A MICRO-EXTRACTION OF VOLATILE METABOLITES FROM BODY FLUIDS FOR ANALYSIS BY HIGH RESOLUTION GAS CHROMATOGRAPHY AND GC-MS

A Thesis

Presented to

the Faculty of the Department of Chemistry

College of Natural Sciences and Mathematics

University of Houston

In Partial Fulfillment

of the Requirement for the Degree

Master of Science

---

by

Michele Lee Stafford

May 1976

To my men,

Bob and Robby

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A MICRO-EXTRACTION OF VOLATILE METABOLITES FROM BODY FLUIDS FOR ANALYSIS BY HIGH RESOLUTION GAS CHROMATOGRAPHY AND GC-MS

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ABSTRACT

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A solvent extraction method for sampling of volatile metabolites from human biological fluids developed by Zlatkis and Andrawes (45) was tested using plasma, urine, breast milk, and amniotic fluid collected from postpartum females and neonate on day 1 or soon after, depending on the fluid. The technique requires only 100 µl plasma, 3 ml urine, 20 µl breast milk, and 500 µl amniotic fluid to be extracted with 500 µl, 1 ml, 100 µl, and 750 µl ether, respectively, after saturation of the fluid with ammonium carbonate. The centrifuge tube containing the mixture is vortexed, centrifuged, revortexed, and centrifuged. The supernant fraction is distributed by a tubular syringe equipped with a 5" 22g needle onto glass wool contained in a tube that can be fitted directly into the injection port. After stripping the ether from the glass wool with helium, the tube is put into the inlet port. The volatile compounds are desorbed at 240°C into a stainless steel pre-column trap submerged into liquid nitrogen. After 15 minutes, gas chromatographic analysis is begun. The procedure is rapid, reproducible, requires only small volumes of biological fluids; thus, its use as a diagnostic test for pathological disorders is promising.

CHAPTER I

INTRODUCTION

INTRODUCTION

For the past twenty years gas chromatography has become increasingly important as an analytical method for mixture separation. The investigation of low molecular weight volatiles was initiated by scientists in a program designed to identify compounds responsible for flavor and spoilage. However, it was not until the publication by Zlatkis (41) that human volatile metabolites were given full consideration.

Some of the early gas chromatographic studies involving volatile metabolites of human urine and plasma were performed by forensic chemists and were used for detection of ethanol and other light alcohols and ketones. Bonnichsen and Linturi (10), for example, used Carbowax 1500 for the analysis of 2 µl urine by direct injection. They reported good sensitivity, but detected only ethanol, methanol, acetone, and acetaldehyde.

As early as 1963, Loney and co-workers (23) analyzed the volatiles in body fluids of cows maintained on control diets. The headspace volatiles of urine, blood, and milk were analyzed by G.C. They detected a few alcohols and ketones and found that the concentration increased in the order; milk, blood, urine. They published additional studies in 1966 (5). Finally, Bassette and Ward (6) evaluated the merit of headspace gas chromatographic analysis of biological fluids, reporting it to be reliable qualitatively and quantitatively. They were quick to point out, however, that the headspace procedure still had room for modification.

With the advent of high resolution open-tubular columns, volatile analysis by gas chromatography became more sophisticated. Teranishi and colleagues (35) utilized flavor techniques for breath and urine. The procedure involved collection of (1) exhaled breath, or (2) volatiles swept from urine heated at 80°C with helium onto a precolumn submerged in an isopropyl alcohol dry ice bath. The chromatograms resulting from analysis of 200 ml urine contained a large number of constituents in high concentration.

Further advances were made in G.C. headspace volatile analysis when Zlatkis (42) developed a new procedure for sample collection. A porous polymer adsorbent (Tenax) contained in a pyrex tube which could be fitted directly into the injection port was used to trap urine headspace volatiles, exhaled breath, and volatile air organics. The procedure is reproducible and reduced the total analysis time to less than 4.5 hours. Later publications by Zlatkis used this method for comparison of volatiles in urine collected from control and diabetic subjects. Significant differences were observed (43). Zlatkis also used the method for gas chromatographic analysis of headspace volatile from 10-15 ml plasma and serum. Politzer et al (26) used the porous polymer for analyzing headspace volatiles of homogenized lung, brain, and liver.

Novotny and co-workers (24) used the porous polymer adsorbent to trap body fluid volatiles for gas chromatographic analysis. But the procedure was modified to include glass capillary columns, to show that some of the less volatile constitutents can be eluted by using thin-film stationary phases. Headspace volatiles were collected onto Tenax by

sweeping with helium a 100 ml aliquot of 24 hour urine, 15 ml serum, or 11 ml cerebrospinal fluid heated to 100°C. Glass capillary columns are advantageous due to inertness and ability to control film thickness, as evidenced by Novotny's work.

Liquid-liquid extraction of volatiles appeared as early as 1965, again in the food industry. A series of three papers by Rodin et al, Silverstein et al, and Rodin et al (27,31,28) reported analysis of an ether extract of 24 gallons of pineapple juice. After concentration by distillation under reduced pressure, analysis was carried out by G.C. Flath and Forrey (15) used a liquid-liquid extraction of pineapple volatiles using isopentane and sodium chloride, but reduced the volume of juice to 9 gallons. They reported 21 compounds not previously reported, however, the extraction time was 24 hours.

Zlatkis and Liebich were first to extract human urinary volatile compounds (41). The method involved ether extraction (24 hours) of 450 ml of a 24 hour urine sample with subsequent concentration by distillation under reduced pressure with further concentration to a final volume of 15 µl by allowing the sample to sit open on the bench overnight. They reported reproducible profiles from samples collected on different days from one individual, and also qualitatively similar profiles from different subjects.

Stoner et al (34) developed a liquid-liquid extraction procedure for plasma specimens of patients with uremic syndrome. The method requires 15 ml plasma which is vortexed with 20 ml of ether in a 50 ml centrifuge tube three times, and concentration in a fractionation column of their design. They reported the method to be "quick, efficient, and routinely applicable".

The solvent extraction technique for biological fluids was further modified. Zlatkis and Andrawes (45) used 100 µl serum extracted with 1 ml ether and 0.05 g ammonium carbonate vortexed and centrifuged. This procedure is a modification of the technique used by Horning (20) in the analysis of drugs in biological fluids. The ether was put onto glass wool, stripped with helium, and the volatiles desorbed into a precolumn trap submerged in liquid nitrogen. After a 10 minute desorption time, the precolumn was put in series with the G.C. column for high resolution analysis. The technique is reproducible, rapid, requires small sample volumes, and therefore is desirable as a routine clinical diagnostic test for pathological disorders which exhibit significant changes in volatile profile when compared to screened normals.

CHAPTER II

STATEMENT OF PROBLEM

STATEMENT AND SIGNIFICANCE OF PROBLEM

Until recently, the sampling of volatiles for analysis by gas chromatography has been lengthy and required large sample volumes. Volatile profiles have been proposed as possible diagnostic tools for pathological disorders (41,42,22). However, large sample size requirements and prolonged analysis time of techniques developed to date have prevented its routine use.

Headspace techniques require large volumes of sample and sweeping for collection into a precolumn, as used in food analysis (23,5), or onto porous polymer absorbent for subsequent precolumn trapping which takes at least one hour, so that total analysis time is four hours or better. These methods of headspace volatile analysis have also been applied to biological fluids and tissues (35,42,43,44,24,25).

Solvent extraction of volatiles have also been employed in the food industry (27,31,28,15) and for human biological metabolite analysis (41,34). However, until a recent work by Zlatkis and Andrawes, human fluid specimens for extraction required 450 ml of a 24 hour urine and 15 ml of plasma to obtain respectable chromatograms.

This work is a continuation of Zlatkis and Andrawes' using a micro-extraction method of volatile compounds to be analyzed by high resolution gas chromatography. The human biological fluids studied are plasma, urine, breast milk, and amniotic fluid, all collected from

postpartum females and neonates within 24 hours of or soon after birth, depending on the fluid type.

The extraction requires as little as 20 µl breast milk, 100 µl plasma, 500 µl amniotic fluid, and 3 ml urine, using for the largest volume only one ml ether solvent. The extraction is facilitated by salting out the volatiles with enough ammonium carbonate to saturate the biological aqueous phase. The ether extract is transferred onto glass wool contained in a tube which fits directly into the injection port. After stripping of the ether with helium, the compounds are desorbed at 240^{o}C onto a precolumn submerged in liquid nitrogen, and after 15 minutes desorption period, gas chromatographic analysis begun.

The micro-extraction technique for human biological fluid volatile analysis requires only small volumes of fluids, which are usually attainable, is reproducible, and cuts analysis time to three hours total, at most. Its use as a rapid diagnostic test for pathological disorders is promising.

CHAPTER III

EXPERIMENTAL

EXPERIMENTAL

The availability of biological samples collected from mother-infant pairs narrowed this study to the specificity of those body fluids collected on or soon after the day of birth. The fluids analyzed are maternal plasma, urine, breast milk, and amniotic fluid and, where available, a complete set of an individual mother, and neonatal plasma and urine. The samples were kept at -14°C until analyzed.

Due to the variable concentration of volatile metabolites, an isolation procedure had to be developed for each type of body fluid, and still conform to the micro-extraction procedure. Before analysis, the samples were allowed to thaw at room temperature just to the point that no ice crystals could be detected. At this point, no pressure build up from escaping volatiles occurs, so that loss of volatile compounds was minimal.

Plasma (100 µl) is transferred to 12 ml teflon-lined screw capped centrifuge tubes and 0.5 ml glass distilled ether, which had been refluxed for 2 hours over lithium aluminum hydride, is added, after saturation of the plasma layer with ammonium carbonate. It is vortexed for 20 seconds or until a jell formed, then centrifuged for 5 minutes, vortexed again, and re-centrifuged for 10 minutes. Very often the protein layer is not completely separated from the ether layer, but can be eliminated by popping the tube and centrifuging a final time for 5 minutes.

Three ml aliquot of a 24 hour urine sample is required for analysis by gas chromatography. After transferring to a 12 ml teflon-lined screw-capped centrifuge tube, 1 ml of ether is added and enough ammonium carbonate is added to saturate the urine. It is vortexed for 20 seconds or until a jell forms, and centrifuged for 5 minutes, vortexed and centrifuged an additional 10 minutes. The protein is eliminated as above if it does not completely separate with the second centrifugation.

For amniotic fluids, a 500 µl sample is employed as described for plasma, and after saturating with ammonium carbonate 0.75 ml of ether is added. The remainder of the procedure is followed as described for plasmas and urines. In the case of the amniotic fluids, none or very little emulsion remained after the second centrifugation.

Because of the concentration of volatile metabolites in breast milk, only 20 µl are needed for analysis; 100 µl ether was used for extraction by the same procedure described for plasma, urine, and amniotic fluid. Due to the small sample size, no protein layer was formed with breast milk using this procedure.

Following centrifugation, the tube is put into a freezer at -14°C for at least one-half hour to assure no loss of volatiles. After cooling, the ether layer is transferred to a 2.5 ml centrifuge tube equipped with a glass ground top, and stored at -14°C until analyzed.

For analysis by gas chromatography, the ether extract is transferred to a concentration tube. The tube is constructed from 1/4 inch outer diameter Corning pyrex glass cut to 15 mm in length and one end firepolished until it closes to about 1/8 inch. This size tube fits

directly into the injection port of the gas chromatograph used. The concentration tube is filled with 0.5 g pyrex glass wool and conditioned in a heating block at 325°C by flushing with helium (20 cc/min) for one hour to assure that no volatile compounds remain. Glass wool was employed instead of a porous polymer such as Tenax. Even after conditioning of the tube, the ether washed volatile compounds from the Tenax, causing contaminant peaks in the chromatogram. The glass wool in an 0.5 g quantity is sufficient to hold as much as 1.0 ml of ether extract, which is the largest amount used in this work. The transference of the sample is carried out with a 1.0 ml B-D tubular syringe equipped with a 5 inch 22 gauge needle, which made it possible to distribute the sample along the full length of the glass wool.

After the ether extract is put onto the glass wool in the concentration tube, the ether is eliminated by sweeping the tube with helium at a flow of 20 cc/minute 20 psig. The time required for each type of sample varied because of the different volumes of ether required for extraction of the four body fluids. Plasma extract is swept for 5 minutes, while the amniotic fluid extract required 7 minutes for stripping. Urine extracts took 10 minutes, and breast milk, 1-2 minutes. Due to the cooling effect of the evaporating ether, moisture condenses on the outside of the tube and is removed by wiping the entire length of the tube with microwipes.

After stripping of the solvent, the concentration tube is put into the injection port of the gas chromatograph and held flush against the outlet of the port by a spring. The septum in the nut on the inlet

of the port is replaced with a teflon liner. The tube is heated at 240°C with a flow rate of 30 cc/minute of helium flow. This condition sweeps the volatile metabolites from the glass wool into a precolumn trap submerged in liquid nitrogen. The precolumn trap is made of 12" of 1/16" uncoated stainless steel. A 1/16" stainless steel precolumn is chosen because at -196°C during the trapping, a tube of smaller diameter very often plugs. To eliminate the plug, the trap has to be removed from the liquid nitrogen and warmed between the fingers; this often causes a loss of sample. The precolumn is not coated for the fact that it is 1/16" instead of gas chromatographic column material and diameter. Coating would cause a loss in resolution due to difference in diameter and, therefore, a difference in helium flow. The sample trapping time is 15 minutes.

The gas chromatograph used is a Tracor 550 with a modified injection port and flame ionization detector. The column is a 100 m, 0.5 mm i.d. Nickel column coated with Emulphor ON-870 by the procedure described by Bertsch (8). The carrier gas is helium, and the flow rate is 30 cc/minute at the injector port. The detector gases are hydrogen 37 cc/minute, 20 psig; air 100 cc/minute, 40 psig; and nitrogen make-up gas 10 cc/minute, 20 psig.

The same attenuation of 1 x 10 is used for every analysis; thus facilitating direct comparison of the chromatograms. After the 15 minute trapping period, the liquid nitrogen is removed, and the column temperature raised to 70°C for 10 minutes. For the analysis the column was programmed at 2°C/minute to 150°C and then held at 150°C for 50 minutes.

The mass spectrometric analysis was carried out on an LKB 9000. A source temperature of 190°C was set with the Variac, however, the filament heated the source to 210°C during the analysis. The separators were kept at 150°C. A pressure of 10^{-6} torr is on the source and 10^{-5} on the separators. The samples are prepared as described above for the respective body fluid, except that larger amounts are required. The volumes of ether extract required are 2.5 ml for urine, 1.5 ml for plasma, 1.5 ml for amniotic fluid, and 1-1.5 ml for breast milk, which corresponds to the extracting of 7.5 ml urine, 0.3 ml plasma, 1.0 ml amniotic fluid, and 0.3 ml breast milk. By comparing the total ionization current output to the gas chromatogram, an estimated 75-90% of the volatile metabolites are lost to the vacuum system upon passing through the separators to the source. Even with this high sample loss, discernable mass spectra was obtained since only nanogram amounts are required.

The actual procedure employed for the GC-MS analysis is basically the same as for gas chromatography. A heating block and concentration tube described by Zlatkis (42) is placed in series with a 12" x 1/16" stainless steel precolumn and the gas chromatographic column. Between the precolumn and the G.C. column, a teflon micro-needle valve is placed so that the precolumn and flash heating block can be open to atmosphere for insertion of the concentration tube and still allow the separator vacuum to be pumping on the G.C. column. This configuration saves a tremendous amount of time and the resolution is preserved. If the needle valve is omitted, each time the concentration tube is

inserted into the heating block the separator must be vented to the atmosphere. After desorption and trapping of the volatiles, the entire system must be pumped down to operating status costing 10-15 minutes, which results in loss of the light ends and peak broading.

CHAPTER IV

RESULTS AND DISCUSSION

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The original objective of this work was to compare the profiles of volatile metabolites of body fluids among individuals. Because of the availability of plasma, urine, breast milk, and amniotic fluid, the volatile profiles of mother-infant pairs were investigated. The micro-extraction procedure was to be tested out on these biological fluids in hopes that with success it could be used in the future as a diagnostic procedure.

Plasma Volatiles

A unique profile of volatile compounds was observed in the first few samples of postpartum plasma that were analyzed, which differed from the profiles of normal adults which had been published by Zlatkis et al (45). As can be seen in Figure 1, peaks at 23 and 40 minutes retention time are elevated.

The first observations suggested a contaminant in the analysis. With this in mind, a gas chromatographic blank was run, which includes gas chromatograph, fuel lines, tanks, fuel gases, carrier gas, and concentration tube with 0.5 g of glass wool. Figure 2 shows quite clearly that the G.C. system could be eliminated as a source of any contaminants in the chromatogram, for the blank is the attenuation 1 x 10, as each sample chromatogram.

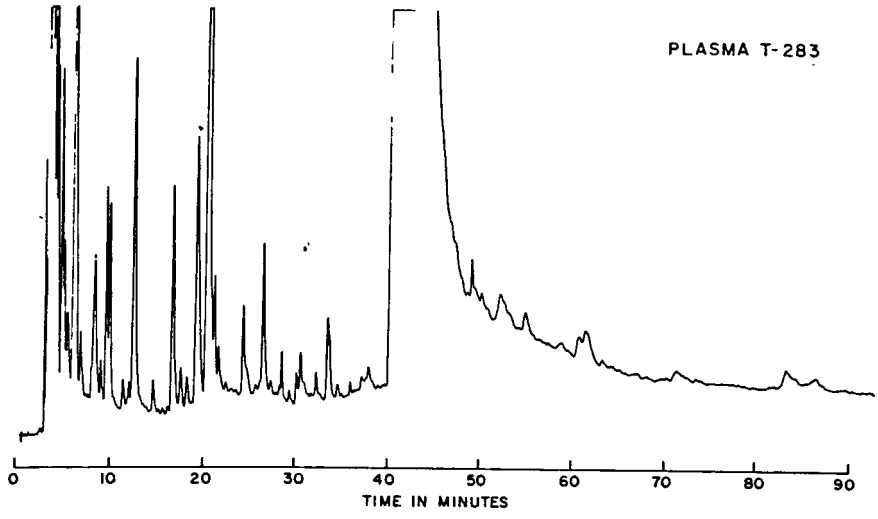
FIGURE 1

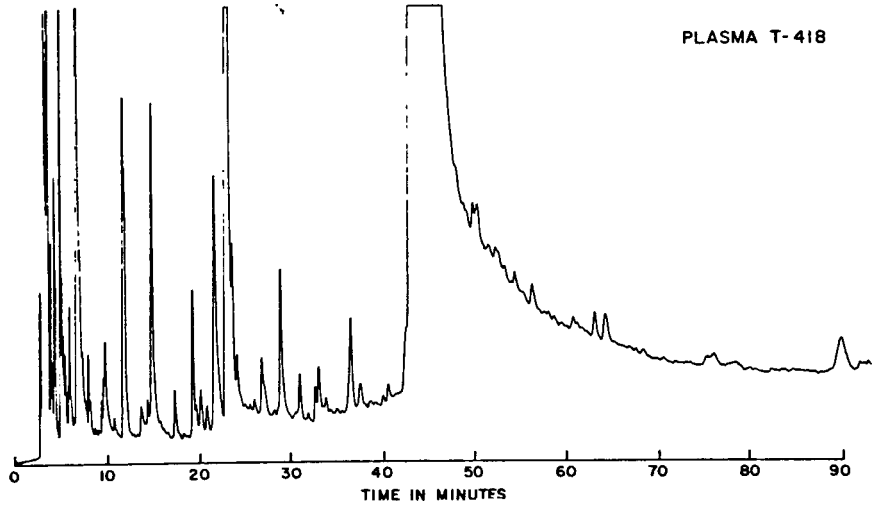
Volatile Plasma Metabolites of Three Individual Postpartum Females.

Samples Collected on Day of Birth.

Gas Chromatographic Conditions as specified in Experimental

Section of Text.


PLASMA T-283
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


PLASMA T-418
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

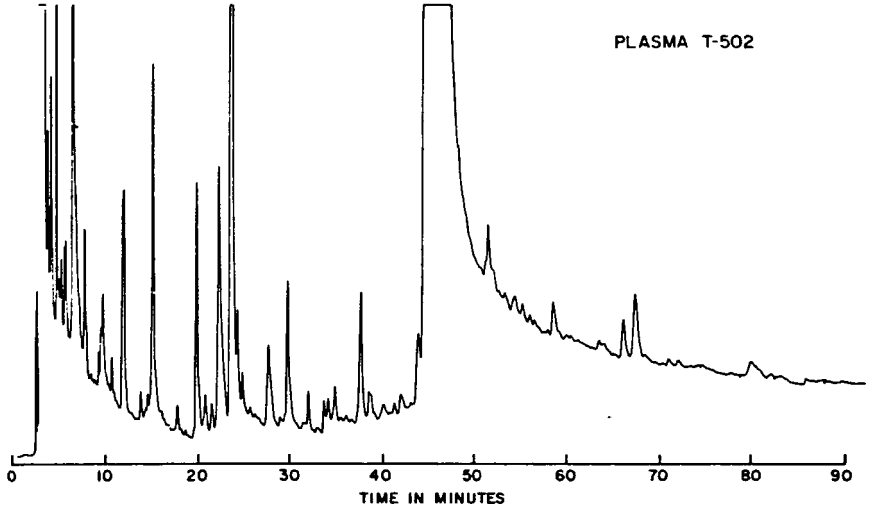

PLASMA T-502
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

FIGURE 2

Blanks of Gas Chromatograph:

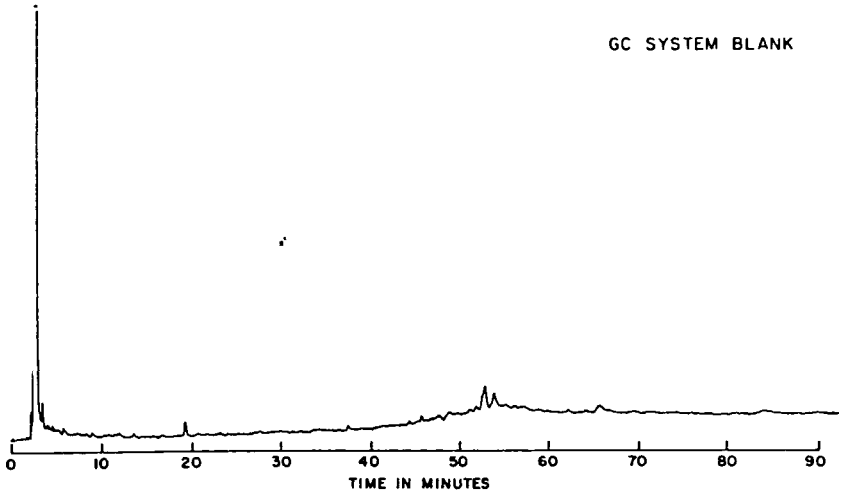
1) GC System

2) Ammonium Carbonate-Ether Blank

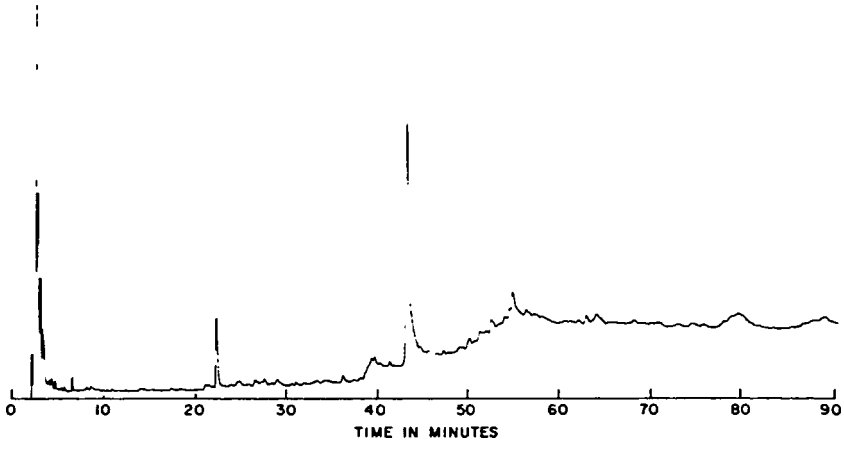
3) Heparin-Ether Blank

Gas Chromatographic Conditions as specified in Experimental Section of Text.

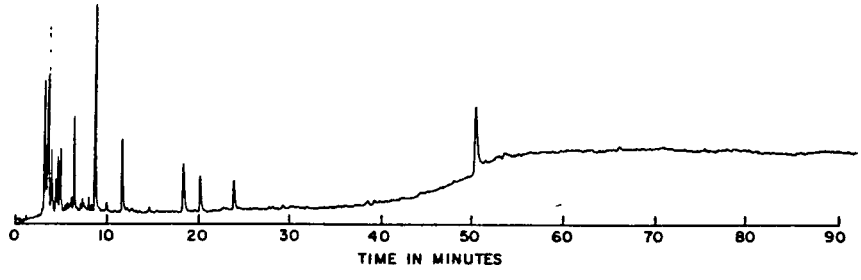
GC SYSTEM BLANK



$(NH_4)_2CO_3$ - ETHER BLANK



HEPARIN-ETHER BLANK



The ether and ammonium carbonate were also tested in conjunction with the G.C. system. Five hundred µl of ether, which had been refluxed for 2 hours over lithium aluminum hydride, was put into a centrifuge tube as described in the experimental section, and an amount of ammonium carbonate equal to that used for plasma extraction was added and the remainder of the procedure followed exactly. The second chromatogram in Figure 2 shows the results. Even though peaks at 23 and 40 minutes appear, the ammonium carbonate-ether-G.C. system could be eliminated as a source of contaminants because the amounts do not approach the concentration of the two peaks appearing in the plasma volatile profile.

Because the samples available were plasmas instead of serum, it was suspected that the heparin might be a source of the elevated peaks at 23 and 40 minutes in the ethereal extract. By definition (37), serum is whole blood minus cellular elements and fibrin, and is formed by allowing the specimen to coagulate naturally. Plasma, on the other hand, is whole blood minus cellular elements only, formed by placing the whole blood into a tube containing heparin and centrifuging. The test was carried out by adding enough refluxed ether to wash a heparinized tube the size used for sample collection. The final volume brought to 25 ml equaled that amount of ether that would be needed if the entire plasma specimen obtained from the heparin tube were extracted. From the 25 ml, an 0.5 ml aliquot was analyzed by G.C. in the manner described for plasma analysis. It can be seen from the third chromatogram in Figure 2 that no contaminants of any significant size are eluted at 23 or 40 minutes. This eliminates the heparin-ether-G.C. system combination as a source of contamination.

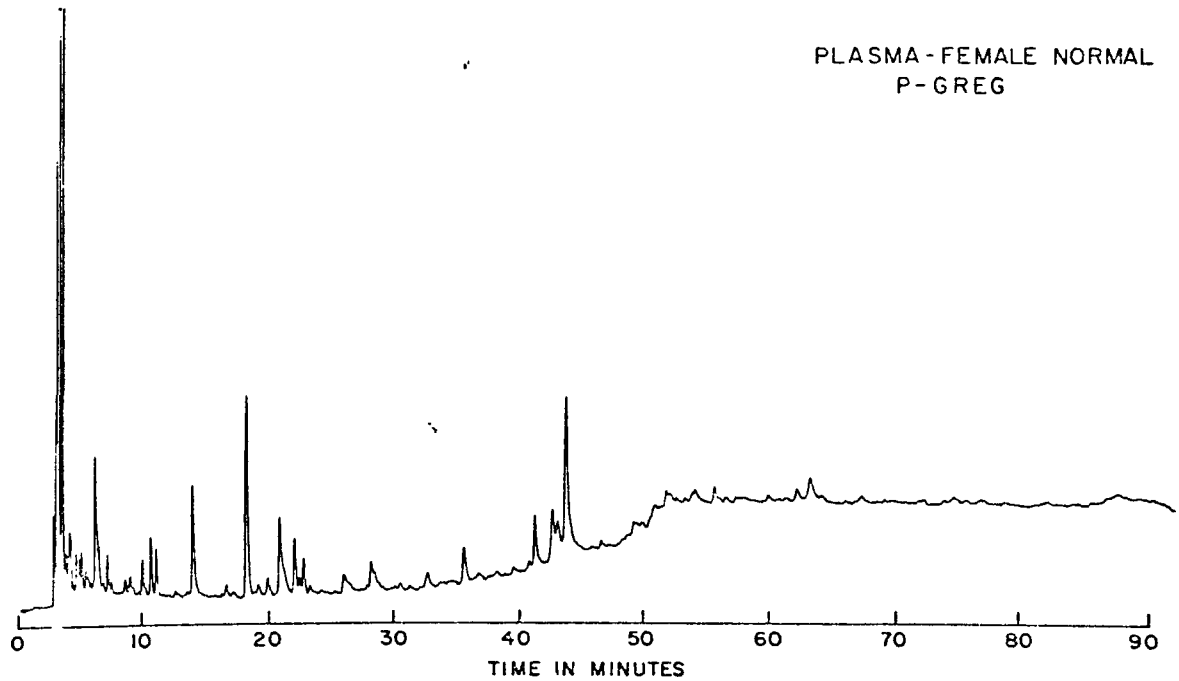
Plasma samples from control female adults who were not on any medication, including birth control pills, were obtained and analyzed in the manner described for plasma volatiles of the postpartum females. It can be seen at once from Figure 3 that no abnormal elevation of any peaks occur in the chromatogram. By analyzing a postpartum plasma, then a normal and another postpartum and comparing profiles, it was concluded that these markedly elevated peaks at 23 and 40 minutes in the postpartum plasma volatile chromatogram were not contaminants, but were indeed volatile compounds extracted from the plasma. Furthermore, the compounds appeared in each and every chromatogram in varying concentrations, but in much larger quantities than in the profiles of normal females.

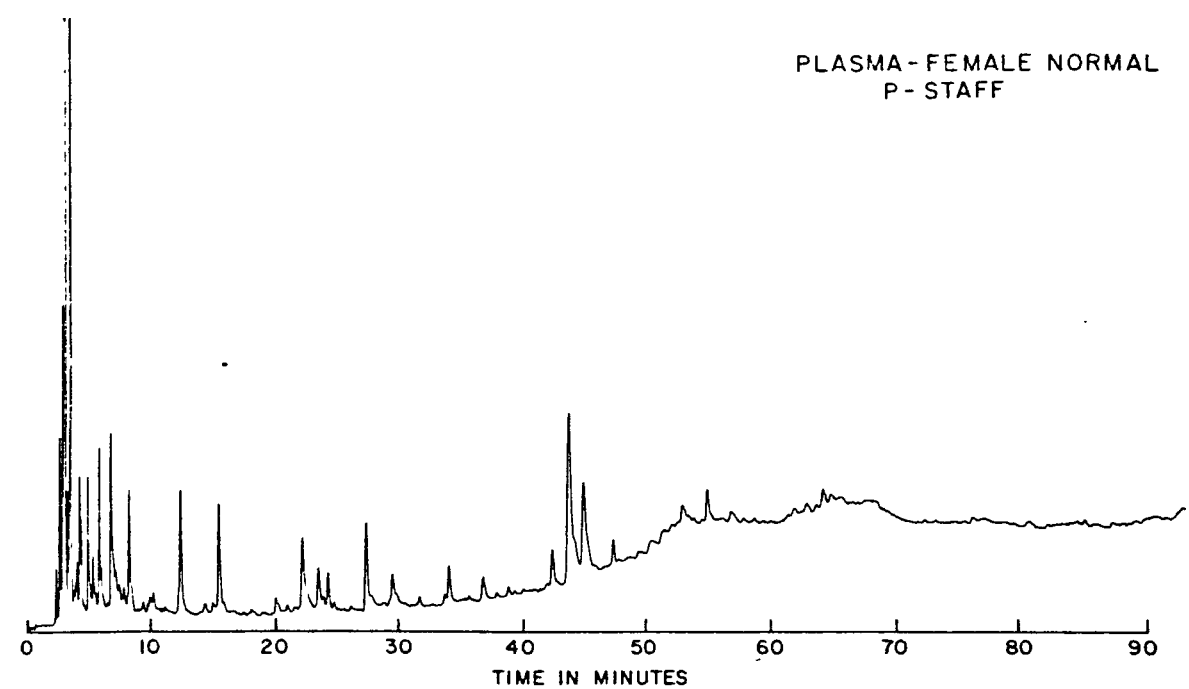
A literature search revealed that many metabolic processes are accelerated during pregnancy, labor, and delivery which could result in elevation in plasma volatiles. During the course of a normal labor and delivery in a hospital, the mother is subjected to a standard procedure which includes immediate I.V. of a 5-10% solution of dextrose and water. The drip is continued throughout labor and delivery. As the course of labor progresses, the contractions become harder and more frequent and are accelerated after the "water is broken", whether naturally or by the obstetrician. This increased contraction strength and frequency brings about an elevation in plasma lactate and pyruvate, which are products of muscular activity during labor (4). Also, hypoglycemia is often reported to occur in otherwise normal labor. The fall in blood sugar indicated by a rise in lactate and pyruvate could be caused by

FIGURE 3

Volatile Plasma Metabolites of Two Non-Pregnant Non-Postpartum Females.

Gas Chromatographic Conditions as specified in Experimental Section of Text.


PLASMA - FEMALE NORMAL
P - GREG
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


PLASMA - FEMALE NORMAL
P - STAFF
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

lack of food (in the final week, and especially upon initiation of labor, it is suggested that food intake be kept to a minimum or avoided completely), and utilization of glucose or dextrose I.V. during labor (3). The mass spectrometric analysis of the plasma volatiles, however, indicated that the peaks at 23 and 40 minutes were not lactate and pyruvate.

The mass spectral analysis did reveal that the compound in the plasma chromatogram occurring at 40 minutes is benzyl alcohol. Comparison of the fragmentation pattern and intensities of the E.I. spectra to that obtained from CRC Atlas of Spectral Data and Physical Constants for Organic Compounds indicated that the elevated compound is benzyl alcohol. A molecular ion of 108 could have included numerous compounds, but the possibilities could be narrowed quickly. Further examination of the spectra revealed a large m/e 77 and 79, which is indicative of an aromatic ring. The ions at m/e 91 represent a tropylium ion, thus a $-CH_2-$ or -N- must be adjacent to the ring. Due to an even mass, nitrogen can be eliminated in the elemental formula. Some of the possibilities conforming to the above observations are three cresols, phenyl methyl ether, or benzyl alcohol. Alcohol spectra usually has a large M^+-1 fragment, which is the case for this unidentified compound, so cresol and benzyl alcohol are good possibilities. The high abundance of ions at m/e 77 (M^+-31) could occur for phenyl methyl ether or benzyl alcohol, representing the loss of $-OCH_3$ and $-CH_2OH$, respectively. However, phenyl methyl ether would not give an ion at m^+-1, but would give a M^+-15, which is not present in the spectra. The base peak at m/e 79 on the spectrum

is formed by a loss of 29 amu. Cresol could possibly lose 29, but would also lose 15 (CH_3-), leaving m/e 93, which is not present as stated above. The m/e 91 corresponding to M^+-17 is a characteristic loss of OH· for alcoholic compounds. Benzyl alcohol, conforming to the above patterns, is the choice. And comparison to CRC Atlas confirms that benzyl alcohol is, indeed, the elevated peak at 40 minutes in the plasma volatile metabolite chromatogram.

To further prove the identity of benzyl alcohol, a sample of a mother's plasma, chosen at random from those used for this study, was extracted as described in the experimental section for plasma. The ether extract was dried with a nitrogen stream. Some of the more volatile compounds were lost, but the peak at 40 minutes was eluted from the column at 150°C, thus its vapor pressure such that it would not blow away at room temperature. The residue was dissolved in 100 µl pyridine and 50 µl acetic anhydride and heated at 60°C for 15 minutes. The sample was then analyzed by GC-MS. The molecular ion went up 42 amu's, indicating the acetylation of a free alcohol. An m/e at 108 (M^+-42) is the loss of $O{=}C{=}CH_2$ with an H^+ migration. The ions at m/e 90 and 91 are formed by the loss of $HO\overset{O}{\overset{\|}{C}}CH_3$ and $\cdot O\overset{O}{\overset{\|}{C}}CH_3$, respectively. Also, the m/e 77 and 79 appear in the ester resulting from the loss of $\cdot CH_2O\overset{O}{\overset{\|}{C}}CH_3$ in the case of 77, and this plus an H^+ migration in the case of 79. It is, therefore, concluded that the compound is, indeed, benzyl alcohol.

The mass spectral analysis of the peak at 23 minutes retention time gave a molecular ion of 106. An intense ion at m/e 77 indicated that a phenyl ring is present, and the high abundance of ions at M^+-1

(m/e 105) suggested that the compound might be an alcohol or aldehyde. Since the molecular weight of this compound is two less than the molecular weight of benzyl alcohol (the peak eluted at 45 minutes) it was concluded that this compound was benzaldehyde. Further proof of benzaldehyde was obtained by analyzing a benzaldehyde standard by the gas chromatographic procedure used for plasma samples. The retention times for the standard and suspect peak in the plasma were identical. To assure that the benzaldehyde had not undergone oxidation to the acid, mass spectral analysis was performed on the standard and confirmed benzaldehyde. When the E.I. spectra of the compound occurring at 23 minutes in the chromatogram was compared to the spectrum of benzaldehyde obtained from CRC Atlas of Spectral Data and Physical Constants for Organic Compounds, they were found to be identical.

It was possible that benzaldehyde and benzyl alcohol elevation were formed from a preservative in the 5% dextrose solution used for intravenous infusion. A sample of 5% dextrose water was obtained from the hospital from which the biological specimens were received. The dextrose solution was analyzed by G.C. in the same manner as the plasma samples. The resulting chromatogram was blank with the exception of several peaks in the first five minutes, thus excluding a preservative as a source of the elevations.

The above data suggests that benzyl alcohol and benzaldehyde in such high concentrations are products of a metabolic pathway involved with the deliverance of the fetus; and may be the result of a hormonal stimulation.

The plasma volatile profiles of the newborn infant (day 1) also contained large peaks that were eluted at 23 and 40 minutes. Further,

as Figure 4 shows, the concentration of benzyl alcohol is higher in the neonatal than in the maternal plasma in most instances. The two peaks were identified as benzaldehyde and benzyl alcohol by GC-MS. With the exception of these two compounds, the concentration of volatile metabolites is lower in the infant than in adult plasma.

Recent studies (7,12) have confirmed that the state of stress is accentuated during labor more than any other time during pregnancy. Because the marked elevation of benzaldehyde and benzyl alcohol occurred in both the mother and newborn, and the fact that childbirth is a stressor, the possibility that stress could cause such a phenomena had to be considered. Plasma specimens were obtained from emergency room patients immediately upon admission to the hospital and analyzed according to the procedure described for plasmas. When the volatile profiles obtained from a patient with cardiac arrest and an automobile accident patient are compared to the profile of a postpartum female, it was found that the large peaks eluted at 23 and 40 minutes were present only in the postpartum female.

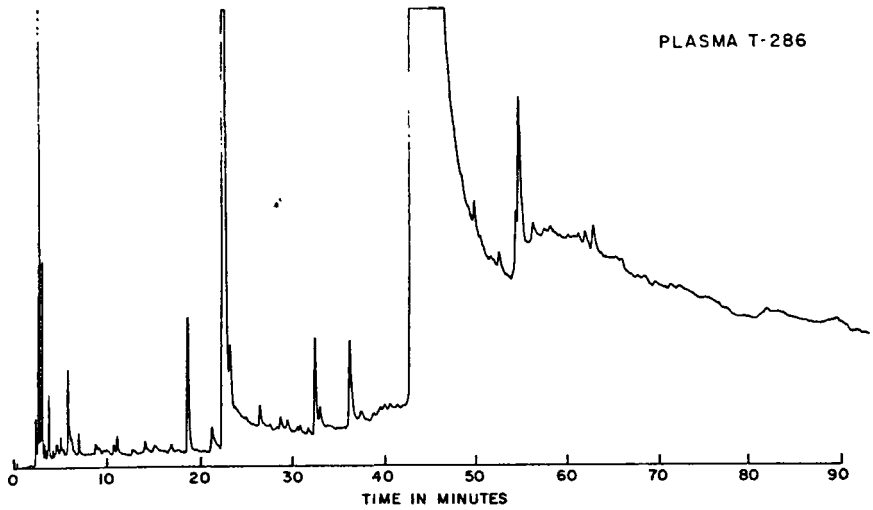
By considering the above information collectively, it was concluded that the increased excretion of benzaldehyde and benzyl alcohol was associated with the process of labor and delivery and not generalized stress. Whether or not these are products of hormonal stimulation or purely the products of the exercise involved with labor and delivery would be difficult to discern.

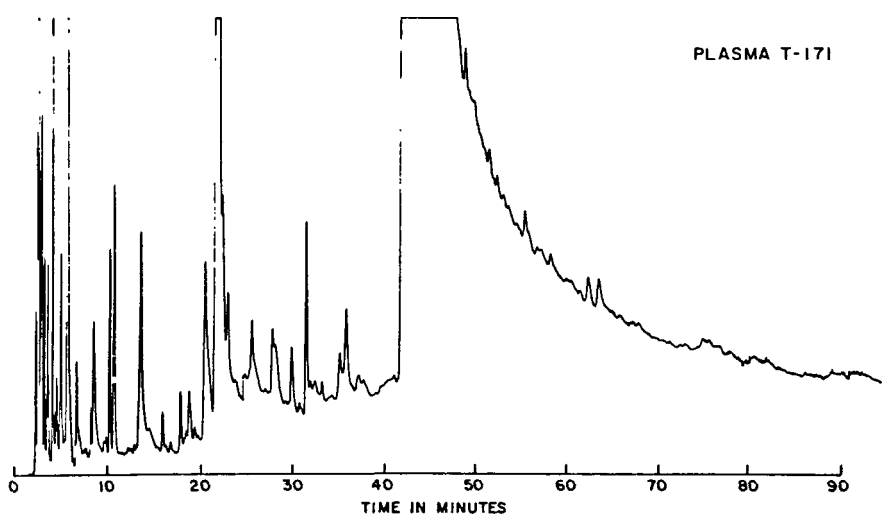
Many of the physiological changes which occur during pregnancy are the acceleration of the metabolic processes to accommodate the new

FIGURE 4

Volatile Plasma Metabolites of Three Newborns. Samples Collected on Day of Birth.

Gas Chromatographic Conditions as specified in Experimental Section of Text.


PLASMA T-286
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


PLASMA T-171
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

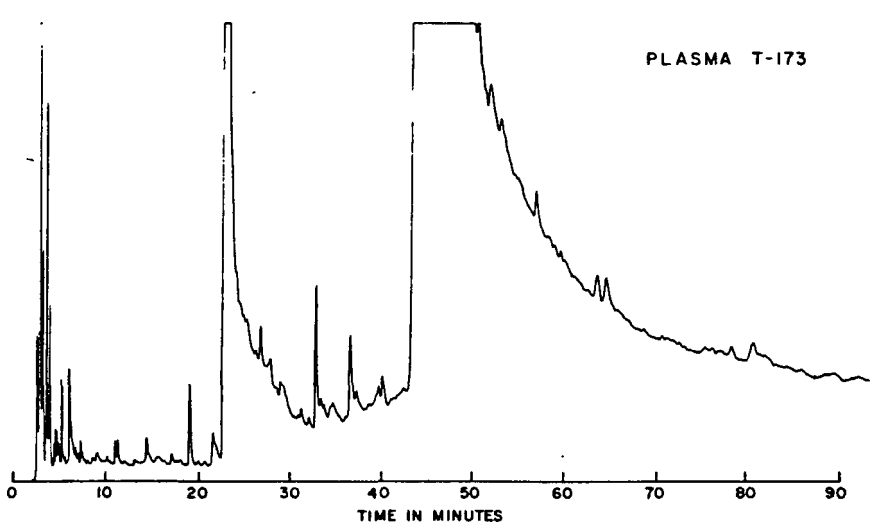
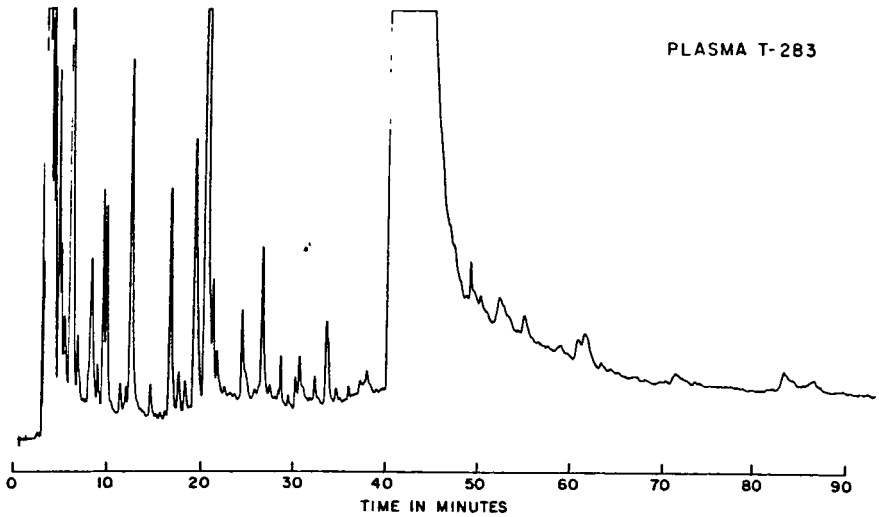
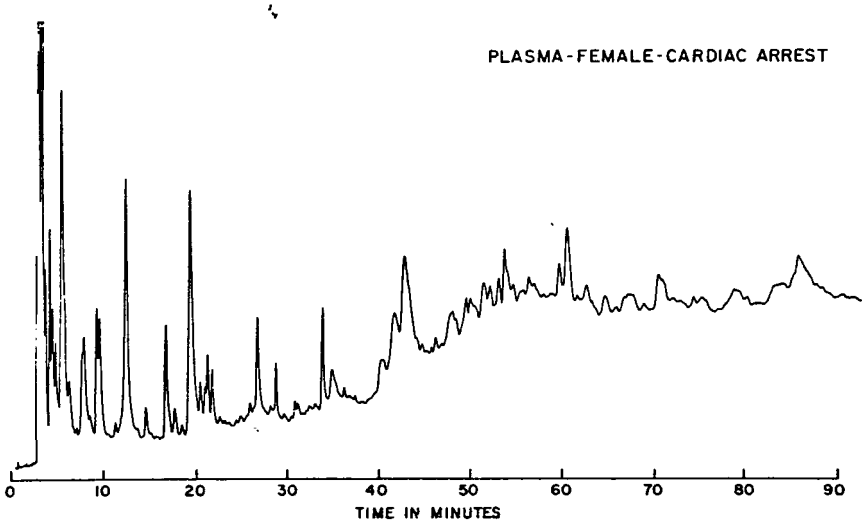

PLASMA T-173
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

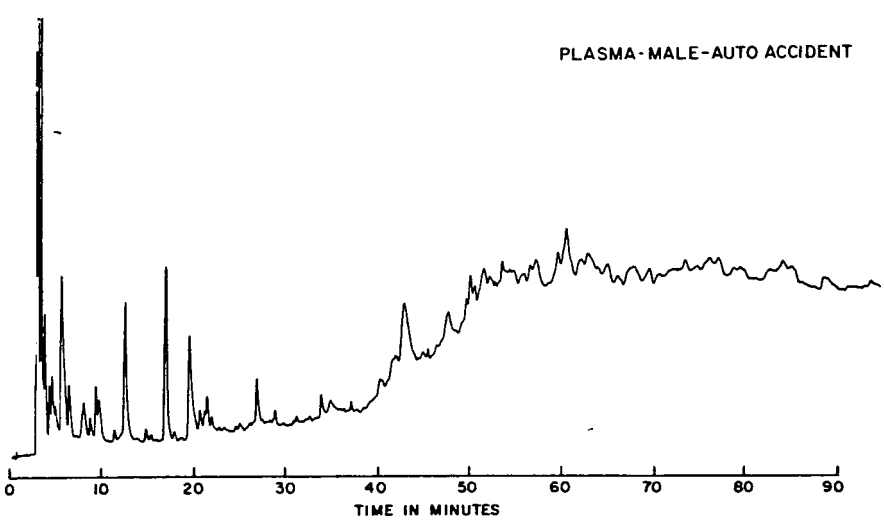
FIGURE 5

Volatile Plasma Metabolites of a Postpartum Female, a Female Cardiac Arrest Patient, and a Male Auto Accident Patient. Stress Patient Specimens Collected upon admission to emergency room.

Gas Chromatographic Conditions as specified in Experimental Section of Text.


PLASMA T-283
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


PLASMA-FEMALE-CARDIAC ARREST
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


PLASMA-MALE-AUTO ACCIDENT
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

tissue growth. One of the more important changes occurs in the endocrine system where the basal metabolic rate increases; the actual size of the thyroid gland increases and there is enlargement of the adrenal glands which progresses throughout pregnancy. Nitrogen balance increases with gestation due to the increased catabolism of serum proteins.

All of the above physiological changes which occur in the maternal system reach a maximum upon initiation of or during labor. They all contribute to the fetal growth as well as the maternal well-being. The preservation of gestation, however, is carried by progesterone. The elevation of progesterone during pregnancy is to maintain uterine growth, keep the muscle fibers at rest, and improve uterine blood flow. The exertation of progesterone near term is at the placental level. Thus, from the third month on, the sedating effect of progesterone on the uterine-muscle fiber by neutralizing the effect of oxytocin maintains gestation.

One important factor in initiation of labor is the imbalance of progesterone and estrogen. Total estrogens are present throughout pregnancy in the form of estriol, estrone and estradiol. Just before parturition, estrone and estradiol increase by 100 fold, whereas estriol, having been higher in concentration throughout the duration, increases 1000 fold. The source of estrogens seems to be the placenta. Degeneration of the placenta reduces its capacity to synthesize progesterone and it is left defenseless against uterine exciting agents such as estrogen. The mechanism suggested is that the rise in estrogen sensitizes the uterus to oxytocin. Studies of blood levels of oxytocin show it is

rapidly inactivated and occurs as quick discharges rather than sustained levels, suggesting that its function is one of sustaining rather than triggering labor (28,34).

New research reveals that myometrical catecholamine content is modified by action of estrogen indicating that this may be the mechanism involved with the initiation of labor (32). The catecholamines are released from the adrenal medulla upon stimulation by estrogen and the sustained increased concentration of estrogen continues to stimulate adrenal medulla secretion of the catecholamines. Urinary catecholamines in non-pregnant women expressed as equivalents of nor-epinephrine in μg/hr is 12.5. In women in the first and second trimester, values are 13.2 and in women in the third trimester (near term) values reach 18.5, a 50% increase, with even further increases during labor. However, plasma levels of epinephrine and nor-epinephrine were not increased during the third trimester, in labor, and postpartum (39). With this in mind, a proposal can be made as to the origin of the elevation of benzaldehyde and benzyl alcohol in plasma of postpartum gravid and neonate.

The catecholamines are synthesized from amino acids phenylalanine and tyrosine in the adrenal medulla, and released in response to stimulation. In the initiation of labor, estrogen rise is the stimulas. The fate of the catecholamines is storage by tissues, degradation, or excretion in the urine. One product of the amino acid decarboxylation is phenyl ethyl amine (18). Phenyl ethyl amine has received only minor attention; however, a recent report by Borison and coworkers in conjunc-

tion with Fischer suggests that it may be a mediator of the central nervous system in actions of amphetamine (11). The concentrations in the central nervous system can increase the amount of phenyl ethyl amine after administration of a suitable agent, such as amphetamine.

In labor estrogen in increased concentrations could be the agent which increases phenyl ethyl amine secretion in the central nervous system. An analogy could be drawn for the large concentration of circulating phenyl ethyl amine to that of catabolic pathways followed by known catecholamines. A metabolic pathway which is described as minor shortens the chain and the resulting compound undergoes oxidative deamination by monoamino oxidase. It follows that the aldehyde formed is metabolized to an acid by aldehyde dehydrogenase or to an alcohol by alcohol dehydrogenase. The factor contributing to the conversion of the aldehyde to larger amounts of alcohol is unknown; no acids were found by mass spectral analysis. The aldehydes have rarely been reported in tissue or urine. Figure 6 shows the steps for proposed catabolism of phenyl ethyl amine (9,30,30a).

Since the adrenal medulla excretion increases progressively throughout pregnancy, the possibility that the aldehyde and alcohol could be on the rise is questionable. A sample of urine obtained during the second trimester pregnancy was available. It was prepared as for the postpartum plasma volatiles. The analysis of the volatiles is shown in Figure 7, and benzaldehyde and benzyl alcohol are not present. In fact, the second trimester volatiles more nearly approximate that of a control female plasma. There is, however, something at 35 minutes

FIGURE 6

Proposed Fate of Circulating Phenyl ethyl amine During Labor and Delivery.

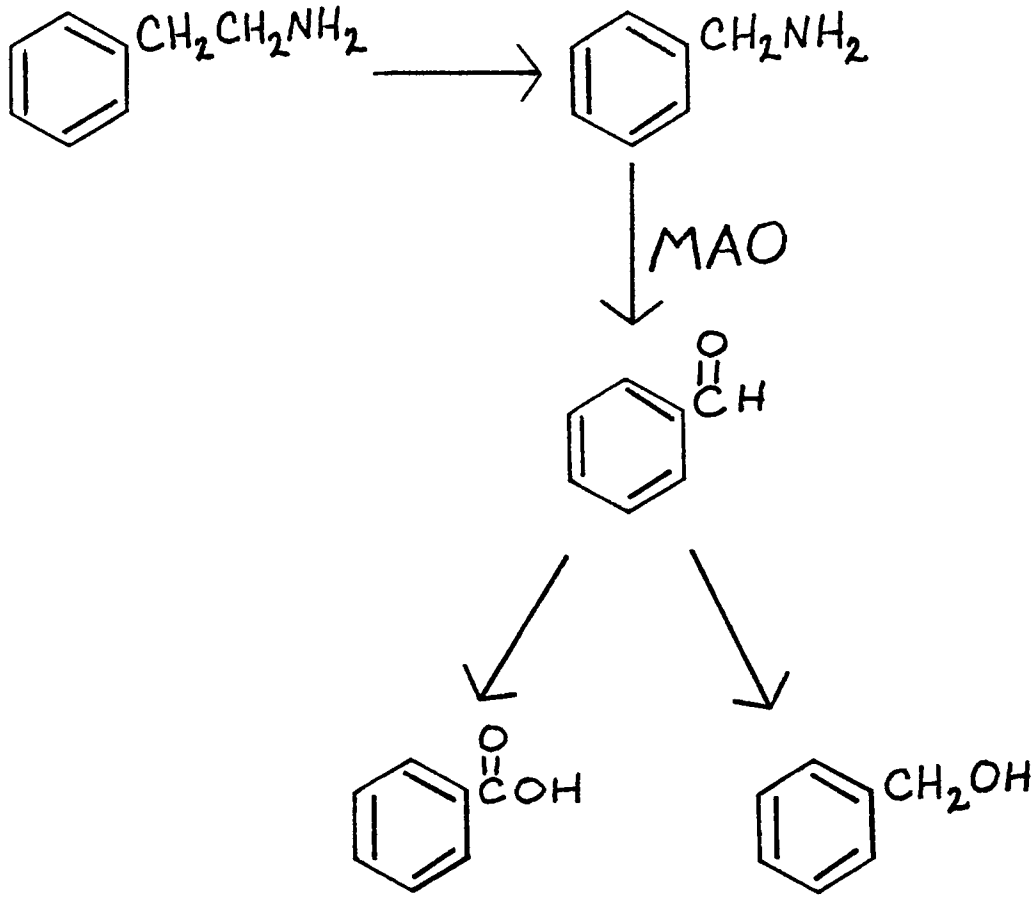
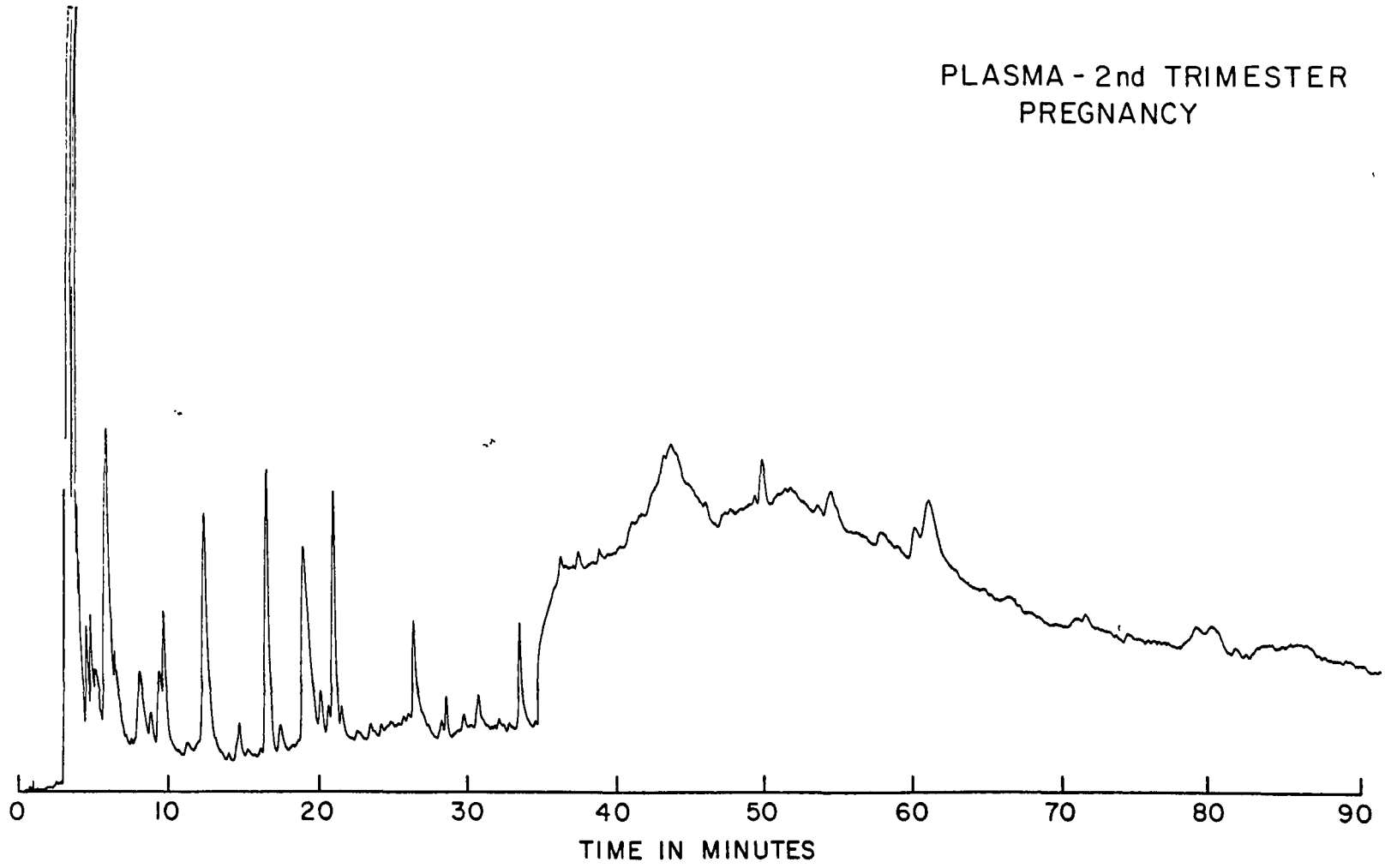

CH2CH2NH2
CH2NH2
MAO
O
CH
O
COH
CH2OH

FIGURE 7

Volatile Plasma Metabolites of a Second Trimester Pregnancy.

Gas Chromatographic Conditions as specified in Experimental Section of Text.


PLASMA - 2nd TRIMESTER
PREGNANCY
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

retention time which was not resolved. At first this tailing peak was thought to be an artifact, however, after re-running the entire procedure three times it was concluded that it is a true compound which is being adsorbed on the column. Unfortunately, no mass spectrum could be run.

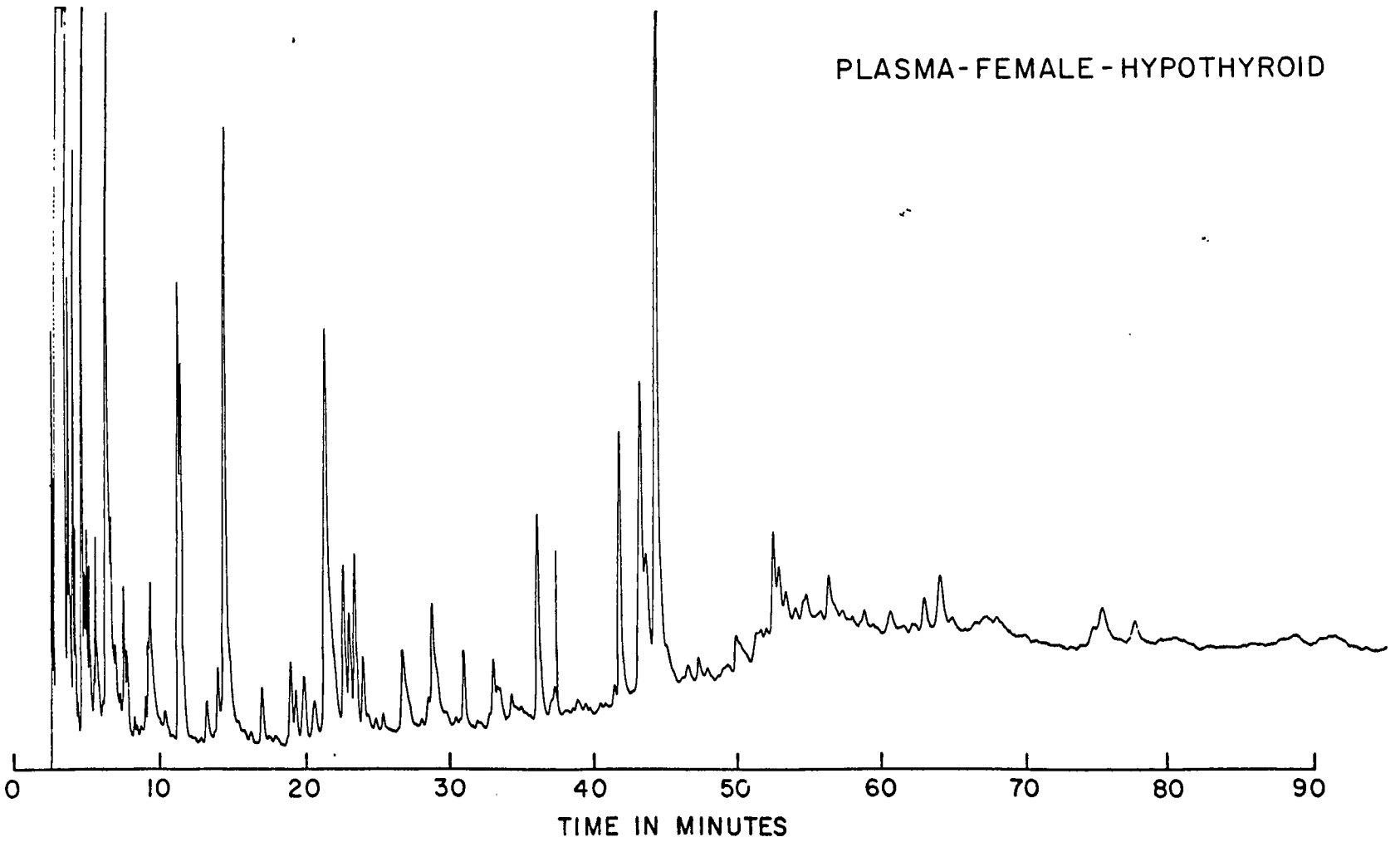
One plasma sample was obtained from a female hypothyroid patient under treatment and analyzed as described for plasma volatiles in the experimental section. Persons whose thyroid gland functions at a slower than normal rate are treated with drugs which speed the metabolism to that point approaching normal. Figure 8 shows the analysis of volatile compounds in the plasma of this patient. The hypothyroidal treated plasma has a higher concentration of volatile compounds than obtained in the control female plasma.

Volatile compounds characteristic of plasma identified by mass spectral analysis were ketones, aldehydes, and alcohol. Table I and II list the volatile compounds found in higher concentrations in a postpartum maternal-neonate pair and a control female, respectively. Benzaldehyde and benzyl alcohol are present in all types of plasma volatiles extracted with ether and ammonium carbonate and analyzed by gas chromatography. These include postpartum and neonate and could be the product of metabolized circulating phenyl ethyl amine.

FIGURE 8

Volatile Plasma Metabolites of a Hypothyroid Patient Treated by Chronic Drug Administration.

Gas Chromatographic Conditions as specified in Experimental Section of Text.


PLASMA-FEMALE-HYPOTHYROID
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

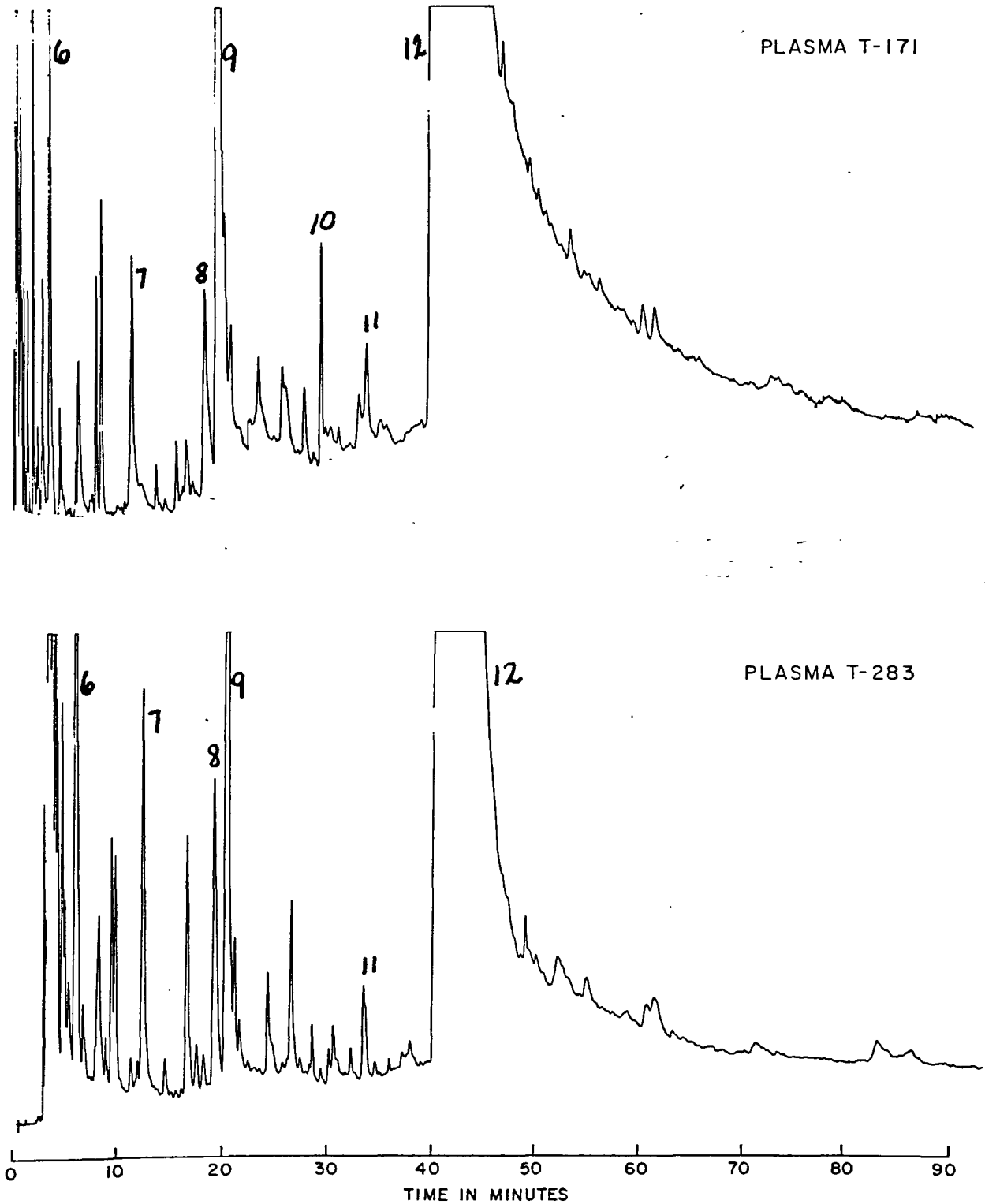


TABLE I

Plasma Volatiles of Postpartum Neonatal-Maternal Pair
Tentatively Identified by Mass Spectral Analysis

6. 5-acetonyl-2-methyl-furan	9. benzaldehyde
7. ethyl cyclopentanone	10. 1-cyclohexan-2-buten-4-ol
8. ethyl cyclohexanone	12. benzyl alcohol

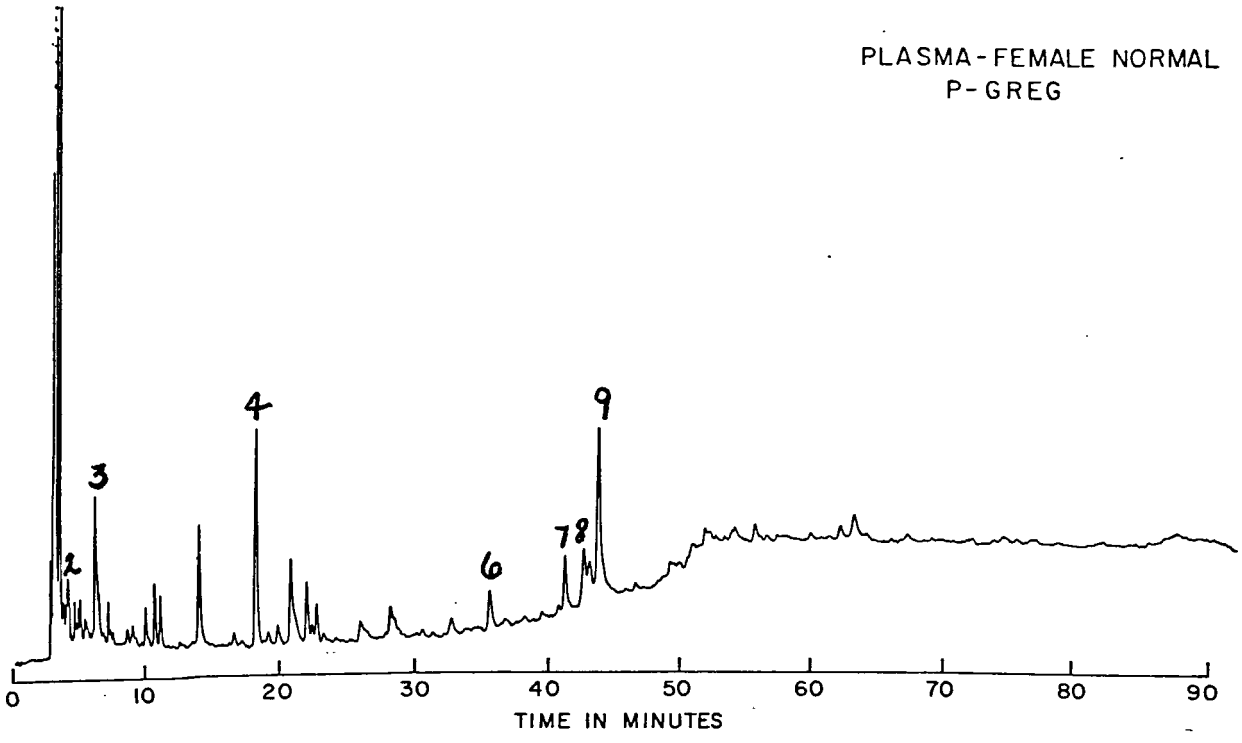


TABLE II

Plasma Volatiles of a Normal Female
Tentatively Identified by Mass Spectral Analysis

2. isopentylamine	7. benzyl alcohol
3. pentanyl cyclohexene + acetone	8. 2-hexanyl furan
4. 2-nor-butoxyethanol	9. tyramine
6. 2-methylpentanyl-cyclohexene	

Urinary Volatiles

Profiles of urinary volatiles were also obtained from normal and postpartum females. Figure 9 shows the results of the analysis. Variance in urinary volatile metabolites can be seen by comparing the profiles of the four individuals. Differences are presumably due to dietary habits and degree of physical activity. The greater concentration of volatile metabolites at longer retention times of the postpartum is probably due to work incurred during labor and increased metabolism. Two characteristic volatile metabolites, at 25 and 53-55 minutes, are present in the postpartum urines. The mass spectra were weak due to loss of the sample in the vacuum system. The peak eluting at 25 minutes was tentatively identified as a mixture of 2-methyl-4-hexene and ethyl cyclohexane. The peak at 50 minutes was 2-vinyl furan. Later samples were not available from the mothers, so that a comparison with the normal urinary profile was not possible. According to texts of obstetrics and gynecology, a period of 6-8 weeks is required for all physiological and biochemical processes to approximate the nonpregnant state (3).

Figure 10 shows the chromatograms of the volatile metabolites from three neonatal urines. Because the samples were taken on day one very few metabolites are present. This could be due to the fact that for the first 12 to 24 hours of life, the newborn is fed only sugar water. Significant large peaks occurred at 25 and 50 minutes retention time, as in the maternal urines. In addition, a peak at 40 minutes was present in most chromatograms and was determined to be benzyl alcohol. The compound at 25 minutes is an off scale peak in every case for the

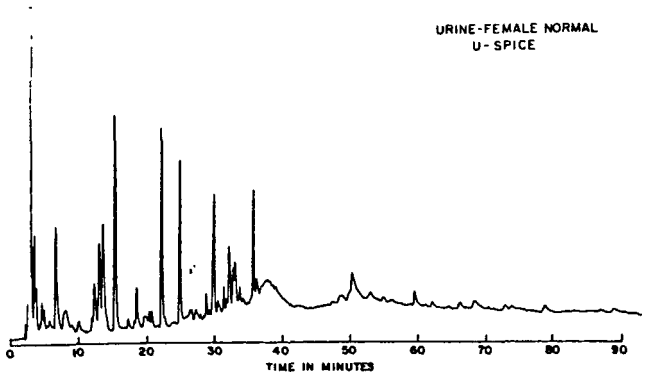
FIGURE 9

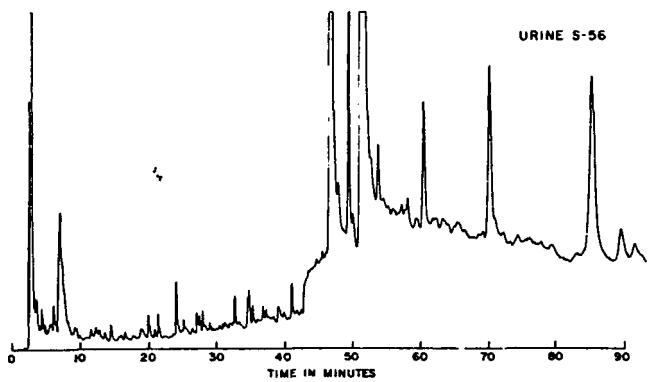
Volatile Urinary Profile of a Control and Three Postpartum Females.

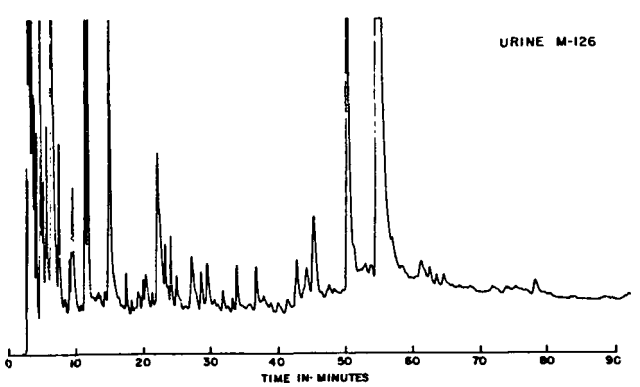
Postpartum samples collected on day one.

Gas Chromatographic Conditions as specified in Experimental

Section of Text.


URINE-FEMALE NORMAL
U-SPICE
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


URINE S-56
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


URINE M-126
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

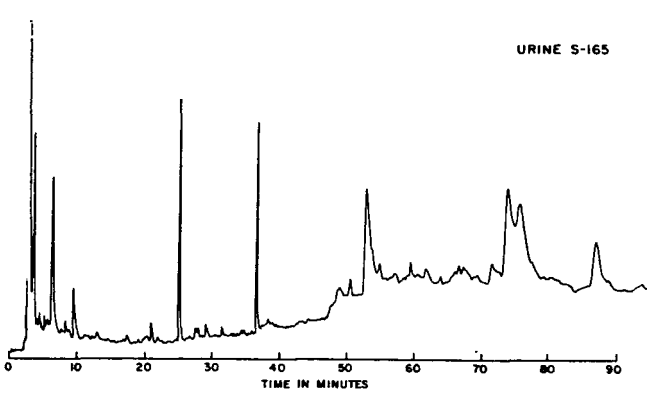
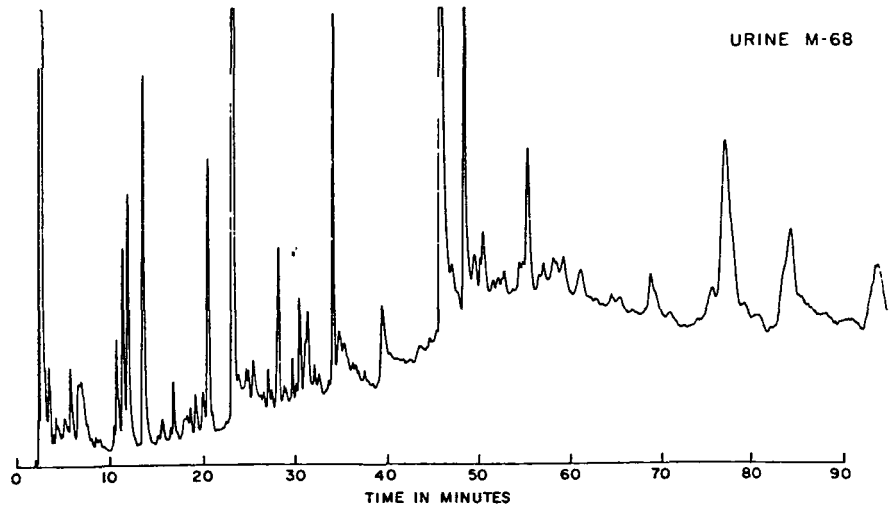
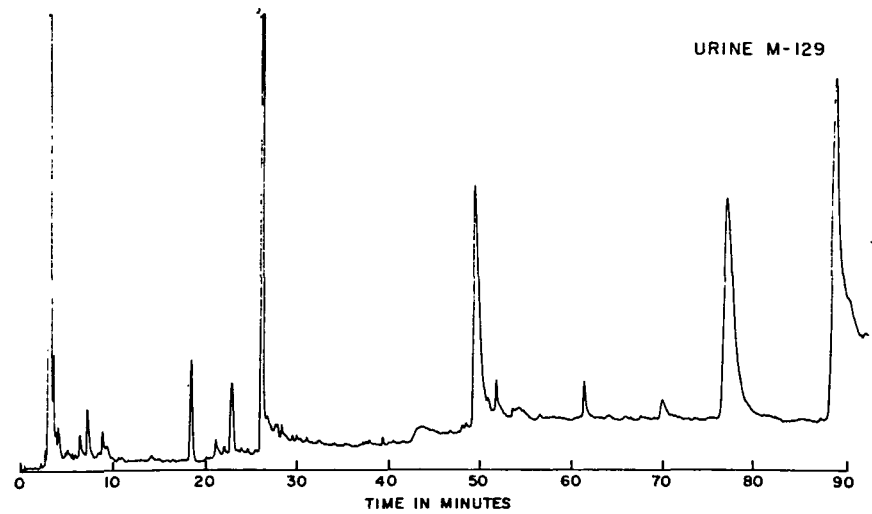

URINE S-165
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

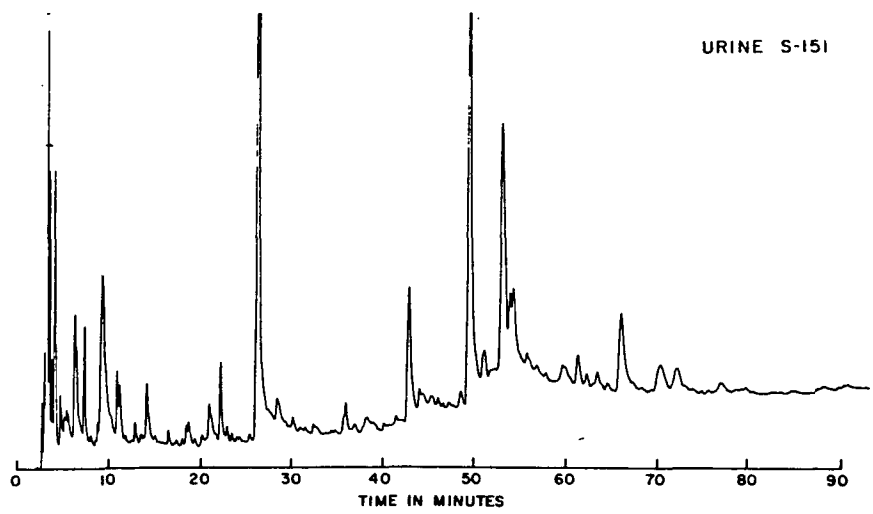
FIGURE 10

Volatile Urinary Profiles of Three Newborns. Samples collected within 24 hours of birth.

Gas Chromatographic Conditions as specified in Experimental Section of Text.


URINE M-68
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


URINE M-129
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


URINE S-151
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

neonate volatiles regardless of how concentrated the remainder of the chromatogram. The peaks at 25 and 50 minutes were tentatively identified by mass spectral analysis to be a mixture of 2-methyl-4-hexene and ethyl cyclohexane and 2-vinyl furan, respectively. No possible explanation as to the rise of these compounds could be found. Urinary volatiles also are characterized by ketones, aldehydes, and alcohols. Table III lists the volatile compounds found in higher concentrations in the neonatal urine.

The experimental procedure for extraction of urine with ether to obtain the volatile metabolites requires a large volume of urine. When a comparison of urinary volatiles obtained by ether extraction and headspace using equal amounts is made, the chromatograms for headspace are far more concentrated. The reason for this is not fully understood, but could be partly due to the fact that the ethereal extract is polarity-partition dependent, whereas headspace is vapor pressure dependent. It was found, however, that more volatile compounds in higher concentrations can be extracted from 3 ml of a 24 hour urine using 2 x 0.5 ml ether rather than once with 1.0 ml. The chromatograms shown in this work, however, follow the original procedure. The reproducibility for two smaller extractions is somewhat higher than that for one large extraction.

Figure 11 shows the urinary volatile metabolites of a control, a postpartum, and a pregnant female, respectively. The pregnancy urine sample was collected late in the third trimester and analyzed. A comparison of the chromatograms indicates that the third trimester urine more nearly approximates the postpartum.

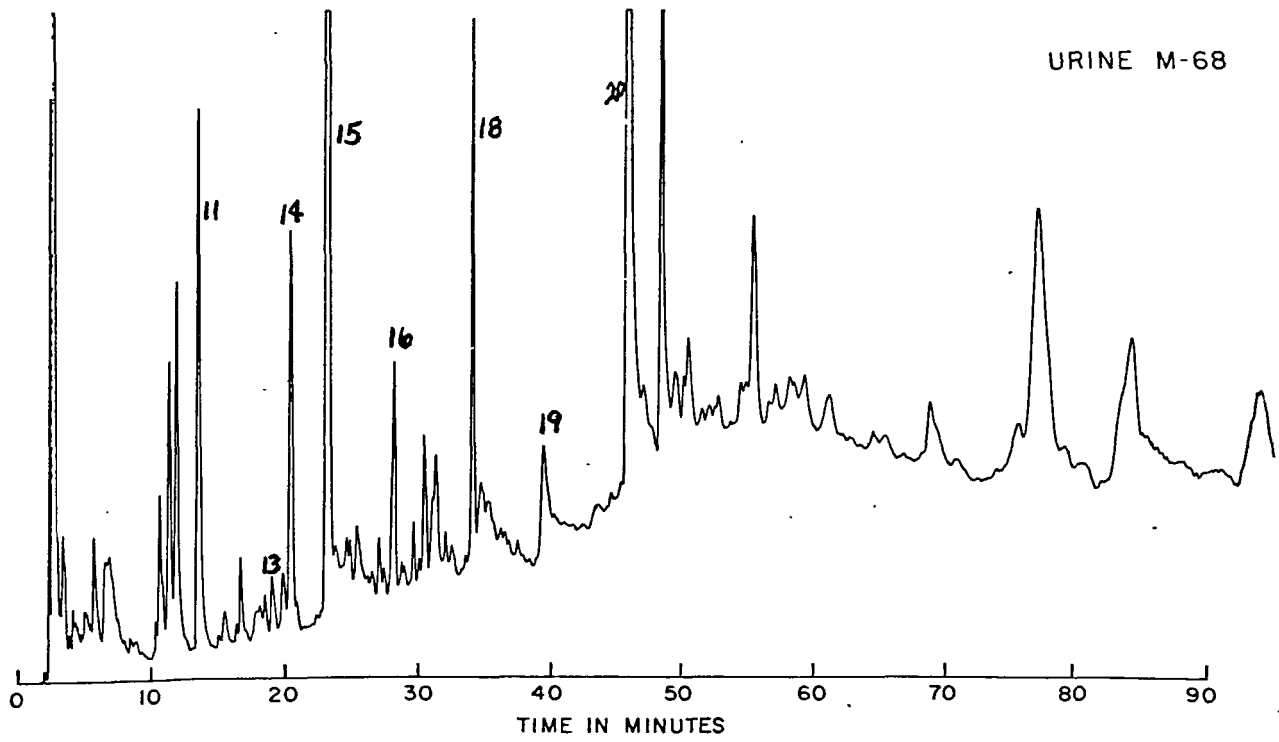


TABLE III

Urinary Volatile Metabolites
Tentatively Identified by Mass Spectral Analysis

11.	acetonylfuran	18.	acetic acid
13.	acetophenone	19.	benzyl alcohol
15.	2-methyl-4-hexene + ethyl cyclohexane	20.	vinyl furan
16.	hexanol		

FIGURE 11

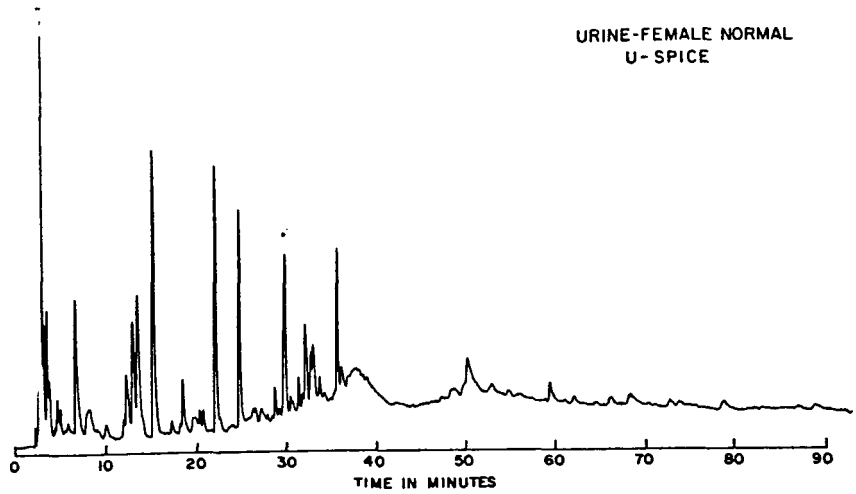
Comparison of Volatile Urinary Metabolites of:

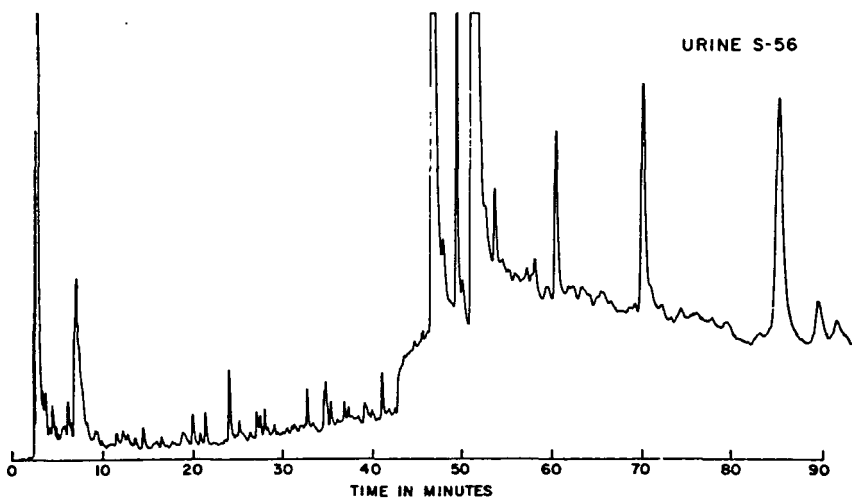
1) Non-Pregnant Non-Postpartum

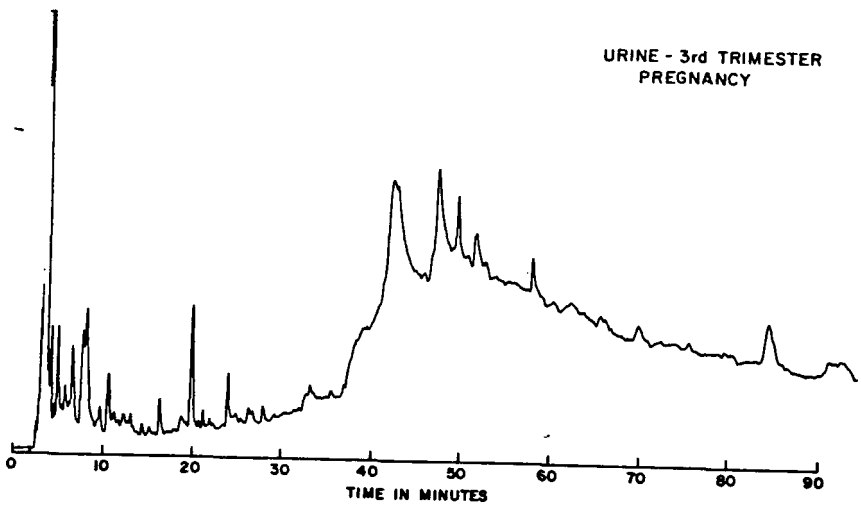
2) Postpartum

3) Third Trimester Pregnancy

Gas Chromatographic Conditions as specified in Experimental Section of Text.


URINE-FEMALE NORMAL
U-SPICE
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


URINE S-56
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


URINE - 3rd TRIMESTER
PREGNANCY
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

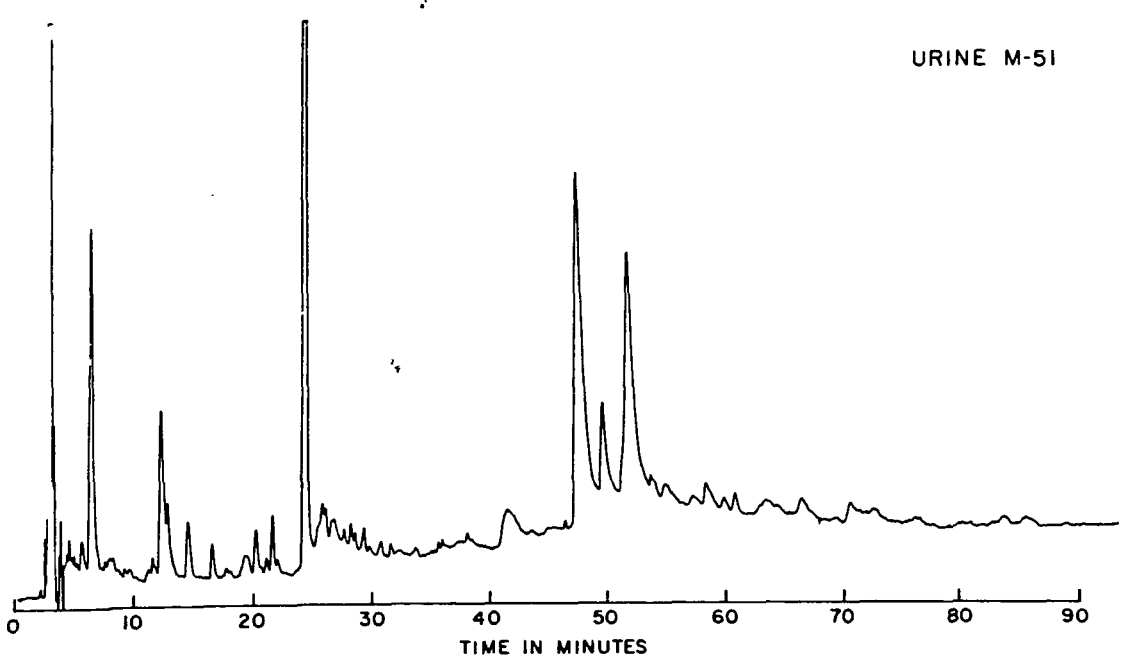
The analysis of urinary samples from newborns is difficult because of the reluctance of parents to give permission to strap a cup to the infant for sample acquisition so that anything more than a routine sample on day one is rarely available. However, several neonate urines were collected a few weeks after birth and analyzed. Figure 12 shows the analysis of the volatile metabolites of a neonatal urine collected on day one and day fourteen. The profiles are incredibly similar, particularly with respect to the more concentrated metabolites at 25 and 45-55 minutes retention time. These chromatograms of samples collected two weeks apart also attest to the reproducibility of this extraction-concentration-desorption technique for volatile metabolites of body fluids for analysis by gas chromatography.

One urinary sample from a female under treatment for hypothyroidism was analyzed. Figure 13 shows the analysis of a control and the hypothyroid patient. Qualitatively, the analyses are similar; however, the hypothyroid metabolite concentration is higher than the normal due to drug administration for speeding metabolic processes. The ratios of constituents within the first 40 minutes are comparable with the variances occurring past 40 minutes. No explanation for the elevation of the doublet at 50 minutes is known. A suggestion is a metabolite excretion stimulated by the chronic administration of drugs for metabolism acceleration.

FIGURE 12

Comparison of Newborn Urinary Volatiles on Day One and Day Fourteen.

Gas Chromatographic Conditions as specified in Experimental Section of Text.


URINE M-51
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

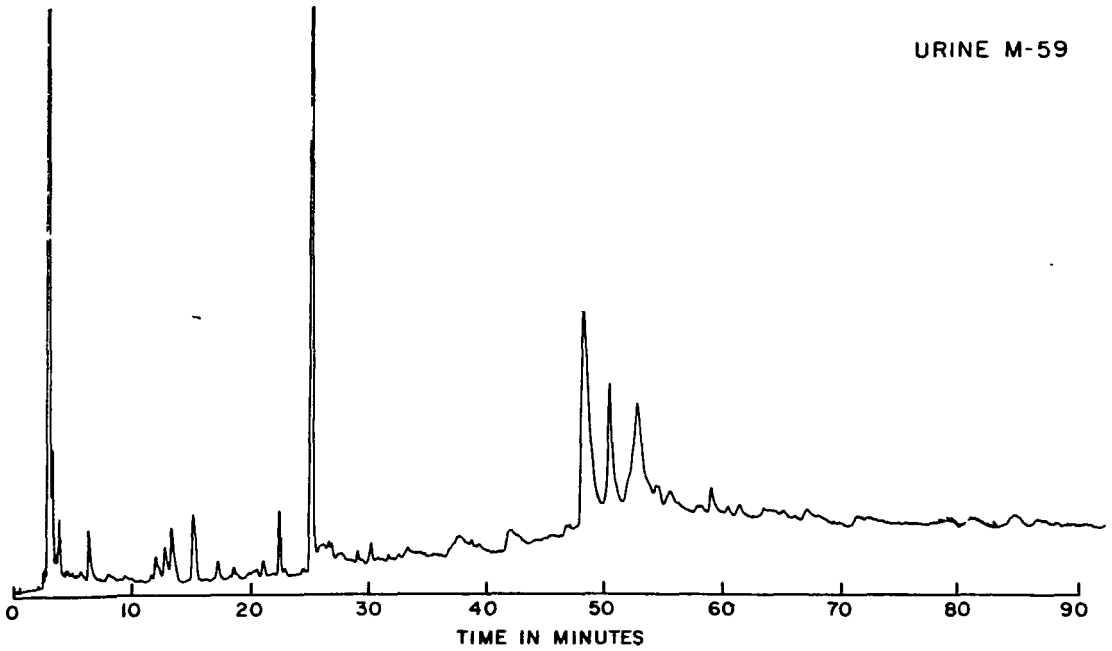
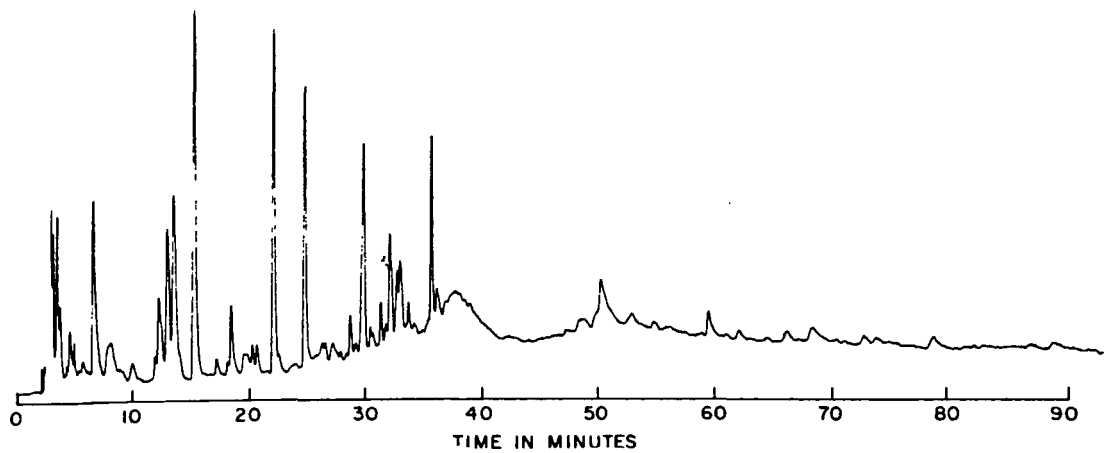

URINE M-59
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

FIGURE 13

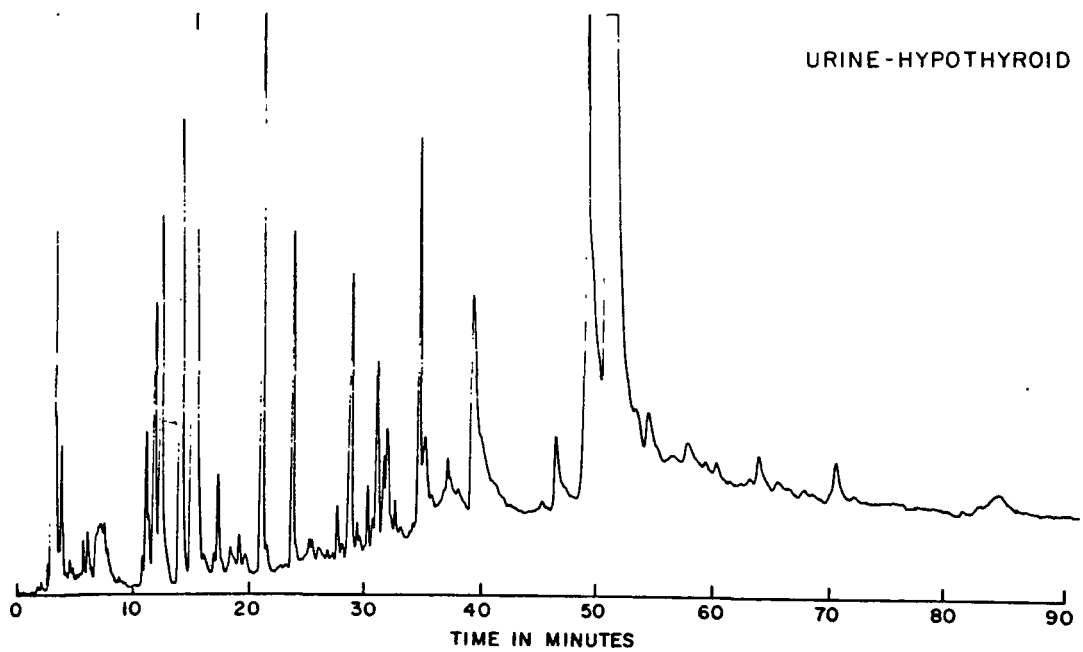
Urinary Volatiles of a Control Female and a Hypothyroid Patient treated by Chronic Drug Administration.

Gas Chromatographic Conditions as specified in Experimental Section of Text.

URINE-FEMALE NORMAL
U-SPICE



URINE-HYPOTHYROID



Breast Milks

The third body fluid to be analyzed was breast milk. The mammary glands are prepared for lactation by progesteronal development of the alveolar epithelium; however, estrogen is reponsible for proliferation of the milk ducts. The ejection of milk is carried out by constriction of the myoepithelial cells in the alveoli and small milk ducts. Actual lactation initiation is caused by decrease of both estrogen and progesterone after birth, withdrawal of placental lactogen, and increases in prolactin and adrenal hormones. The increase in prolactin circulation does not begin to stimulate secretion until the second or third day postpartum. For these first few days postpartum, the secretion is called colostrum. Colostrum contains the nutrients needed by the infant, but not the fats present in milk. After the milk comes in, its production is sustained by stimulus of the removal which causes release of prolactin which produces the required amount of milk. When the milk comes in, it is usually rather painful due to the overabundance, but it quickly adjusts by the requirements of the nursing infant (3).

Figure 14 contains the chromatograms of breast milk volatile components from three postpartum females. The analysis required only 20 µl of milk for extraction with 100 µl of ether. By comparison, the three profiles are almost superimpositional; these were the results of every breast milk analyzed, regardless of collection day. These results are suggestive that human milk does not vary quantitatively for it is the only foodstuff a newborn receives for two to four weeks after birth.

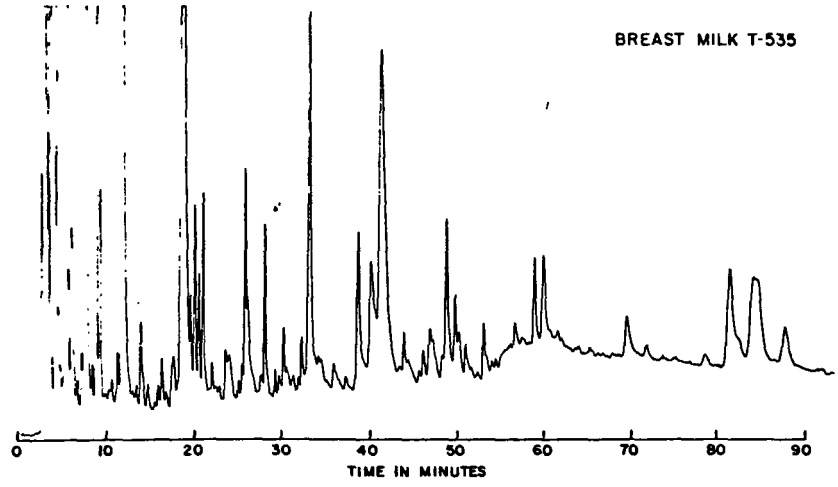
FIGURE 14

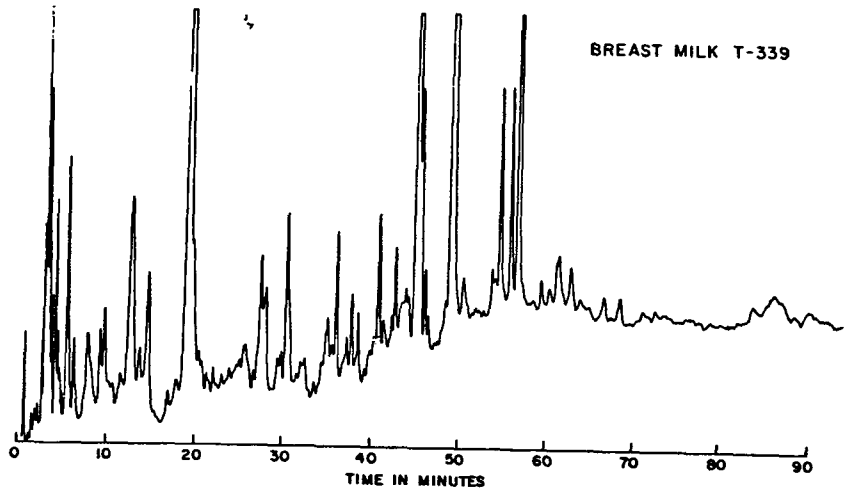
Volatile Profile of Etheral Extract of Human Breast Milks. -

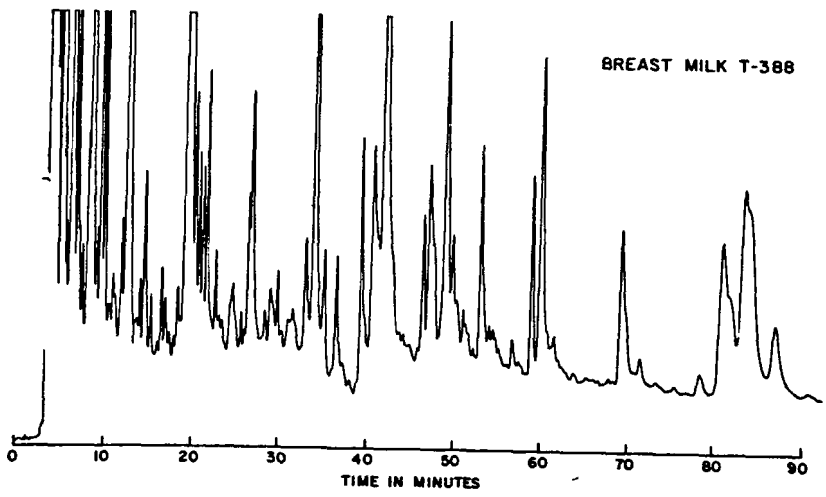
Collected within one week of lactation .

Gas Chromatographic Conditions as specified in Experimental

Section of Text.


BREAST MILK T-535
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


BREAST MILK T-339
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


BREAST MILK T-388
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

In fact, it was not until the last few decades that infants were given any supplement to milk before the age of six months to a year. Characteristic peaks are the five at 20 minutes varying slightly in ratio among individuals. The large peak at 35 minutes, the group of three at 40 minutes, and one peak off scale at 50 minutes are also present in each analysis, as well as the doublet at 60 minutes. Volatile compounds characteristic of human breast milk tentatively identified by GC-MS analysis were furfuryl ketones, aldehydes, and alcohols. Table IV lists the compounds in higher concentrations present in the ethereal extract profiles of breast milk.

Amniotic Fluid

After implantation of the fertilized egg into the endometrium layer of the uterus, the tissue grows in the amniotic fluid contained by the placental sac. Origin of the amniotic fluid is speculative, however, analysis in early pregnancy is similar to the maternal serum, suggesting that it is a transudate of the maternal plasma. As the fetus grows, it secretes fluids into the amniotic fluid and analysis of later samples shows the fluid to be more fetal like (14). Figure 15 shows the volatile analysis of two amniotic fluids. Comparison of these to the chromatograms of Figure 10 shows no similarity of profiles except for sparsity of components. Distinguishing peaks of the amniotic fluid volatile chromatogram are those off scale at 17 and 80 minutes. Of particular interest is the presence of a doublet at 60 minutes, also present in the plasmas and breast milks. Because of the nature and low

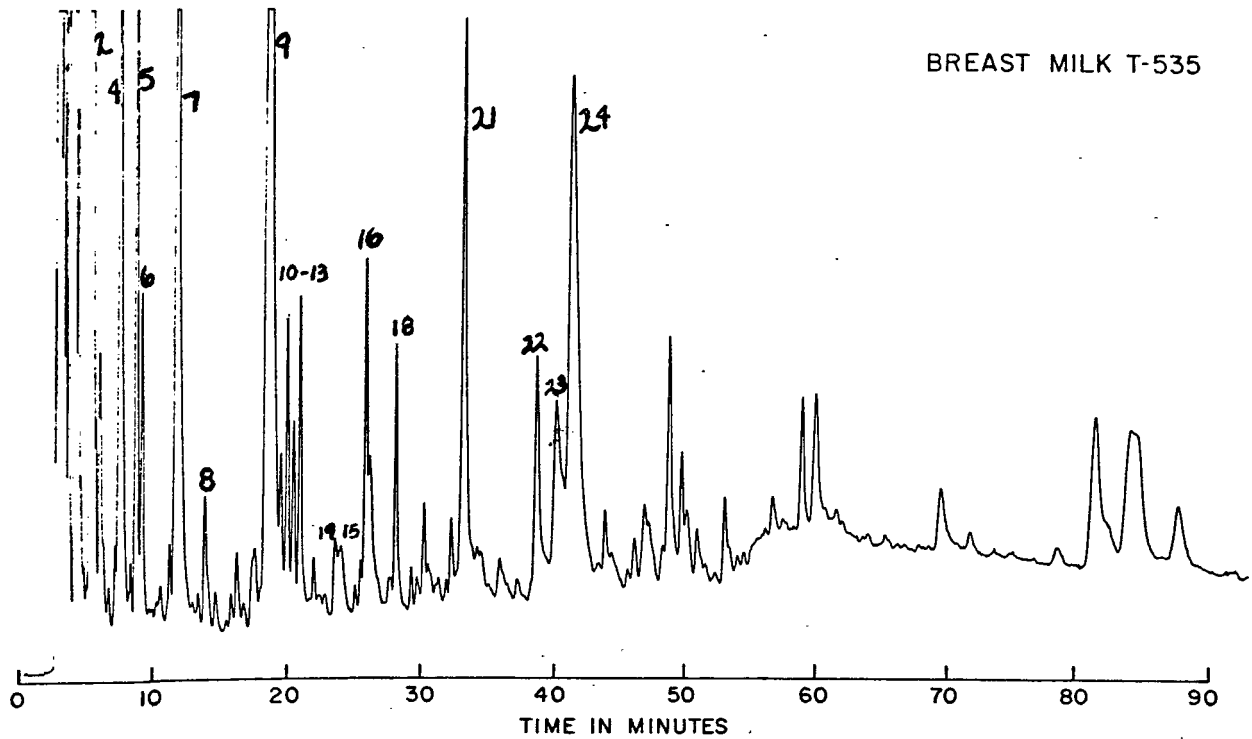


TABLE IV

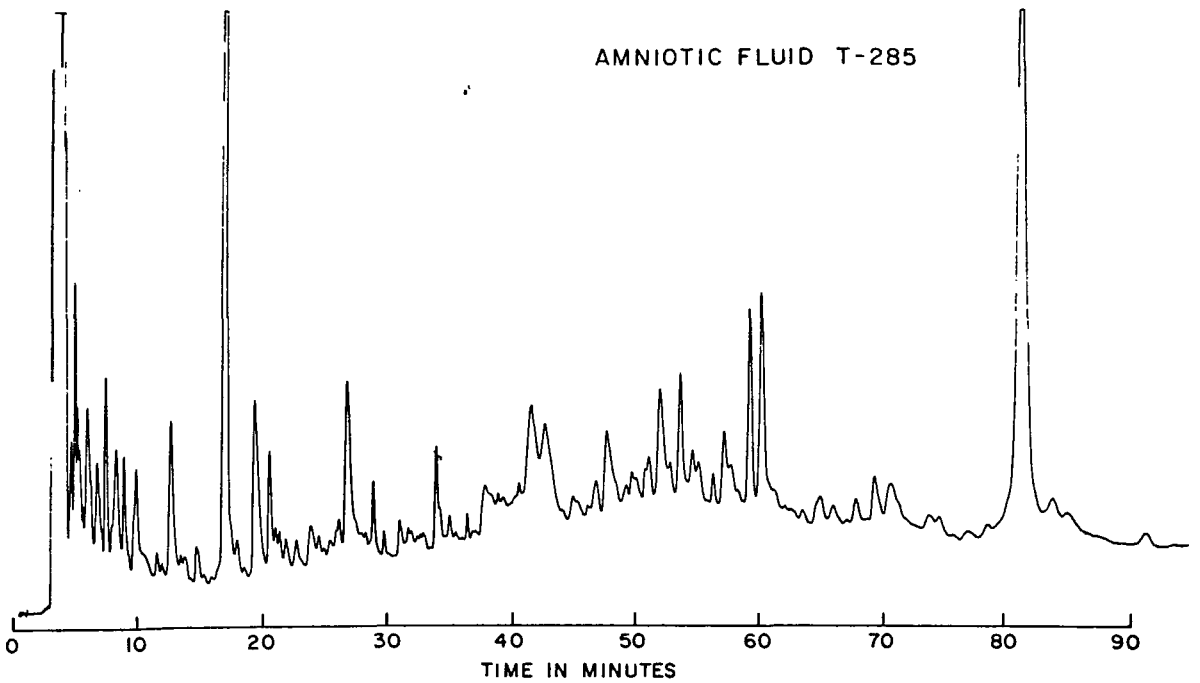
Breast Milk Volatiles
Tentatively Identified by Mass Spectral Analysis

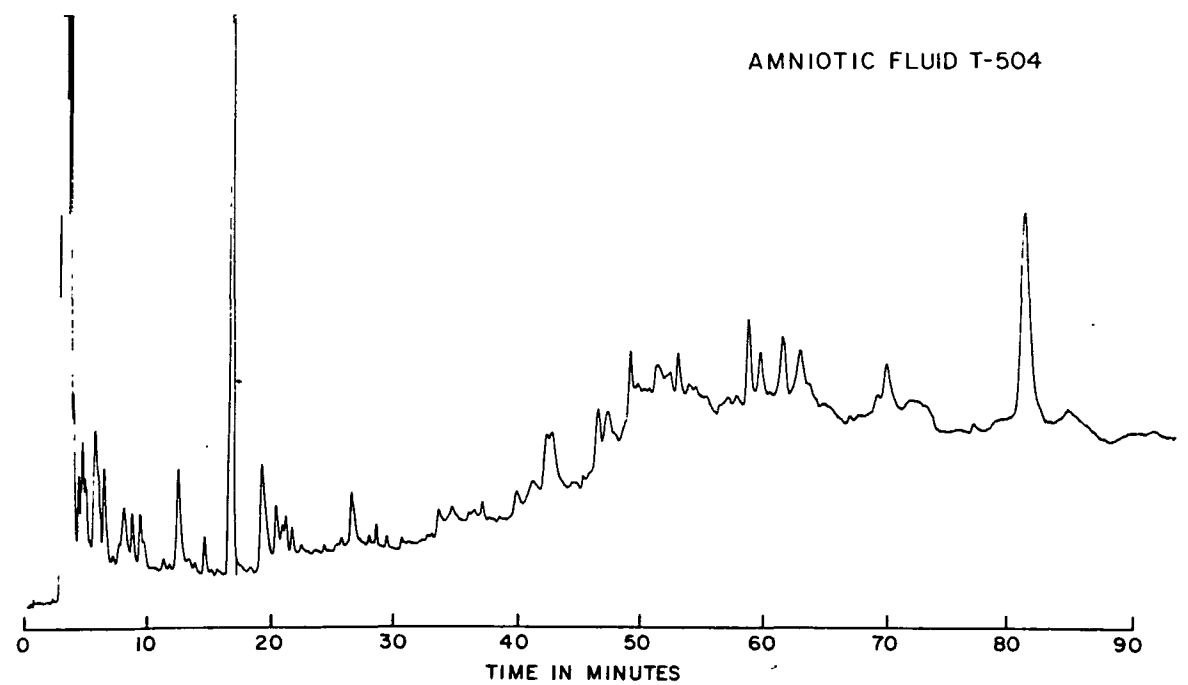
2.	hexanal	12.	methyl hexanal
4.	heptanal	15.	2-methyl-4-hexene
5.	6-methyl-2-heptanone	16.	2-methyl-amylnitrile
6.	2-pentylfuran	18.	hexanol + 4-methyl-1-pentene
7.	ethyl cyclopentanone	21.	4-methyl-1-pentyn-3-ol
8.	acetonylfuran	22.	isomer of 24
9.	ethyl cyclohexenone + ethyl cyclohexanone	23.	pentenyl cyclohexene
10.	methyl cyclopentanone	24.	amino pentanyl furan

FIGURE 15

Volatile Metabolites of Human Amniotic Fluid. Collected at birth from two individuals.

Gas Chromatographic Conditions as specified in Experimental Section of Text.


AMNIOTIC FLUID T-285
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


AMNIOTIC FLUID T-504
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

concentration of amniotic fluid volatiles, mass spectral analysis could not be made.

Volatiles of Body Fluids within an Individual

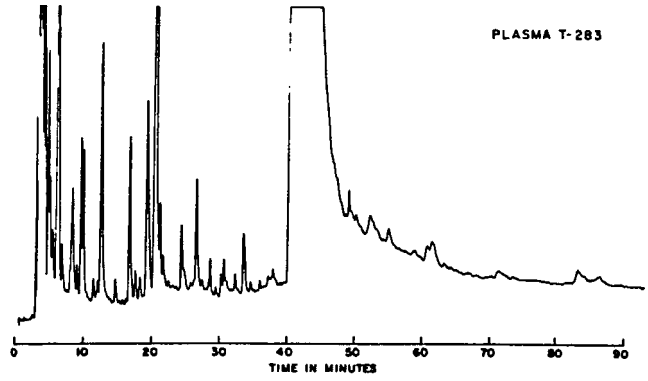
In several cases, all four body fluids studied were available from an individual postpartum female. The analyses were carried out as described earlier. Figure 16 shows the volatile analysis of the body fluids. It appears that the classes of compounds obtained by ether extraction are not equally distributed among the fluids studied, since varying aliquots were required for comparable chromatograms. Benzyl alcohol was present in all fluids analyzed by mass spectrometry. With few exceptions, the volatile compounds varied with the fluid.

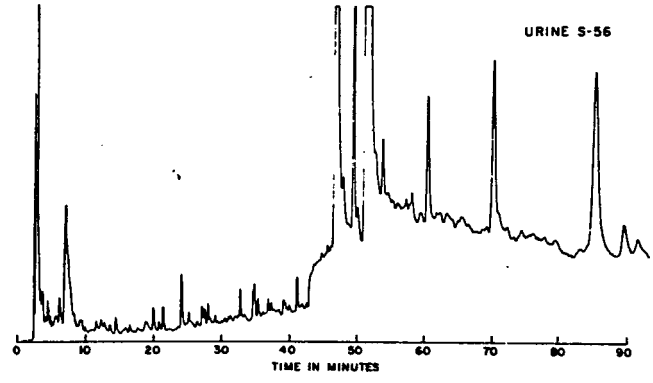
Figure 17 shows the volatiles of body fluids of an individual who delivered by the natural childbirth method. As above, comparison within the individual demonstrates very few similarities. Reports have shown that the volatile profile of diseased patients are significantly altered from normal (41,21). However, comparison of the postpartum in Figure 16 who underwent fetal delivery by anesthetic administration and the natural childbirth postpartum indicates that no alteration occurs from drug administration. The types of drugs used during childbirth are barbiturates and valium for relief of labor pain, and anesthesia for delivery. It must be noted, however, that the drugs are not administered chronically.

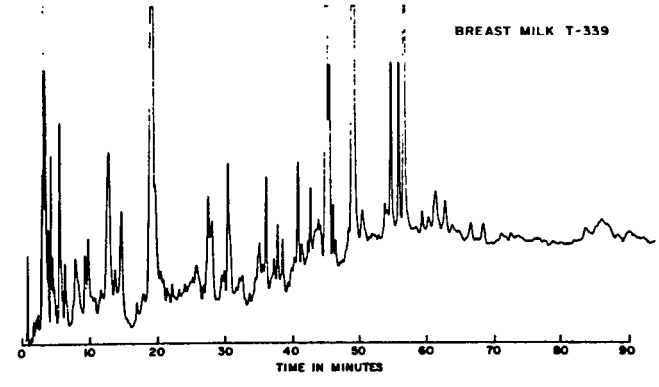
FIGURE 16

High Resolution Gas Chromatographic Profile of an individual Postpartum's Plasma Volatiles, Urinary Volatiles, Breast Milk Volatiles, and Amniotic Fluid Volatiles.

Gas Chromatographic Conditions as specified in Experimental Section of Text.


PLASMA T-283
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


URINE S-56
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


BREAST MILK T-339
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

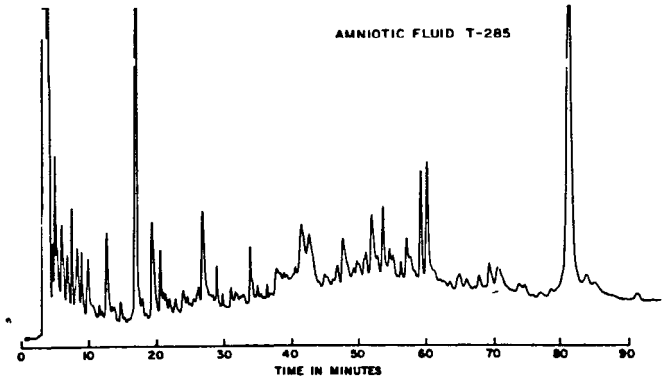
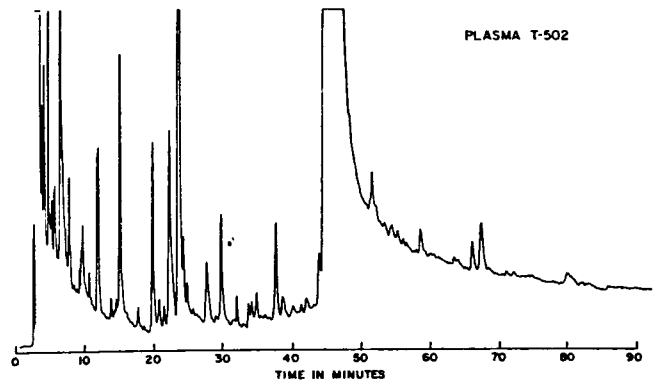
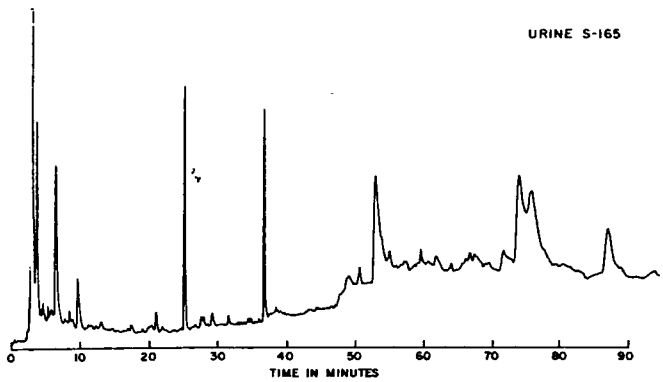

AMNIOTIC FLUID T-285
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

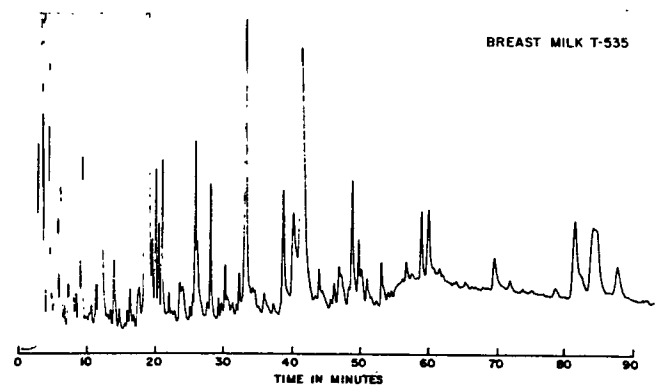
FIGURE 17

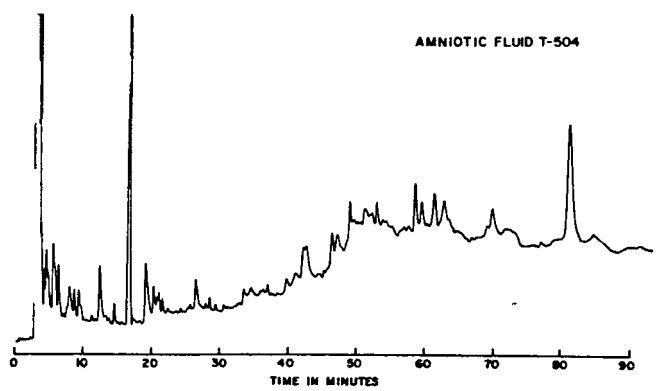
Volatile Metabolites of Four Body Fluids studied from Natural Childbirth Postpartum Female.

Gas Chromatographic Conditions as specified in Experimental Section of Text.


PLASMA T-502
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


URINE S-165
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


BREAST MILK T-535
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


AMNIOTIC FLUID T-504
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

Maternal-Neonatal Pair

In the cases where maternal-neonatal pair plasmas and urines were available, analysis was carried out and the profiles compared. The results shown in Figure 18 indicate that no unique congenital pattern is present to link a maternal-neonatal pair. The urines usually vary with individuals. Since the samples were collected on day one, the benzyl alcohol elevation is evident in plasma of both mother and her baby.

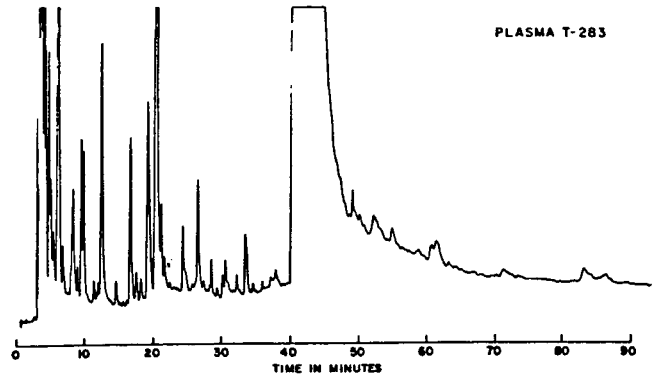
Twins

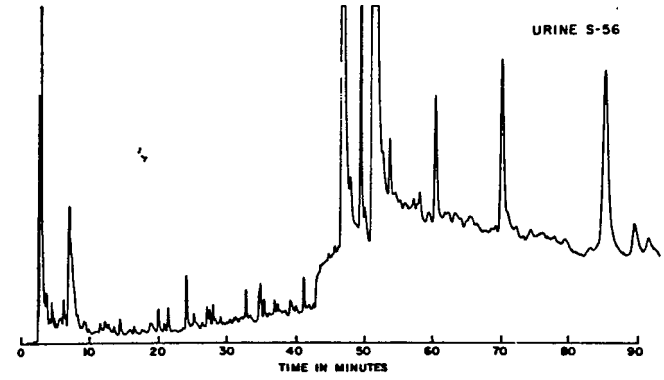
The plasma and urine specimens of several sets of twins were analyzed. Figure 19 shows, once again, that only comparisons as individuals can be made since plasma to plasma are similar and urines vary. Urine S-41 does have a larger number of volatile metabolites than his brother M-51, but no significance can be given. Plasma T-171 and urine M-51 are from baby boy "A" and the remainder from baby boy "B".

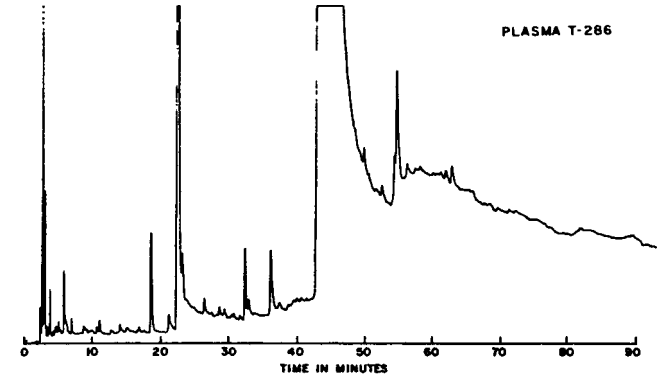
FIGURE 18

Plasma and Urinary Volatiles of Maternal-Neonatal Pair

Gas Chromatographic Conditions as specified in Experimental Section of Text.


PLASMA T-283
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


URINE S-56
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


PLASMA T-286
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

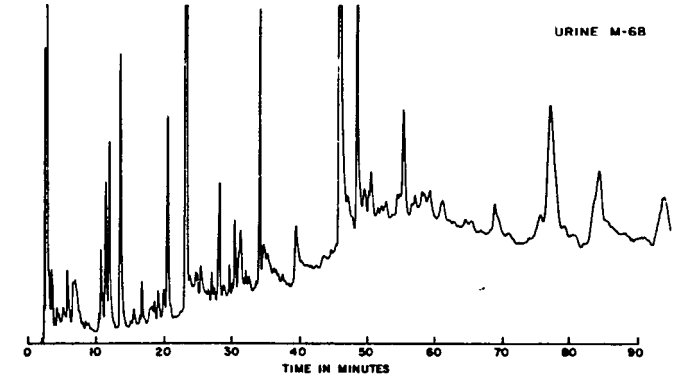
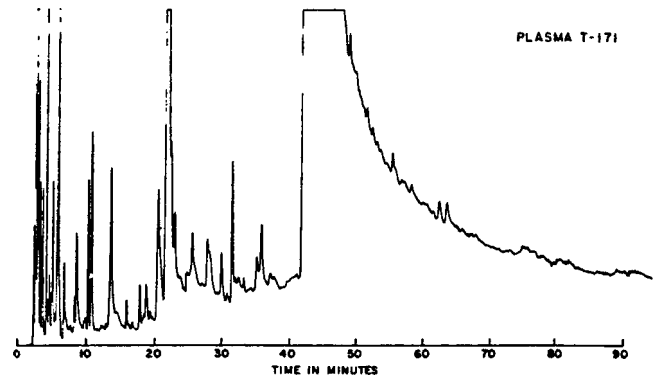
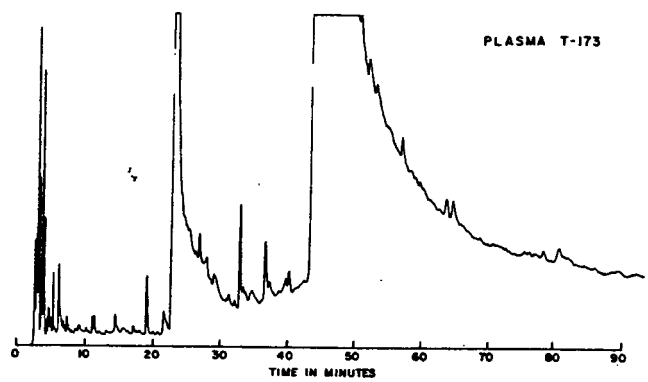

URINE M-68
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES

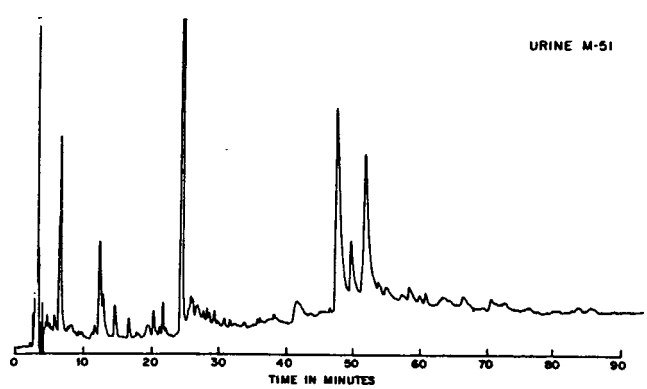
FIGURE 19

Plasma and Urinary Volatiles of Male Twin Neonates

Gas Chromatographic Conditions as specified in Experimental Section of Text.


PLASMA T-171
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


PLASMA T-173
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES


URINE M-51
0
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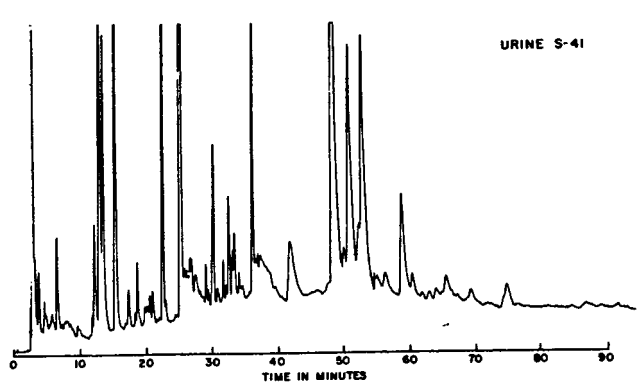
90
TIME IN MINUTES


URINE S-41
0
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TIME IN MINUTES

CHAPTER V

CONCLUSION

CONCLUSIONS

A micro-extraction procedure for the analysis of volatile metabolites by high resolution gas chromatography has been studied. Its use as a diagnostic procedure for human pathological disorders is plausible, since it requires small sample volumes, which are usually attainable, and is rapid. The body fluids tested chosen by availability were plasma, urine, breast milk, and amniotic fluid. A total of 22 plasmas, 30 urines, 9 breast milks, and 6 amniotic fluids were analyzed.

The plasma volatiles of all individuals tested are very similar. The postpartum mother and newborns had elevated amounts of benzaldehyde and benzyl alcohol, proposed as metabolites of the catabolism of phenyl ethyl amine secreted in large amounts during labor due to estrogen stimulus. Even though the volatile constituent content is the same, the remainder of the metabolites are also more concentrated than normals, presumably due to the metabolism acceleration incurred during pregnancy, labor, and delivery.

The urinary volatile metabolites obtained from different individuals are unrelated, presumably due to varied dietary habits and degree of physical activity. Breast milk volatiles of different postpartums are almost identical regardless of day of collection after lactation begins. Amniotic fluid volatile concentration is very low due to volume contribution by fetal excretions.

A comparison of body fluid volatiles within an individual indicates that the metabolites are not equally distributed. One interesting aspect of this study is that in a comparison of all four body fluid volatiles of two postpartum females, one delivering with the aid of anesthetic and the other natural childbirth, no obvious differences are detected. Due to the infrequency of natural childbirth deliveries of patients willing to release specimens for analysis, only one such series was analyzed, so that any definite statements that drug administration does not change the volatile profile would require additional study. It must also be kept in mind that the drug administration during labor, delivery, and for a short postpartum period is not chronic, but only taken as needed. Bassette and co-workers (5), using headspace analysis for urine, blood, and milk in cows, reported volatile metabolite concentration to increase in the order milk, blood, and urine. This study, using the extraction procedure, obtained values in reverse order for human specimens.

The ethereal extraction procedure is facilitated by a salting out of the volatile metabolites with ammonium carbonate. Those characteristic are ketones, aldehydes, and alcohols. The method needs additional study as many variables exist. Some suggestions would be to use the same concentration tube and glass wool for each analysis, and to be certain that helium flow for ether solvent stripping remain continuous throughout. It would be advantageous to keep samples and centrifuge cold at all times and assure that equal amounts of ether be removed upon extraction. In this study, due to limited time, many different tubes were used, but

the glass wool weight remained the same. The volume of ether recovered varies with protein concentration among individuals; larger protein content causes thicker emulsions which are difficult to separate by centrifugation. Even with the above drawbacks, the ethereal extraction of volatiles from small volumes of plasma, urine, breast milk, and amniotic fluid for analysis by high resolution gas chromatography can be obtained reproducibly.

The micro-extraction method of volatile metabolites for analysis by gas chromatography is promising as a diagnostic tool in the medical field. A direct profile comparison of a potential pathological disorder patient to that of screened normals could detect disease early and possibly reduce fatality rates. Several areas in which the method could possibly aid early disorder detection are cancer and epilepsy. Failure of this method is the reluctance of potential patients to volunteer for analysis.

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