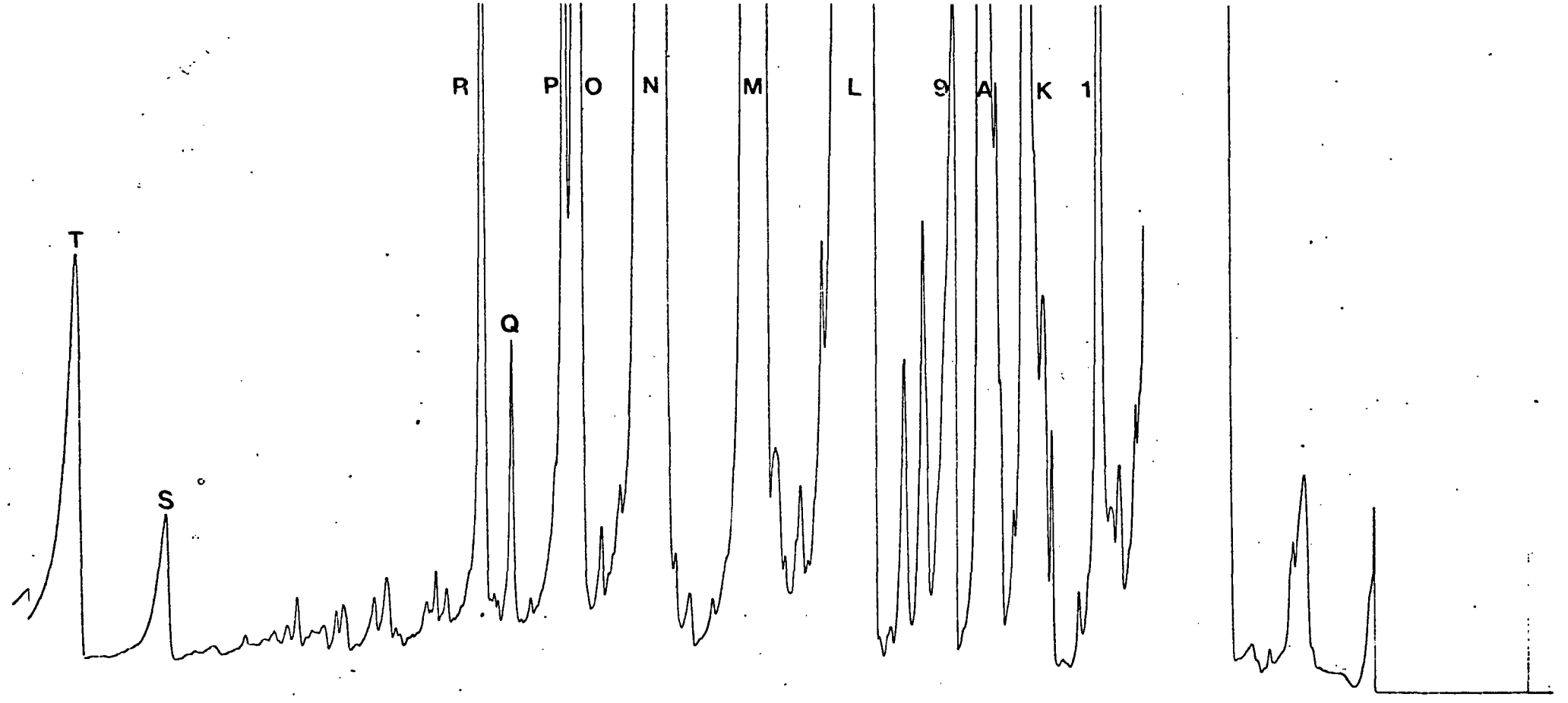

T
S
R
Q
P
O
N
M
L
K
1

FIGURE 19

COMPONENTS IN DIABETIC URINE

CHARACTERISTIC COMPONENTS OF DIABETIC URINES

CYCLOHEXANONE

$C_4 - C_5$ ALCOHOLS

OCTANOLS

PYRAZINES

FIGURE 20

A, B, AND C

DIABETICS ON PHENFORMIN (ORAL AGENT)

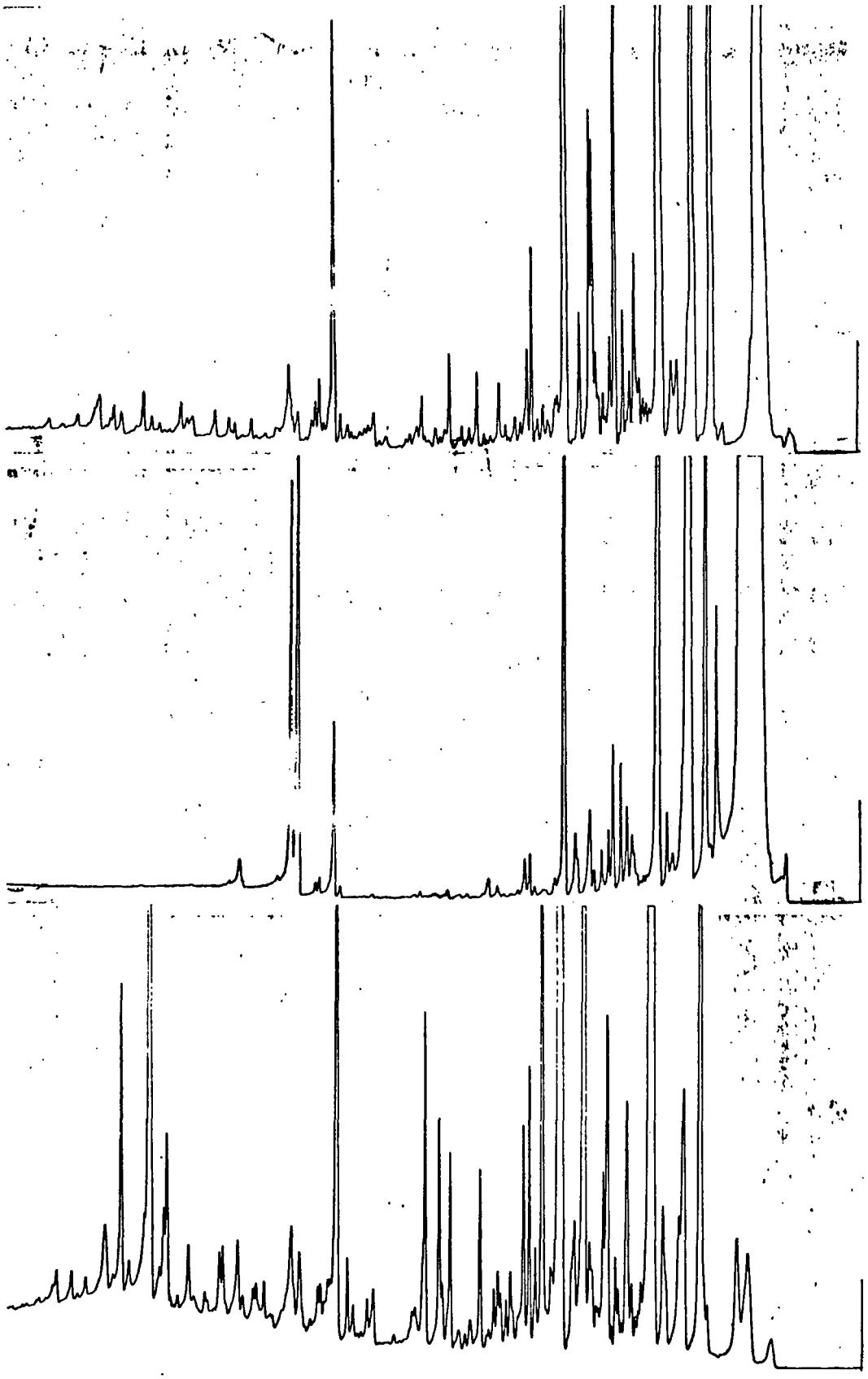


FIGURE 21

DIABETIC GROUP

A. DIABETIC ON INSULIN

B. DIABETIC ON PHENFORMIN AND CHLORPROPAMIDE (ORAL AGENTS)

C. DIABETIC (NO THERAPY)

D. DIABETIC ON CHLORPROPAMIDE (ORAL AGENT)

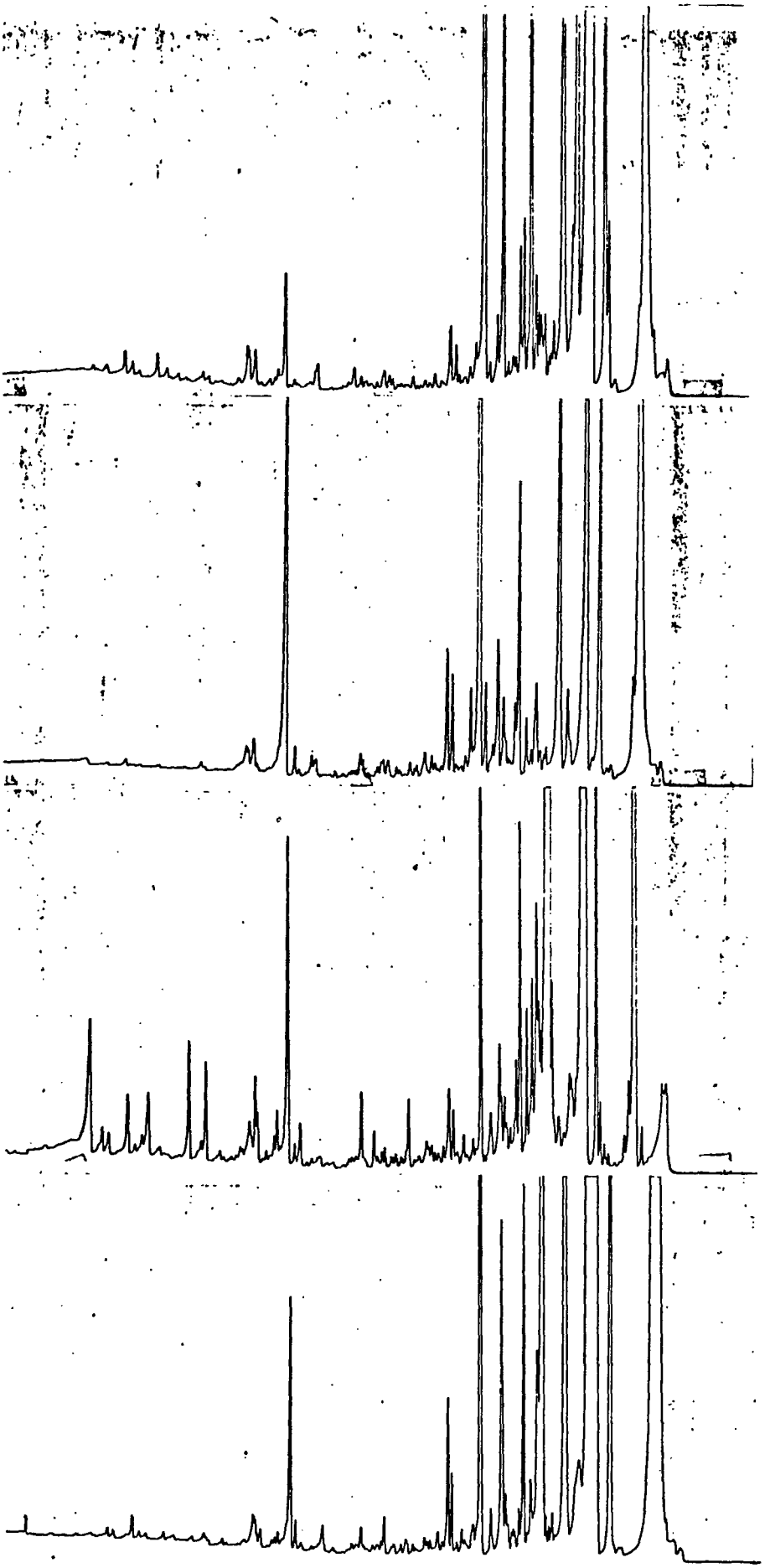


FIGURE 22

A. DIABETIC ON INSULIN

B. DIABETIC ON INSULIN

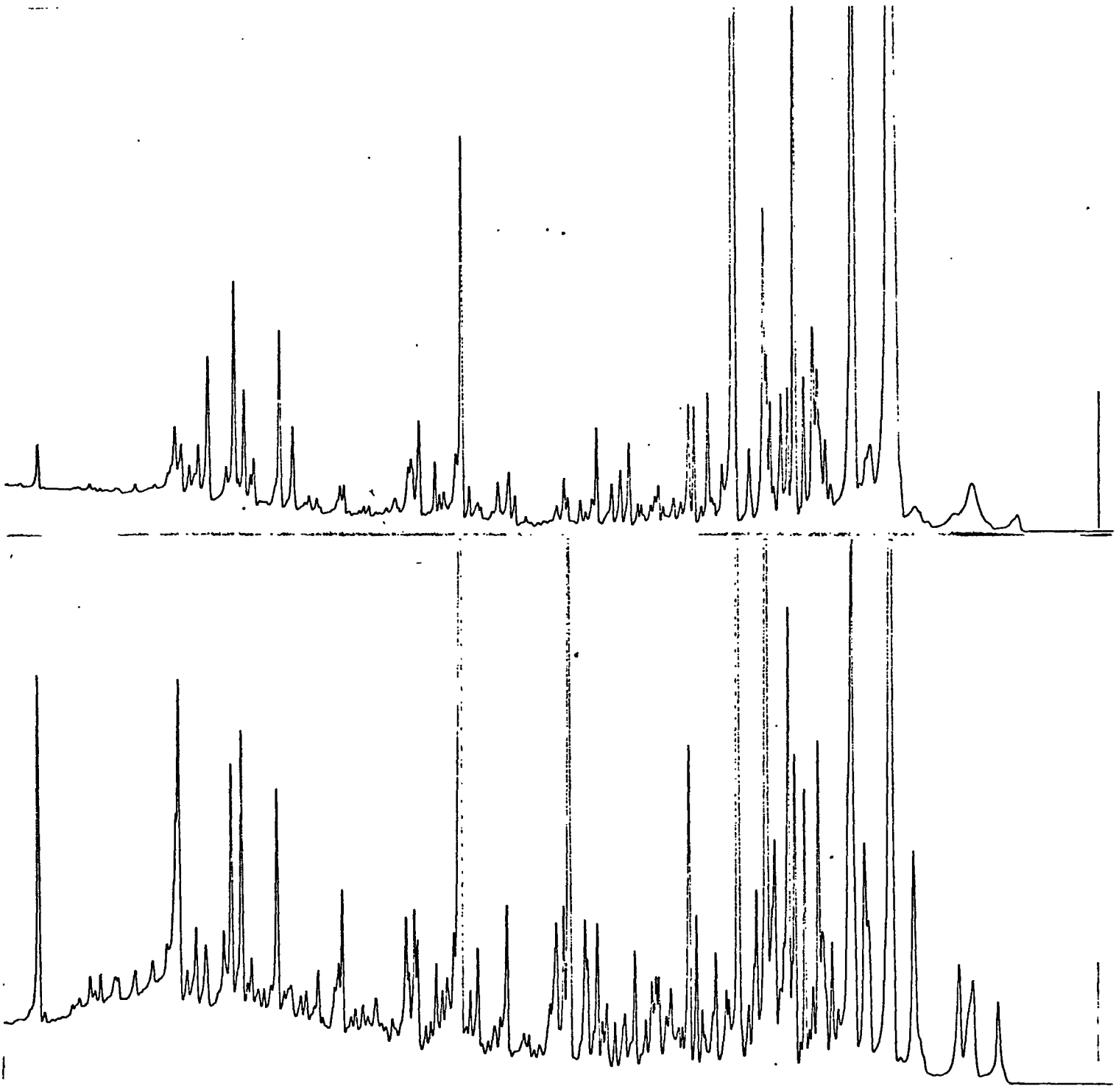


FIGURE 23

A. DIABETIC, ACROMEGALY, NO THERAPY

B. DIABETIC, ACROMEGALY, TREATED

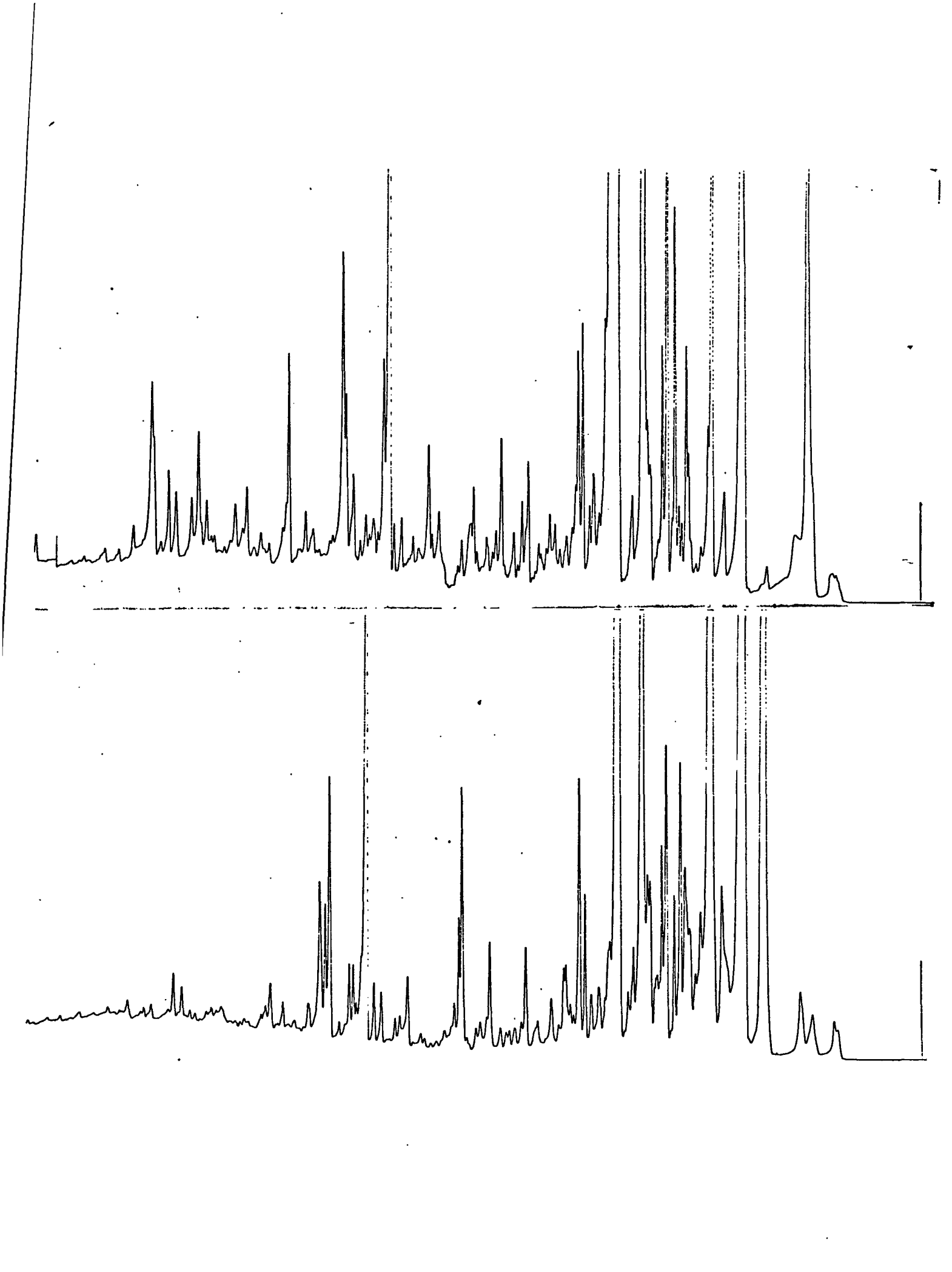


FIGURE 24

VOLATILES IN URINE OF TWO

MEMBERS OF THE SAME FAMILY

A. MOTHER- DIABETIC ON THERAPY

B. DAUGHTER- DIABETIC , NO THERAPY

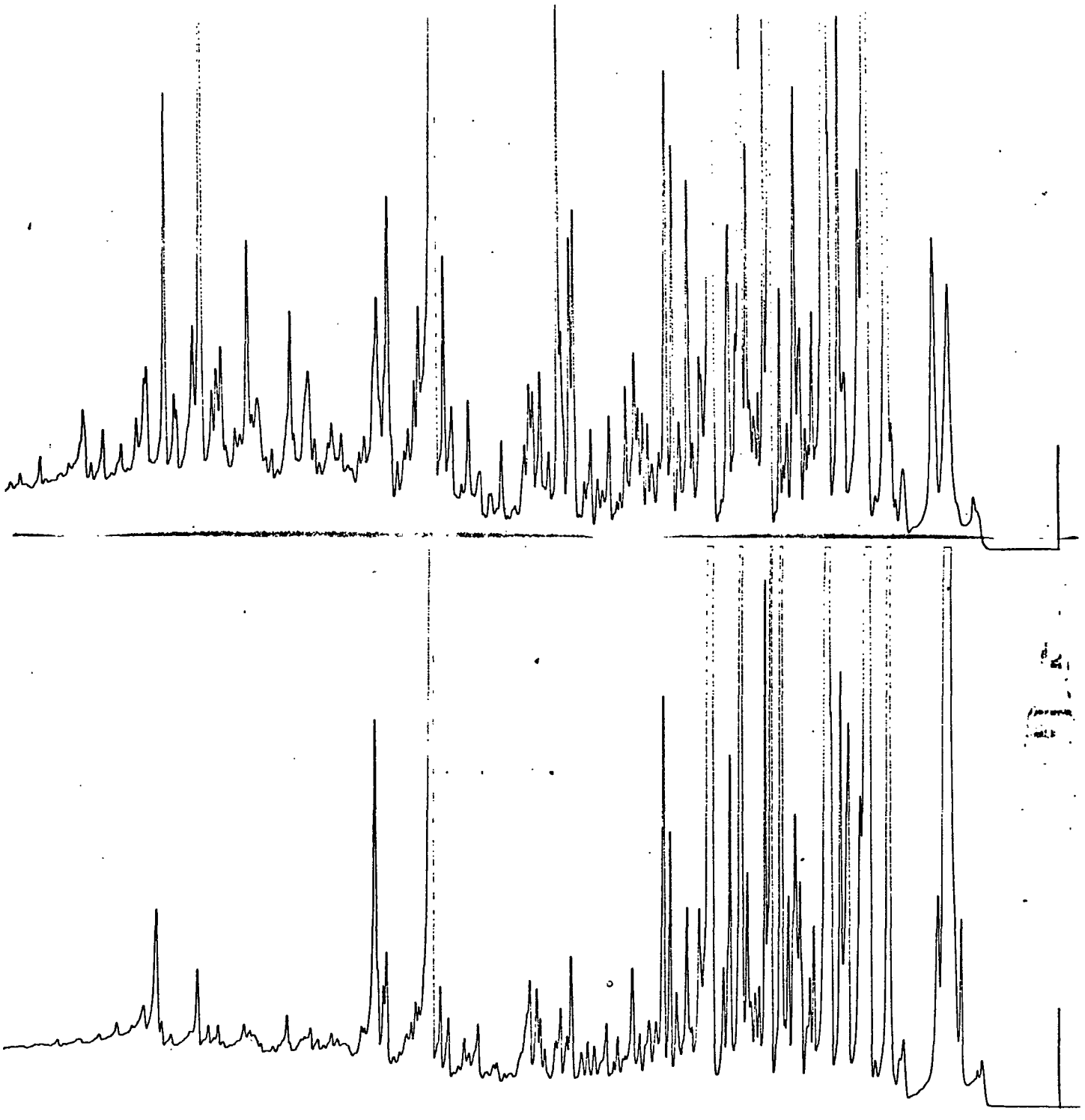


FIGURE 25

VOLATILES IN URINE OF TWO
MEMBERS OF THE SAME FAMILY

A. FATHER- DIABETIC ON THERAPY

B. SON - DIABETIC ON THERAPY

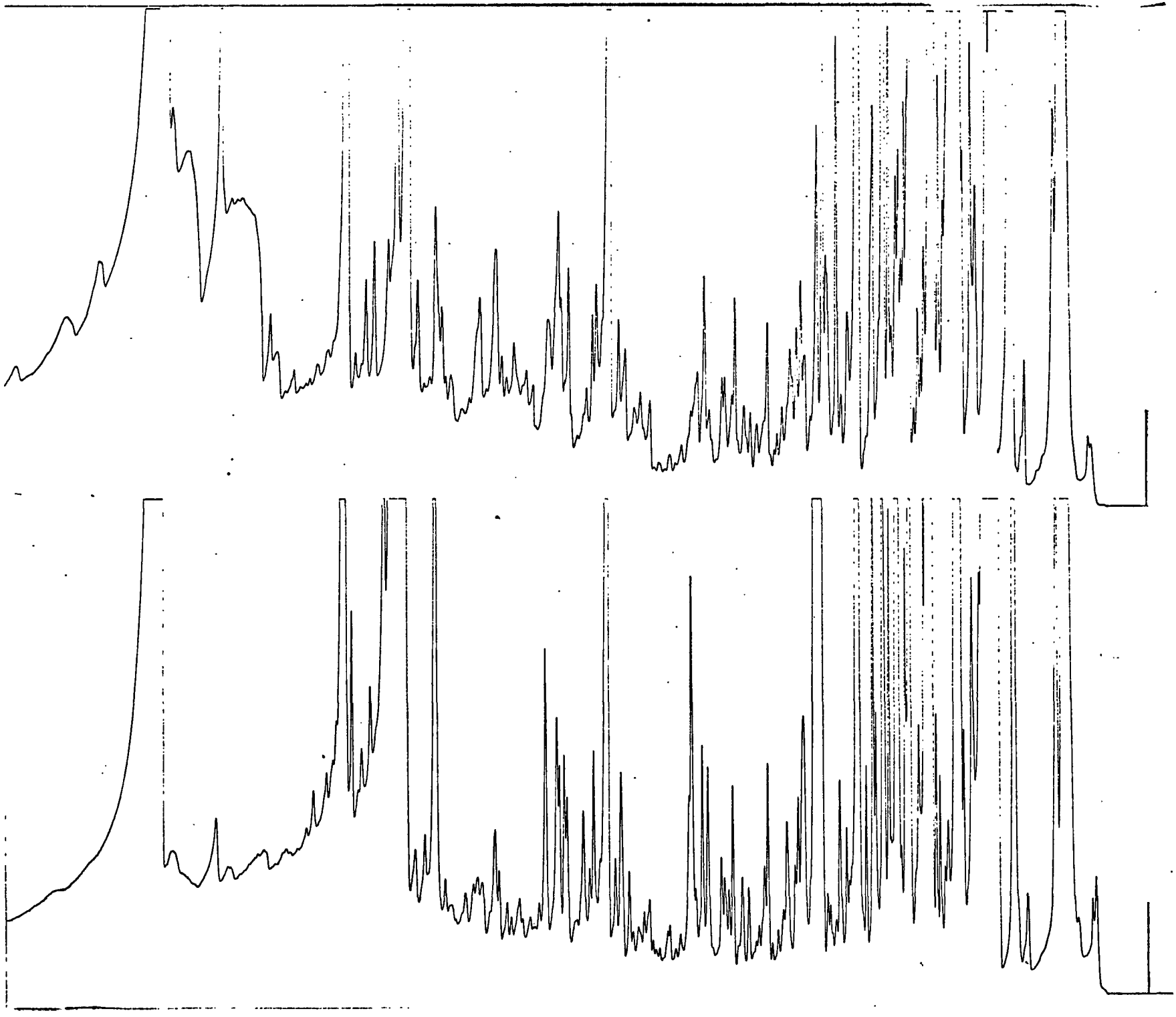


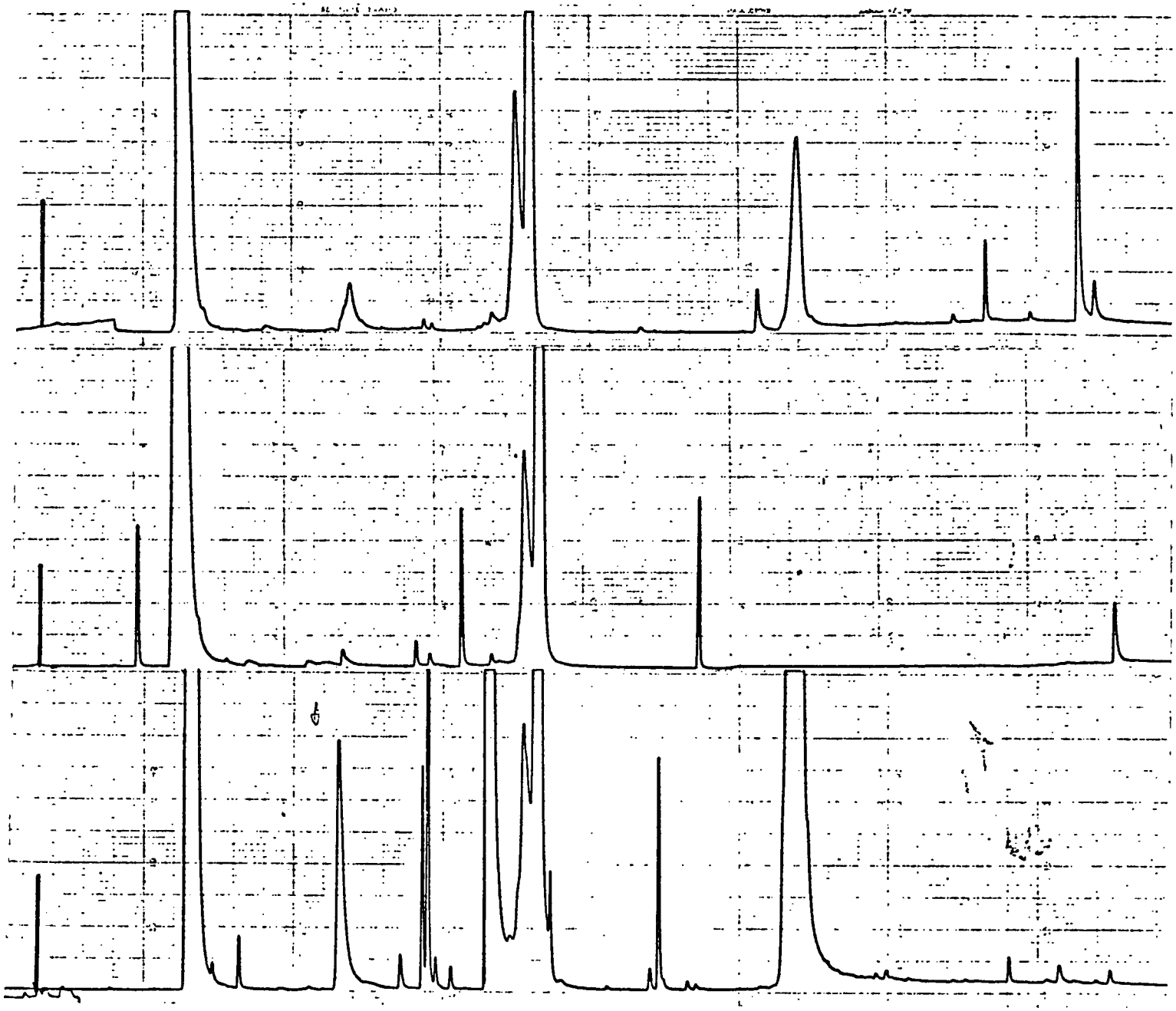
FIGURE 26

SULFUR PROFILES IN DIABETICS

A. DIABETIC ON INSULIN

B. DIABETIC ON ORAL AGENT

C. DIABETIC ON ORAL AGENT



her daughter. The most surprising and interesting thing was the remarkable similarities between each set of profiles.

As in the case of normal urine, selective detectors such as the flame photometric detector for sulfur was used to simplify the profiles of diabetic urine (Figure 26).

C. ORGANIC VOLATILES IN BLOOD.

This project would not have been complete without the investigation of volatiles in blood (serum or plasma). Clinical laboratories depend to great extents on blood as opposed to urine for diagnostic purposes; because, unlike urine in which the composition of volatile material depends on external factors such as, the time of day when the sample is collected and its total volume, blood maintains a more dependable equilibrium with respect to the compositon of compounds found in it.

There are, however, certain drawbacks to the analysis of organic volatiles in blood, most important of which is the limitations imposed on the amount of blood needed per analysis. About 10 ml of blood were needed to afford 3-4ml of serum for analysis and, an extra 10ml of blood for 3-4ml of plasma. The second drawback is that, in addition to the fact that blood contains very small concentrations of volatile material the small amount that had to be used made the amount of volatiles in blood even smaller. Unlike urine, the addition of

ammonium sulphate (salt) and heating of the sample to increase the amount of volatiles, resulted in precipitation or coagulation of the sample and was therefore avoided. The mere heating of the sample (serum or plasma) was sufficient. Table III lists the most important volatiles, found in serum. As expected, most of these substances also show up in urine. The profile of 55 ml of pooled serum is shown in Figure 27. The peak numbers refer to the compounds in Table III. The identification of the compounds was done by comparing the mass spectra with reference spectra and data published in the literature.

As is the case of urine, reproducibility was excellent but unlike urine, profiles of different healthy individuals possessed remarkable resemblance (Figure 28). It could be generally stated that the profiles of volatiles in serum are by far simpler than a corresponding profile of volatiles in urine. Although plasma showed very similar profiles to those of serum (Figure 29), serum was chosen for most of the samples.

The analysis of serum from diabetic patients showed great similarity (Figure 30), however, comparison of profiles from diabetics with those from normals showed certain differences. An advantage of serum over urine is the simplicity of the profiles, which would be of great facility in diagnosing the disease in question or any other disease.

FIGURE 27

VOLATILES IN 55 ml OF POOLED

SERUM FROM HEALTHY INDIVIDUALS

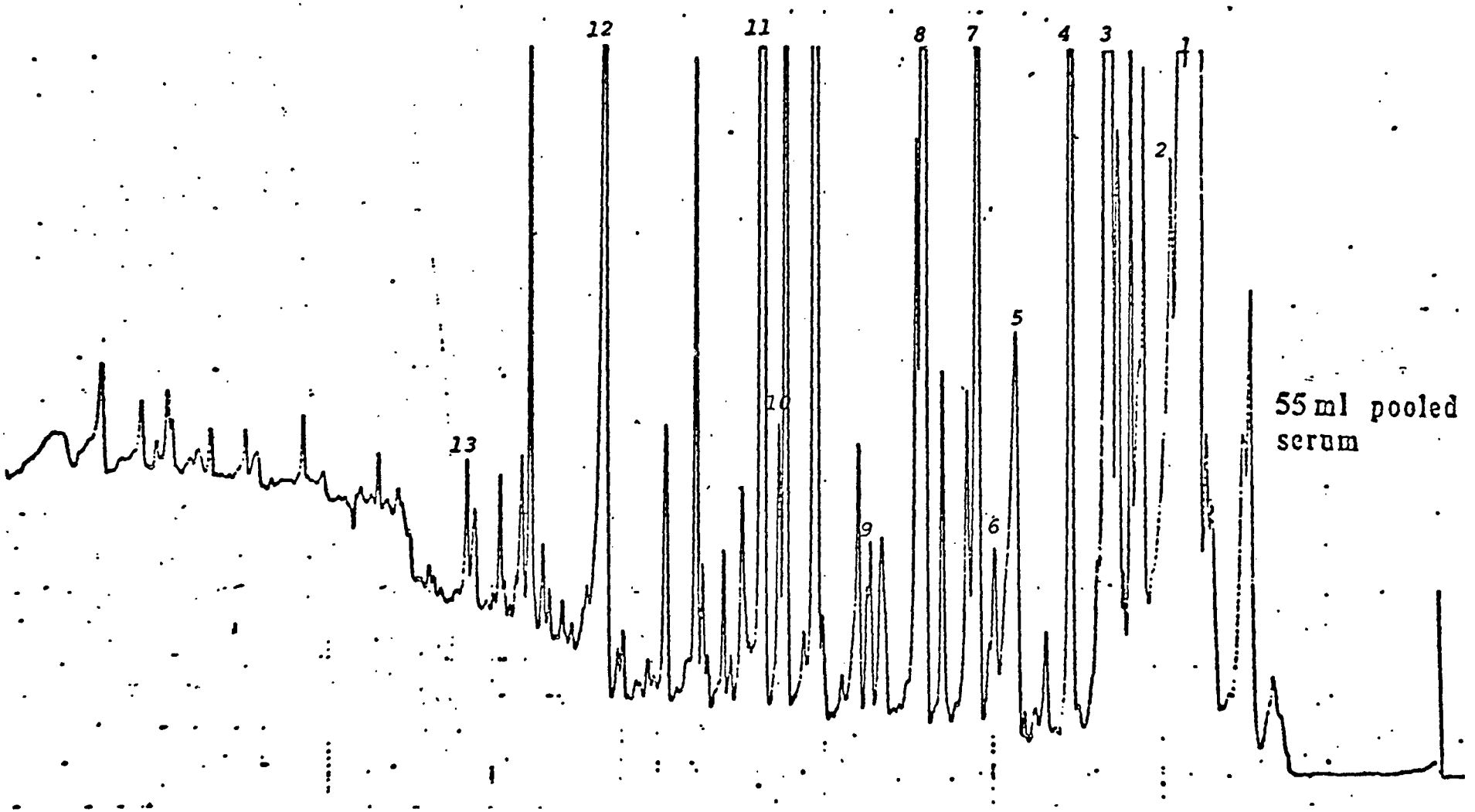

12
11
8
7
4
3
1
2
5
13
10
9
6
55 ml pooled serum

FIGURE 28

VOLATILES IN SERUM OF THREE HEALTHY INDIVIDUALS

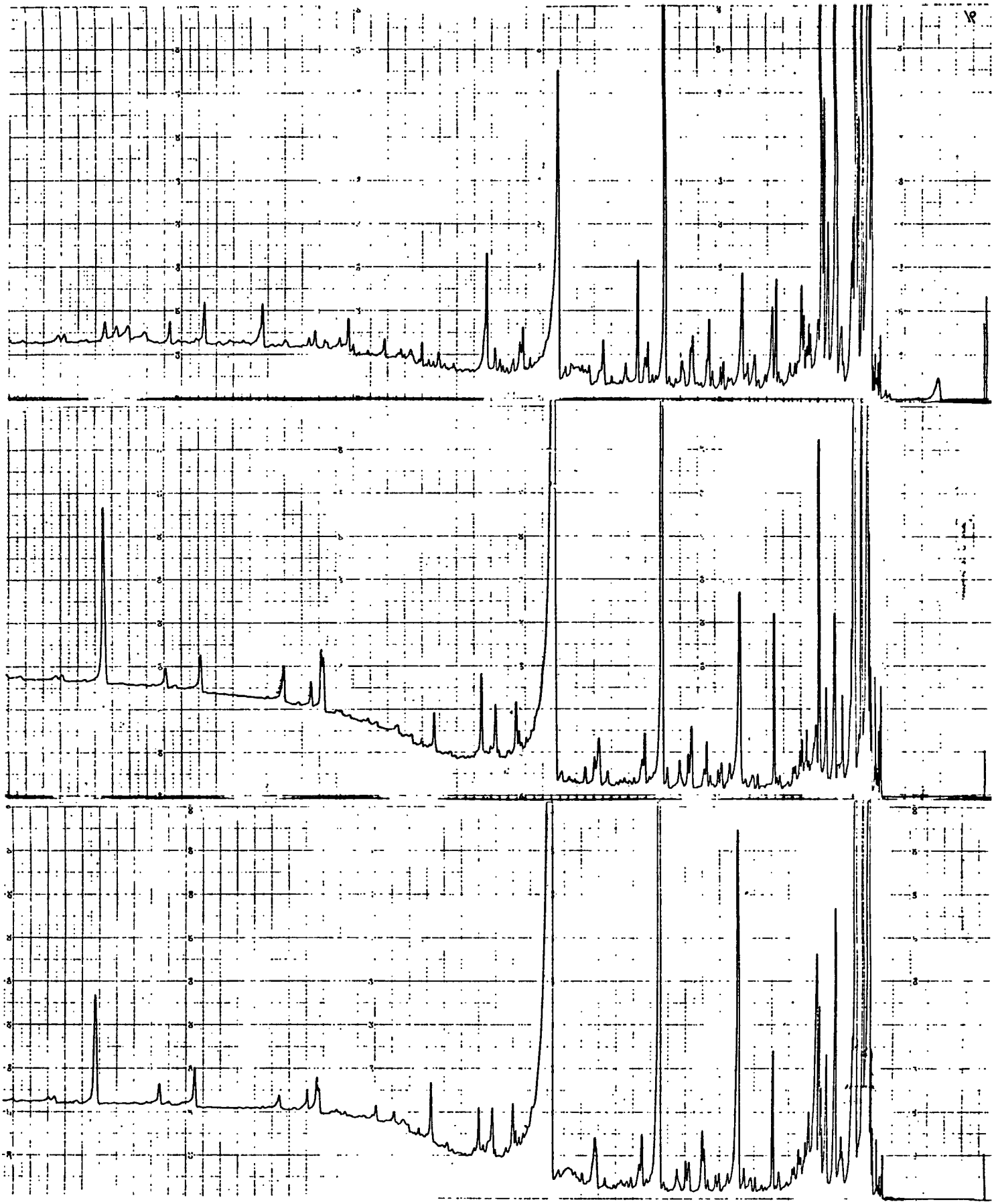


FIGURE 29

PLASMA AND SERUM VOLATILES

A. PLASMA VOLATILES

B. SERUM VOLATILES

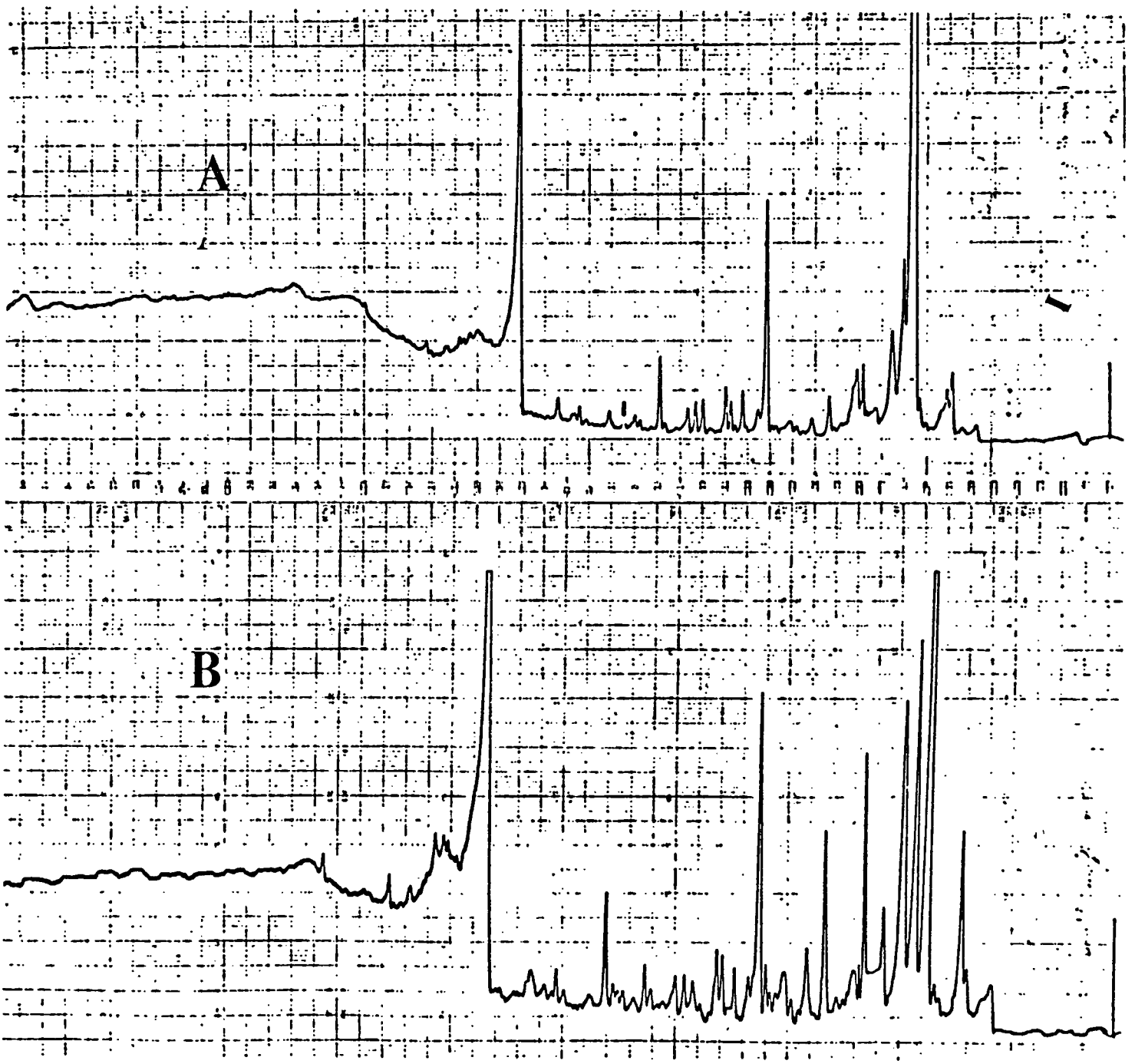

A
B

FIGURE 30

VOLATILES IN SERUM OF TWO DIABETICS

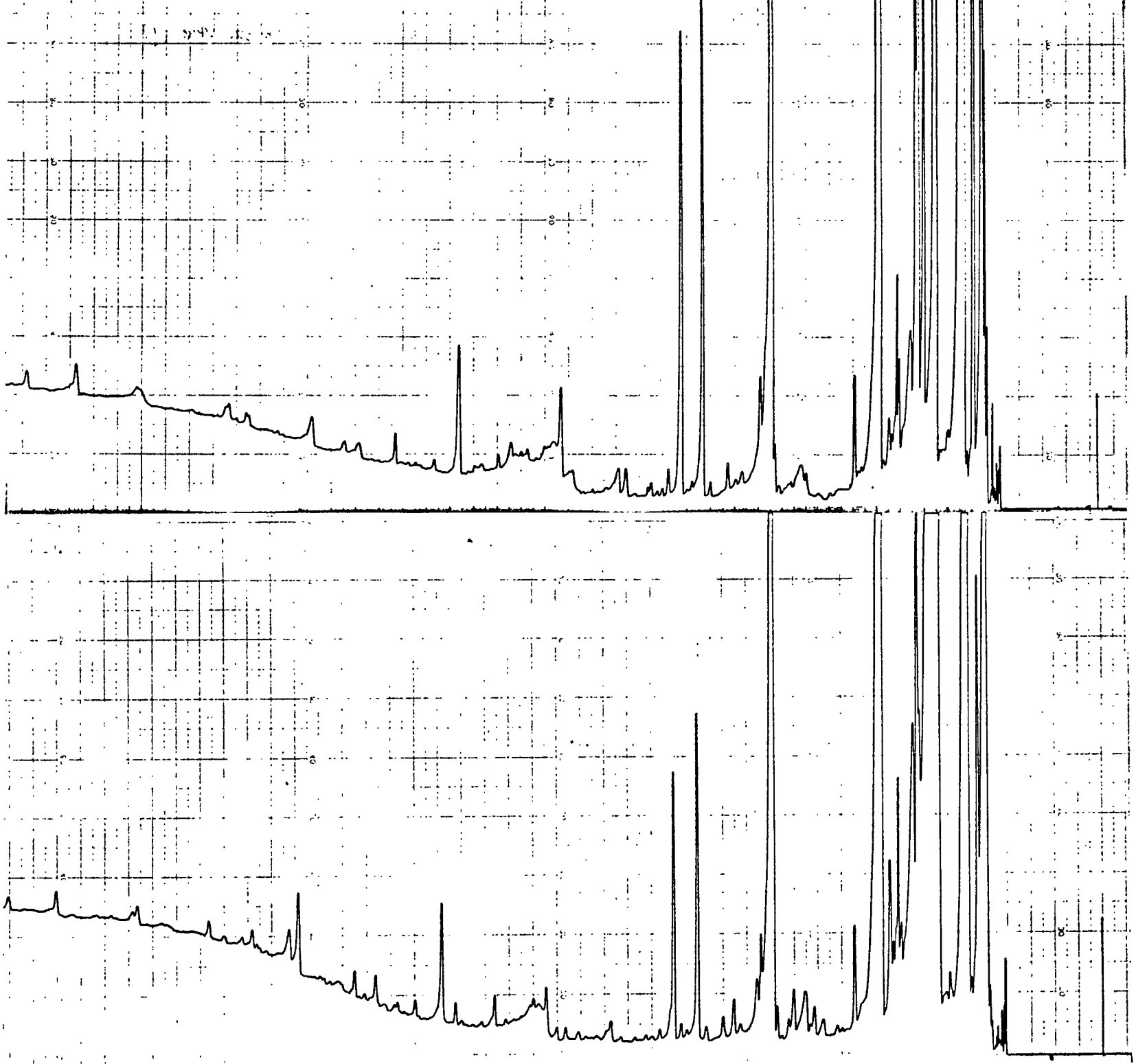


FIGURE 31

VOLATILES IN SERUM OF HEALTHY
AND DIABETIC INDIVIDUALS

A. HEALTHY

B. DIABETIC

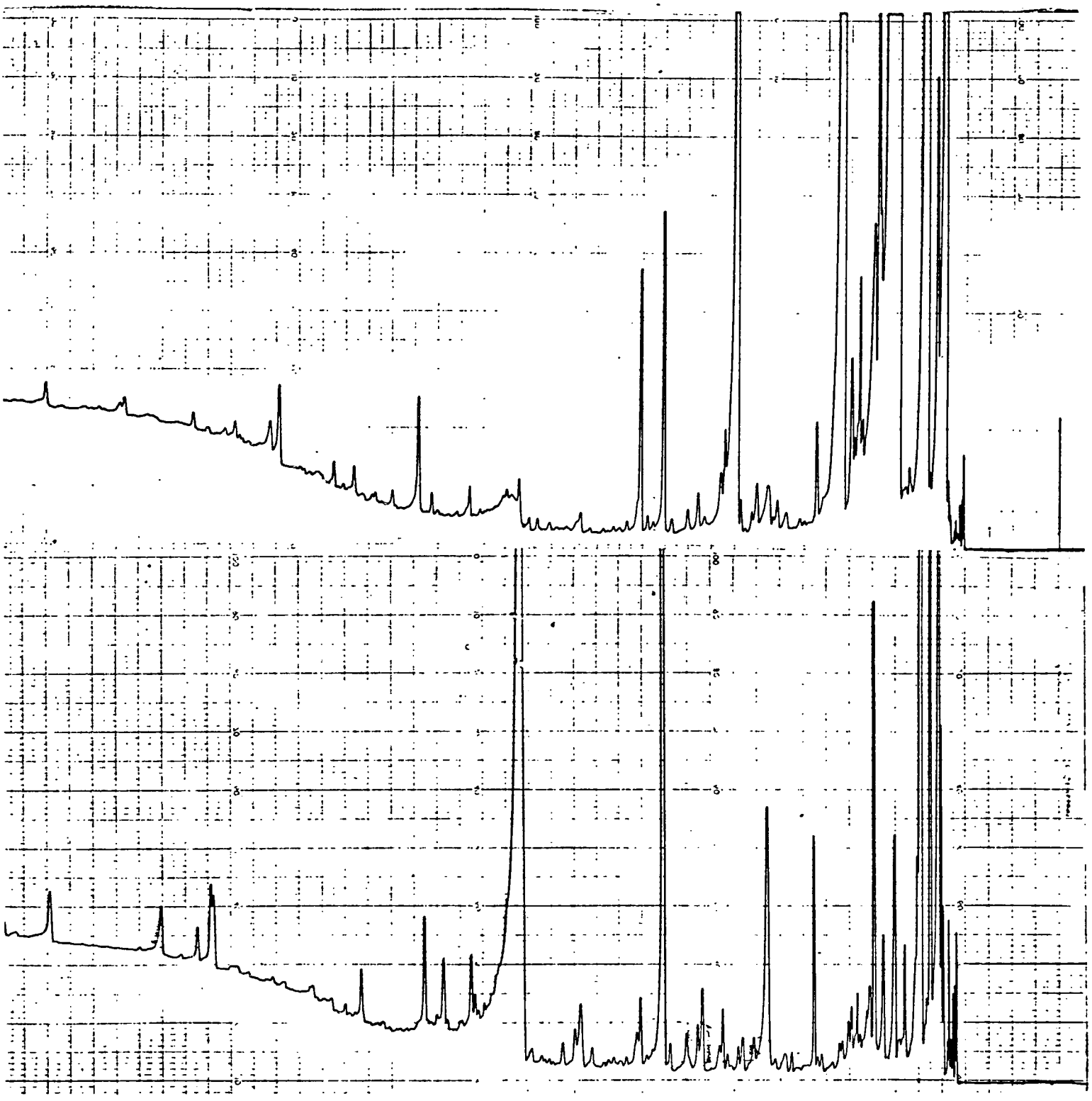


TABLE III

COMPOUNDS IN HUMAN SERUM

Peak Number	Compound	In urine
1	Ethanol	+
2	2-pentanone	+
3	4-methyl-2-pentanone	+
4	Toluene	?
5	Hexanol	+
6	3-penten-2-one	+
7	1-butanol	+
8	2-hexanol	+
9	2-hexanone	+
10	Cyclohexanone	+
11	Limonene	+
12	2-n-butoxyethanol	-
13	Benzaldehyde	+

CONCLUSION

The credibility of a new technique for concentrating and analyzing organic volatiles in biological fluids , with the purpose of diagnosing disease has been tested.

The investigation of volatiles in normal urine with the intention of establishing guidelines for comparison with pathological samples, has shown that profiles from different healthy individuals showed great similarities and, the only differences occuring are in the concentration of certain compounds. Some compounds such as dimethyl sulfone, 4-heptanone, and 2-pentanone invariably occur in high concentration while others such as pyrrole and allyl isothiocyanate are subject to more pronounced changes in concentration. These differences appear to be characteristic of the individual and not a result of variations in diet.

Comparison of profiles of subjects with diabetes mellitus with those of healthy individuals showed striking differences, this includes profiles obtained on FID as well as the more simplified sulfur profiles. However, in general the profiles were considerably less complex than those of normal subjects which made them easily recognizable. The characteristic components in diabetic urine could b classified into four groups: pyrazines, cyclohexanone, lower aliphatic alcohols, and compounds with mass spectrometric fragment m/e 112 (the octanols).

The results obtained from this work together with other data obtained in our laboratories using the same techniques, seem to under-

line the practicality of the method described for concentrating volatile organics in biological fluids and, the usefulness of high resolution gas chromatography and mass spectrometry in clinical chemistry and biochemistry.

BIBLIOGRAPHY

1. Garrod, A.E., "Inborn Errors of Metabolism," Henry Froude, London 1909.
2. Pauling, L., Science, 160, 265(1968).
3. Pauling, L., Robinson, A.B., Teranishi, R., and Cary, P., Proc. Nat. Acad. Sci., 68, 2374(1971).
4. Raab, W.P., Clin. Chem., 18, 5(1972).
5. Zielke, K., Okada, S., and O'brien, J.S., J. Lab. Clin. Med., 79, 164(1972).
6. Thomas, G.H., and Howell, R.R., Clin. Chim. Acta., 36, 99(1972).
7. Mandera-Orsini, F.M., and Volini, F.I., Ann. Clin. Lab. Sci., 2, 40(1972).
8. Leonard, C.A., Stand. Methods Clin. Chem., 7, 267(1972).
9. Horning, E.C., and Horning, M. G., Clin. Chem., 17, 802(1971).
10. Horning, E.C., and Horning, M.G., J. Chromatogr., 9, 192(1971).
11. Hutterer, F., Roboz, J., Sarkozi, L., Ruhig, A., and Baschin, P., Clin. Chem., 17, 789(1971).
12. Jellum, E., Stokke, O., and Eldjarn, L., Ibid., 18, 800(1972).
13. Waterbury, L.D., and Pearce, L.A., Ibid., 18, 258(1972).
14. Wilding, P., Rollason, J.G., and Robinson, D., Clin. Chim. Acta., 41, 375(1972).
15. Young, D.S., Epley, J.A., and Goldman, P., Clin. Chem., 17, 765(1971).
16. Cunningham, G.C., Crit. Rev. Clin. Lab. Sci., 2, 45(1971).
17. Davidson, R.G., and Rattazzi, M.C., Clin. Chem., 18, 179(1972).

18. Thomas, M., and Baldwin, G., J. Clin. Pathol., 24, 320(1971).

19. Burtis, C.A., and Warren, K.S., Clin. Chem., 14, 290(1968).

20. Dalgleish, C.E., Horning, E.C., Horning, M.G., Knox, K.L., and Yarger, K., Biochem. J., 101, 792(1966).

21. Fink, K., and Adams, W.S., Arch. Biochem. Biophys., 126, 27(1968).

22. Horning, E.C., and Horning, M.G., J. Chromatogr. Sci., 9, 129(1971).

23. Jolley, R.L., Warren, K.S., Scott, D.C., Jainchill, J.L., and Freeman, M.L., Amer. J. Clin. Pathol., 53, 793(1970).

24. Jolley, R.L., and Scott, D.C., Clin. Chem., 16, 687(1970).

25. Karoum, F., Ruthven, C.R.J., and Sandler, M., Clin. Chim. Acta., 20, 427(1968).

26. Scott, C.D., Jolley, R.L., Pitt, W.W., and Johnson, W.F., Amer. J. Clin.Pathol., 53, 701(1970).

27. Mrochek, J.E., Butts, W.C., Rainey, W.T. Jr., and Burtis, C.A., Clin. Chem., 17, 72(1971).

28. Zlatkis, A., Oro, J., and Kimball, A.P., Anal. Chem., 32, 162(1960).

29. Novotny, M., and Zlatkis, A., J. Chromatogr. Sci., 8, 346(1970).

30. Novotny, M., and Zlatkis, A., Chromatogr. Revs., 14, 1(1971).

31. Stillwell, W.G., Horning, E.C., Horning, M.G., Stillwell, R.N., and Zlatkis, A., J. Steroid Biochem., 3, 699(1972).

32. Jellum, E., Stokke, O., and Eldjarn, L., Anal. Chem., 45, 1099(1973).

33. Douglas, D.R., Ph.D. Dissertation, University of Houston, (1971).

34. Bengt, T.L., and Widmark, G., Acta.Pharm. Suecica., 6, 479(1969).

35. Teranishi, R., Mon, T.R., Robinson, A.B., Cary, P., and Pauling, L., Anal. Chem., 44, 18(1972).

36. Matsumoto, K.E., Partridge, D. H., Robinson, A.B., Pauling, L., Flath, R.A., Mon, T.R., and Teranishi, R., J. Chromatogr., 85, 31(1973).

37. Robinson, A.B., Partridge, D.H., Turner, M., Teranishi, R., and Pauling, L., Ibid., 85, 19(1973).

38. Pauling, L., Robinson, A.B., Teranishi, R., and Cary, P., Proc. Nat. Acad. Sci., 68, 2347(1971).

39. Zlatkis, A., Lichtenstein, H.A., and Tishbee, A., Chromatographia, 6, 67(1973).

40. Liebich, H.M., Koenig, W.A., and Bayer, E., J. Chromatogr. Sci., 8, 527(1970).

41. Zlatkis, A., and Liebich, H.M., Clin. Chem., 17, 592(1971).

42. Scaulan, R.A., Arnold, R.G., and Lindsay, R.C., J. Gas Chromatogr., 6, 372(1968).

43. Flath, R.A., Forrey, R.R., and Teranishi, R., J. Food Sci., 34, 382(1969).

44. Rushneck, D.R., J. Gas Chromatogr., 3, 319(1965).

45. Heins, J.T., Maarse, H., ten Noever de Brauw, M.C., and Weurman, C., J. Gas Chromatogr. 4, 395(1966).

46. Bartle, K.D., Bergstedt, L., Novotny, M., and Widmark, G., J. Chromatogr., 45, 256(1969).

47. Grob, K., and Grob, G., J. Chromatogr. Sci., 8, 635(1970).

48. Kirsten, W.J., and Mattsson, P.E., Anal. Lett., 4, 235(1971).

49. Dravnieks, A., Krotoszynski, B.K., Whitfield, J., O'Donnell, A., and Burgwald, T., Envi. Sci. Technol., 5, 1220(1971).

50. Dravnieks, A., and O'Donnell, A., J. Agr. Food Chem., 19, 1049(1971).

51. Novak, J., Vasak, V., and Janak, J., Anal. Chem., 37, 660(1965).

52. Dave, B.S., J. Chromatogr. Sci., 7, 389(1969).

53. van Wijk, R., Ibid., 8, 418(1970).

54. Bertsch, W., Ph.D. Dissertation, University of Houston, (1973).

55. Zlatkis, A., Personal Communications.

56. Bertsch, W., Chang, R.C. and Zlatkis, A., J. Chromatogr. Sci., Submitted for Publication.

57. Novotny, M., Lee, M.L., and Bartle, K.D., Anal. Chem., Submitted for Publication.

58. Zlatkis, A., Lichtenstein, H.A.,.Tishbee, A., Bertsch, W., Shunbo, F., and Liebich, H.M., J. Chromatogr. Sci., 11, 299(1973).

59. Zlatkis, A., Bertsch, W., Lichtenstein, H.A., Tishbee, A., Shunbo, F., Liebich, H.M., Coscia, A.M., and Fleischer, N., Anal. Chem., 45, 763(1973).

60. Bertsch, W., Shunbo, F., Chang, R.C., and Zlatkis, A., Chromatographia, in press.

61. Zlatkis, A., Principal Investigator, "Progress Report," Contract NAS 9-12093, December 1973.