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Report No. IITRI-U6012-4
(Final Report)

DETECTION AND IDENTIFICATION OF CHEMICAL SIGNATURES

Ballistic Research Laboratories

Report No. IITRI-U6012-4
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DETECTION AND IDENTIFICATION OF CHEMICAL SIGNATURES

September 16, 1964, through September 15, 1965

Contract No. DA-11-022-AMC-1775(X)
IITRI Project U6012

Prepared by

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FOREWORD

This is Report No. IITRI-U6012-4 (Final Report) on IITRI Project U6012 (formerly C6046), entitled "Detection and Identification of Chemical Signatures." The work was performed by IIT Research Institute for the Ballistics Research Laboratories, Aberdeen Proving Ground, Maryland, under Contract No. DA-11-022-AMC-1775(X). This report covers the period from September 15, 1964, through September 14, 1965.

Personnel who contributed to the research effort included A. Dravnieks, B. K. Krotoszynski, H. Neumann, and J. Frerichs.

Data are recorded in Logbooks C15225, C15351, C15509, C15647, C15665, C15955, C16033, and C16040.

Respectfully submitted,

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ABSTRACT

DETECTION AND IDENTIFICATION OF CHEMICAL SIGNATURES

This investigation was initiated to study human airborne chemical signatures. A novel apparatus was designed and constructed with inner surfaces of glass and Teflon to permit close control of experimental environment and to monitor humidity and oxygen content. An efficient signature collection system was developed, and techniques of sample acquisition and gas chromatographic analysis were established.

The results of signature collection experiments performed on selected human subjects revealed several important features. Seven signature components were found to be common to white, Negro, and Indian males and a white female. It is probable that ethanol, butanol, acetone, and pyruvic and lactic acids are among the common components. Other compounds were found to be common to selected pairs of subjects compared. Some components were characteristic only of a certain individual among those studied and either do not occur in others or exist below the detection limits. Chemical signatures from the same white male individual obtained 14 days apart exhibited large similarities. Less significant similarities were observed between the latter signatures and those from other subjects.

The major fraction of the signature components was found in water condensate. Many of these components were also represented in the equilibration trap. The volatility of the majority of the components corresponded to those of the organic compounds with up to 16 carbon atoms. Characteristic features of airborne chemical signatures can be obtained by the equilibration sample collection technique developed without interference from excess of water vapor. Total production of organics emitted from a resting and partially nude subject, with the effluents in breath included, amounts to 0.5 g/hr. The lowest number of signature components found in effluents from an individual was 24 and the highest was 44. The actual number depends on the resolution of the instrument used and is expected to be considerably higher.

Prospects exist that chemical signatures can be useful not only in the detection of humans but also in the identification of types of humans and individuals. Further improvements in techniques are desired, especially acceleration of the tests and processing of the water condensate and data, so that the gap between the knowledge of the nature of chemical signatures and their utilization for future military devices can be narrowed. It is suggested that the detection of humans should be based on several signature components rather than on one, including those of low volatilities.

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DETECTION AND IDENTIFICATION OF CHEMICAL SIGNATURES

1.0 INTRODUCTION

Many military tasks could become possible if the technology of rapid analysis of air for small amounts of complex mixtures of vapors advances to the point where devices could match or surpass the capabilities of trained animals to detect and identify by odor. Such tasks include the detection and location of hostile personnel, the identification of their nature, the discrimination between friendly and hostile personnel, the tracing of past movements (past trajectory in time and topography) of hostile personnel that started from a location already vacated, and the objective reconstruction of the past activities of captured suspects.

Two major obstacles have hindered the progress toward these long-term objectives. First, the knowledge of the composition and amounts of the complex airborne effluents from humans is very meager, and almost nothing is known about the variation of the effluent composition among individuals, races, sexes, dietary factors, etc. Without such information it is difficult to plan for technologically feasible detection and identification devices. Second, even if this knowledge were available, until recently there was little hope that sufficiently sensitive systems and detectors could become available to detect routinely

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many substances at concentrations of 10^5 to 10^9 molecules per cm^3 of air. Recent advances in detectors promise to lower this technological barrier very significantly. Thus, a microwave-heated argon plasma detector developed by Cooke et al at Cornell University (Ithaca, New York) is claimed to detect less than 5×10^{-15} g of hydrocarbon, corresponding to 10^7 to 10^8 molecules.

Since we are rapidly approaching the required sensitivities, the need to know more about the airborne constituents of human effluents gains in urgency. This project was conducted to accumulate the relevant knowledge.

Humans generate effluents in the form of vapors and particulate matter. The primary sources of effluents are skin, hair, and breath. Vapor effluents from the skin have been studied recently (ref. 1), and pyruvic acid, lower alcohols, and acetone were identified among the components of the vapor. The same studies indicated that similar compounds occur in breath. The particulate effluents are fragments of skin and hair and droplets of saliva. Past studies on the variation of the composition of effluents from individual to individual yielded inconclusive results (ref. 2).

The ability of trained animals to distinguish individuals on the basis of human odors hints that there are differences in the composition of human effluents. Previous studies of human sebum swabbings at IITRI (ref. 3) indicated that analytically detectable differences occur in the composition of sebum when races, sexes, and balding or full-haired males are compared.

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This evidence strongly indicated that human effluents could consist of some common characteristics produced by all humans and, in addition, contain features such as other compounds or different ratios of the concentrations of compounds specific to sex, race, and individual traits. Thus, the airborne chemical signatures (a term used to describe the characteristic compositions of the effluents) should be able not only to provide information on the presence or past presence of a human, but also to describe the individual in more detail.

The objective of this project was to investigate the chemical signatures of several types of individuals and to establish the degrees of similarities and differences in their airborne chemical signatures. Emphasis was placed on the vapor components. To obtain the chemical signatures, new equipment and new experimental methods had to be developed, with emphasis on elimination of vapor-adsorbing and difficult-to-clean components and of artifacts that plague work with minute amounts of complex organic mixtures.

Vapor components of the chemical signatures of humans can originate from metabolic processes in the body and microbiological activity on the surface of the skin, hair, and mouth and from components in diet or produced by digestion of the foods. Additional components can come from the environment in which

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the individual has been previously; extraneous vapors absorb and dissolve in body tissues, skin lipids, etc., and are gradually released into the new environment. Thus, chemical signatures are expected to reflect much information about the individual, his state of health, hygiene, dietary habits, occupation, and environment in which he lives. These complex factors require a comprehensive study that was impractical within the time limitations of the project. Hence, the selection of the human subjects was limited to white females and males of several races.

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2.0 EXPERIMENTAL APPROACH

2.1 Principles of Experimental Approach

Previous work on chemical signatures of explosives (ref. 4) and heated bearings and wire insulation (ref. 5) indicated that avoidance of irrelevant contamination by materials in the sampling and and analysis system is one of the most important requirements in the initial studies of any airborne chemical signature. Plastics, coatings, and sealants themselves contain components that are slightly volatile and can serve as continuous chemical emission sources. In addition, these materials and various atmospheric grease and oil films present on solid surfaces readily dissolve organic vapors occurring in air and release these slowly into pure air or gas streams during the collection and processing of chemical signatures.

In order to avoid artifacts introduced by these effects, it was decided to isolate the human in a high-purity controlled environment for the duration of the sample collection. The isolation system consisted exclusively of inert and easily cleaned surfaces High-pressure tank air, additionally purified by cooled active carbon, was used to supply breathing oxygen. The construction materials were Pyrex glass and Teflon, with the exception of some copper tubing for the air inlets. This system was always thoroughly cleaned with acetone and dried with artificial heat to eliminate a partial transfer of one subject's chemical signature, through adsorption on the walls, to the next

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experiment. To keep the person in the glass cell comfortable, an airflow of 0.75 to 1.0 liter/sec was needed, which is much in excess of the breathing requirement alone.

Several techniques were considered for collection of the human-related effluents from the air flowing out of the cell. The techniques can be subdivided in three principal groups:

(1) Total trapping

(2) Equilibration sampling

(3) Combinations of the above.

In total trapping, components of the chemical signature are adsorbed on a high-surface material or totally condensed by cooling. These traditional techniques have several serious drawbacks. Energies and heats of adsorption of organics on solids, including carbon and other conventional adsorbents, are considerably higher than heats of vaporization. Hence, although trapping by adsorption is efficient, the desorption for analysis is slow and requires heating to several hundred degrees Centrigrade.

Contact with the catlytic surface of the adsorbent at elevated temperatures undoubtedly results in profound changes in the organic components of the chemical signatures, thus distorting their nature. Also, it is practically impossible to obtain a good blank, free of volatile contaminants, from a solid adsorbent at conditions to which the adsorbent must be subjected during desorption of the chemical signature.

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Total condensation by cooling results in condensing water together with the organic components. Since water constitutes several percent of air, the total condensation increases the mole fraction of the organic components by a factor of 30 to 40 only. The great excess of water in the sample makes it impossible, with the present techniques, to analyze such samples for the minor components of the chemical signature, since a sufficiently large water sample cannot be injected into the gas chromatograph.

The equilibration technique of air sampling for organic components consists of bringing the effluent air in contact with thin oils or greases. Airborne vapor of a compound dissolves in the film until an equilibrium is reached between the concentration of the compound in air and in the film, according to the equation (ref. 6):

$$n_c = n_a \cdot \alpha = n_f \frac{62370\ T\ d}{M} \cdot \frac{1}{\gamma} \frac{1}{p}$$

Here n_f and n_a are the concentrations, molecules per cm^3, of the compounds, molecules in the film and in the air; α, the partition coefficient; T, the absolute temperature; d, the density and the molecular weight of the film substance; p, the saturation pressure of the compound in pure form at T; and γ, the activity coefficient of the compound when in the solution in the film.

The task of the film is not to trap all vapors fully, but rather to reflect their concentration in air by collecting the number of molecules until the level of n_f approximated by the above equation is reached. By selecting a film substance that

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has a small α for water and large α for the organic components of the effluent, it is possible to sample the chemical signature while capturing only negligible amounts of water. A solution of the olfactory signature in film results and can be termed an "olfactoprint." The olfactoprint contains a reflection of the chemical signature, analogous to a photoprint, which is the result of the action of photons on a photon-capturing substance.

The concentration of each component at low concentration levels in the film is approximately proportional to this component's concentration in air, but the proportionality factor is $1/\gamma p$. Thus, the lower the volatility of the component, the higher the concentration in film at the same n_a. The activity coefficient is close to unity when the component is chemically similar to the film substance, larger than unity if they are dissimilar, and smaller than unity if a compound formation, including hydrogen bonding, can occur between the vapor component and the film. A typical value for d is approximately 1000 for hexane in di-isodecyl phthalate at 25°C. To minimize water vapor pickup, nonpolar films must be used.

To accelerate equilibration between the film and air, the film thickness should be small, since most of the kinetic hindrance is in the diffusion of the vapor molecules through the film. In the form adapted for the present work, when fast airflow rates were used, the films were placed inside glass tubes wound in a spiral.

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Elution of the vapors from the film requires only a moderate temperature elevation, since the heats of solution are comparable to heats of vaporization. The temperature range is limited by the possibility of decomposition of the film material itself. Thus, as film materials, most esters were unsatisfactory because of continuous slow decomposition or perhaps oxidation or hydrolysis. For the Apiezon L and other film materials used, 80°C was the highest temperature at which vapors generated by the film itself did not interfere with the analysis.

A combination of equilibration and total trapping techniques was the method chosen for this work. The effluent air, at a flow rate of 0.5 to 1.0 liter/sec, is first passed through an equilibration trap in which representative samples of the organic constituents of the vapors accumulate in the films, with a minimum of water vapor. The air then flows through one or more series of cold traps in which water and the organics are condensed. Thus, advantage is taken of the simplicity with which organics can be analyzed in the absence of water and of the high trapping efficiency of the cold traps.

2.2 Apparatus for Study of Chemical Signatures

In order to obtain authentic and adequate samples of human chemical signatures, a special apparatus was designed and constructed in our laboratories. In the design of the apparatus care was taken to provide a simple, reproducible, and safe experimental environment as well as some degree of comfort for

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the human subject. The apparatus consists of three units: the airflow train, the glass cell assembly, and the chemical signature collectors. A description of the units and their functions follows.

2.21 Airflow Train

The components of the airflow train and their arrangement in the system are illustrated in Figure 1. These parts include:

(1) A source of hospital-grade breathing air.

(2) A high-precision shielded spherical float flowmeter, individually calibrated with air, purchased from the Cole-Parmer Company, Chicago, Illinois. The flowmeter covers the range from 3000 to 77,000 ml of air per min and exposes the fluid to glass, Teflon, and polypropylene only; the fluid does not contact the acrylic protection tube.

(3) One 4000-lb pressure gauge and two 100-lb pressure gauges.

(4) Four blunt-point needle valves.

(5) A refrigerated active carbon filter.

(6) A glass column, with a 2-in. ID and 3 ft long, packed with ceramic fragments.

(7) Two hygrometer sensors connected to a meter through a multiselector switch. The direct-reading electrohygrometer (Lab-Line Instruments, Inc., Chicago, Illinois) indicates relative humidities from

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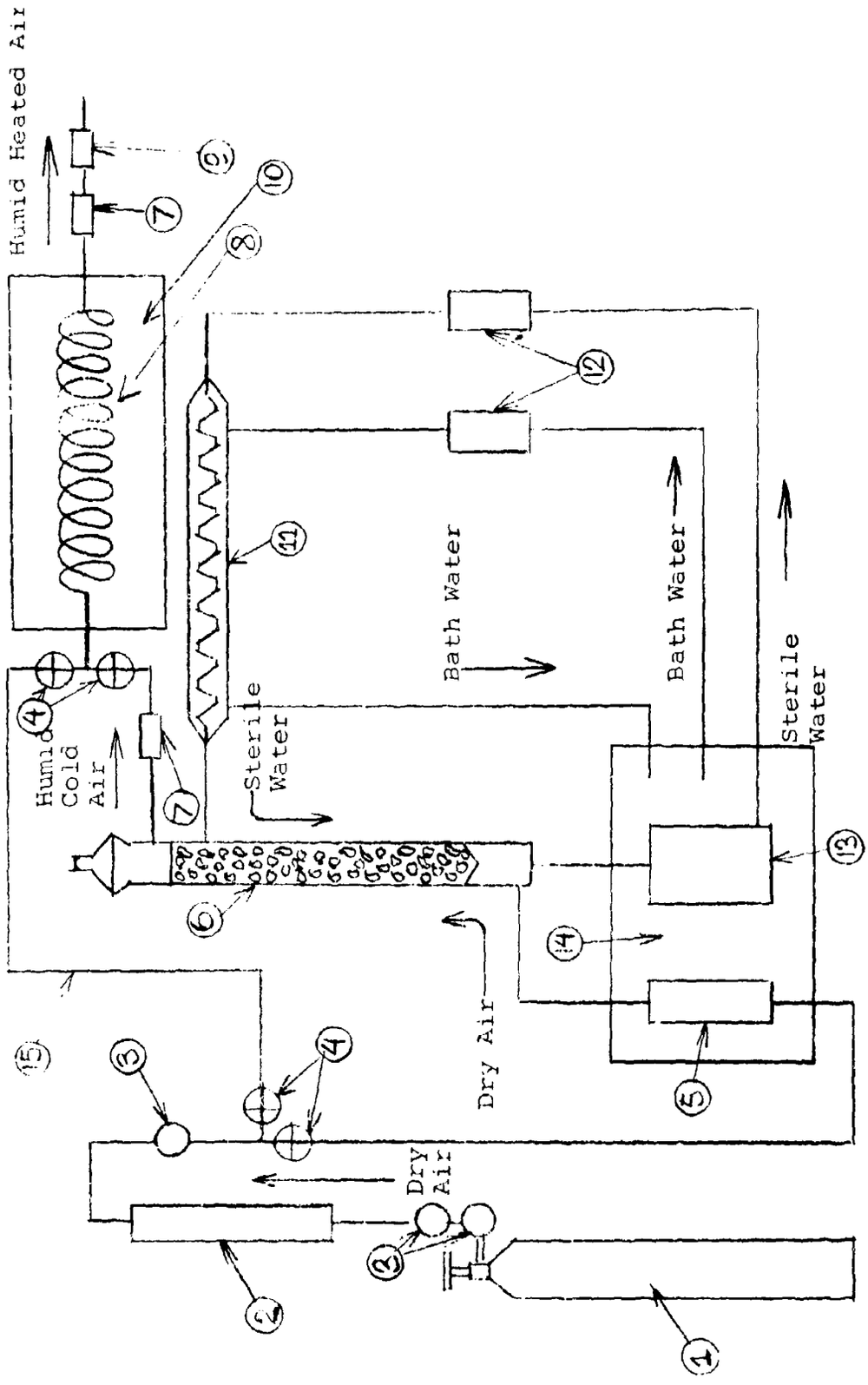

Figure 1

AIRFLOW TRAIN

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30 to 95% with an accuracy of ±5%. Humidities can be measured under temperature conditions ranging from 32 to 180°F.

(8) Coiled copper tubing with a 1/4-in. OD and 150 ft long.

(9) A Weston dial thermometer.

(10) A heating Magni Whirl water bath (made by the Blue M Electric Company, Blue Island, Illinois, and purchased from the Cole-Parmer Company, Chicago, Illinois) with operating temperatures ranging from room to 100°C and temperature uniformity within ±0.25°C. The bath is equipped with dual microtrol settings for ready selection and duplication of any two particular temperatures. In addition, it utilizes a magnetic stirring feature consisting of an electromagnet, a timing mechanism to pulse the magnet, and a stainless steel perforated plate moving up and down as the timing mechanism energizes and deenergizes the magnet.

(11) A Graham coil-type glass condenser.

(12) Two oscillating pumps (Cole-Parmer Company, Chicago, Illinois).

(13) A glass sterile water reservoir.

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(14) A Magni Whirl refrigerated bath (manufactured by the Blue M Electric Company, Blue Island, Illinois, and purchased from the Cole-Parmer Company, Chicago, Illinois). This bath is constructed from the Magni Whirl water bath with the addition of a compressor system, and it can be operated in the range from 0 to 100°C with the temperature constancy within $\pm$0.2°C.

The airflow train provides the glass cell assembly with pure air at a constant flow rate and with a particular relative humidity and temperature. For this purpose, the dry air at a measured flow rate is passed first through the refrigerated carbon filter to remove the odorous compounds. The pure precooled air is then saturated with water in its upward passage through the vertical glass column, which is constantly exposed to a downward flow of sterile water cooled to the air temperature. The water-saturated air is finally heated to the desired temperature during its passage through the long copper tubing kept at this temperature. Before the air is allowed to enter the glass cell assembly, its relative humidity and temperature are recorded.

The water used in the air saturation process is delivered to the humidifier column by means of the oscillating pump from the reservoir kept in the refrigerated bath. Also, the bath liquid used for cooling the coiled condenser is continuously circulated by the pump.

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2.22 Glass Cell Assembly

The glass cell assembly consists of two main parts, the glass pipe and the loader; its schematic diagram is shown in Figure 2.

The glass pipe (Figure 2, No. 1), made in England by Q.V.F. Limited and purchased from Fred S. Hickey Corporation, Schiller Park, Illinois, is 60 in. long and 18 in. in nominal ID, and has a minimum wall thickness of 0.12 in. It supports a maximum internal working pressure of 6 psi.

To minimize the danger of breakage, the pipe was uniformly supported by means of a polyurethane foam cast (Figure 2, No. 4) made at IITRI from a foam of density 4 lb/cu ft. A sheet of neoprene, 1/8 in. thick, was placed on the outer surface of the pipe before cast fabrication so that the pipe could be removed if necessary.

To permit the attachment of the airflow train to the glass pipe, one end of the pipe was provided with a glass reducer (Figure 2, No. 2), 18 in. over 2 in. and 16 in. long. The reducer remains connected to the glass pipe throughout the experiment. The other end of the glass pipe was permanently equipped with a 12-in. glass pipe section (Figure 2, No. 3) to increase the usable length of the glass system.

The glass pipe reducer and the pipe section are connected to the pipe by means of cast iron backing flanges held together

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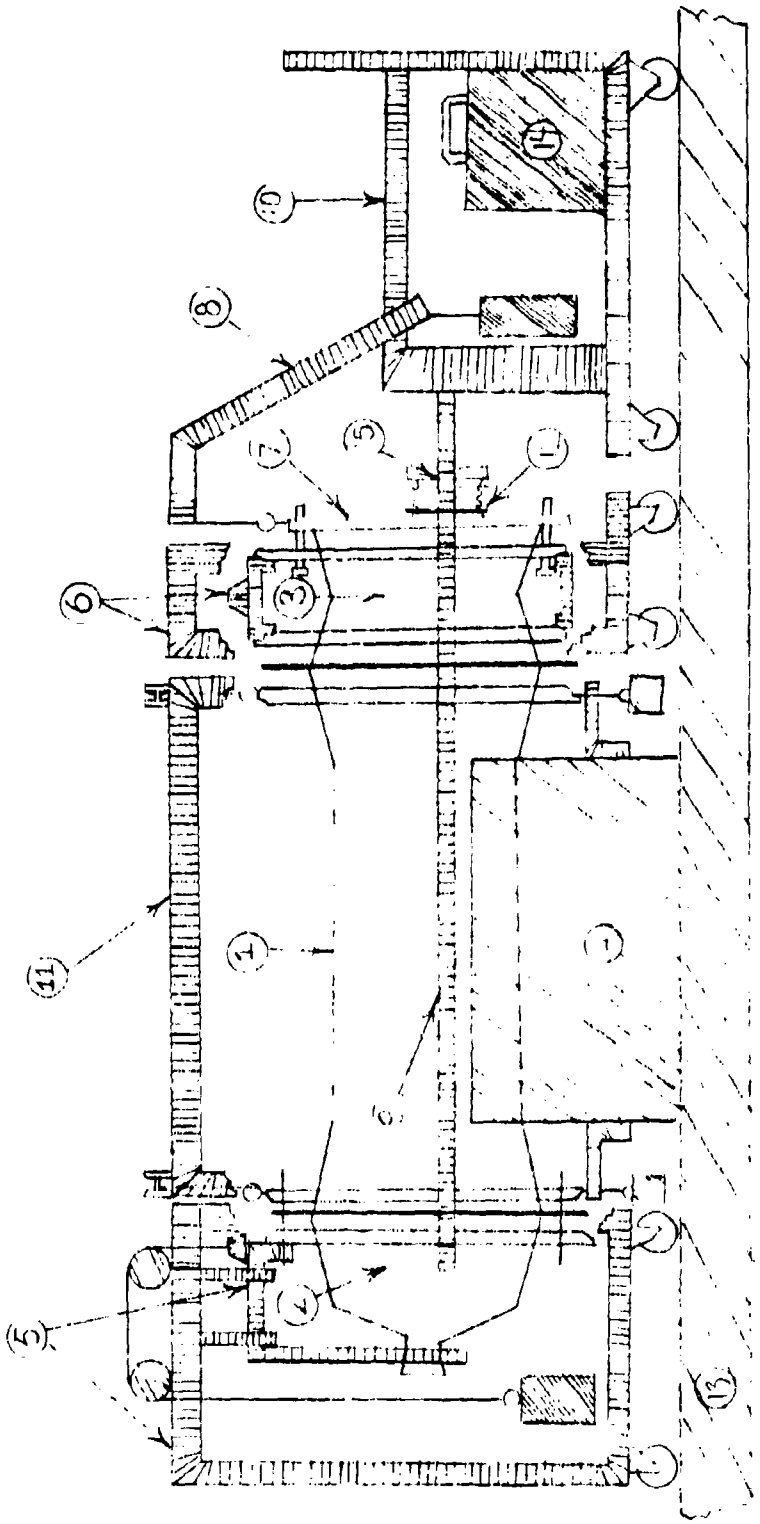

Figure 2
GLASS CELL ASSEMBLY

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by metal bolts and nuts. To eliminate glass-to-glass and metal-to-glass contact, compressed asbestos fiber gaskets, 1/16 in. thick, covered with Teflon gasket sheaths, also 1/16 in. thick, and asbestos inserts are used in each joint. The stresses induced upon the glass pipe by the weight of the metal flanges, the pipe reducer, and the section are excluded by using specially designed and constructed metal supports (Figure 2, No. 5, 6, 8, and 11). The principle underlying the construction of the supports utilizes a simple arrangement of counterweights, pulleys, and nylon ropes. The supports of the reducer and the extension can be easily rolled on the floor (Figure 2, No. 13).

The loader assembly (Figure 2, No. 10, and Figure 3) was designed and constructed to introduce and support a human subject in the glass cell and to remove the subject at the end of the experiment. The assembly was designed to minimize the man-to-glass contact and to avoid exposing the air to surfaces other than glass and Teflon. The principal parts of the assembly include a stretcher (Figure 3, No. 9), a Teflon-coated aluminum cover plate (Figure 3, No. 7), a 4-in. Teflon bellows (Figure 3, No. 12), a cover-plate support (Figure 3, No. 8), and a movable counterweight (Figure 3, No. 14).

The stretcher consists of a welded rectangular Teflon-coated frame construction made from 1-in steel pipe, 75 in. long, and 13-5/16 in. wide. The metal frame is covered with a network of strips of Teflon to provide the human subject with some degree

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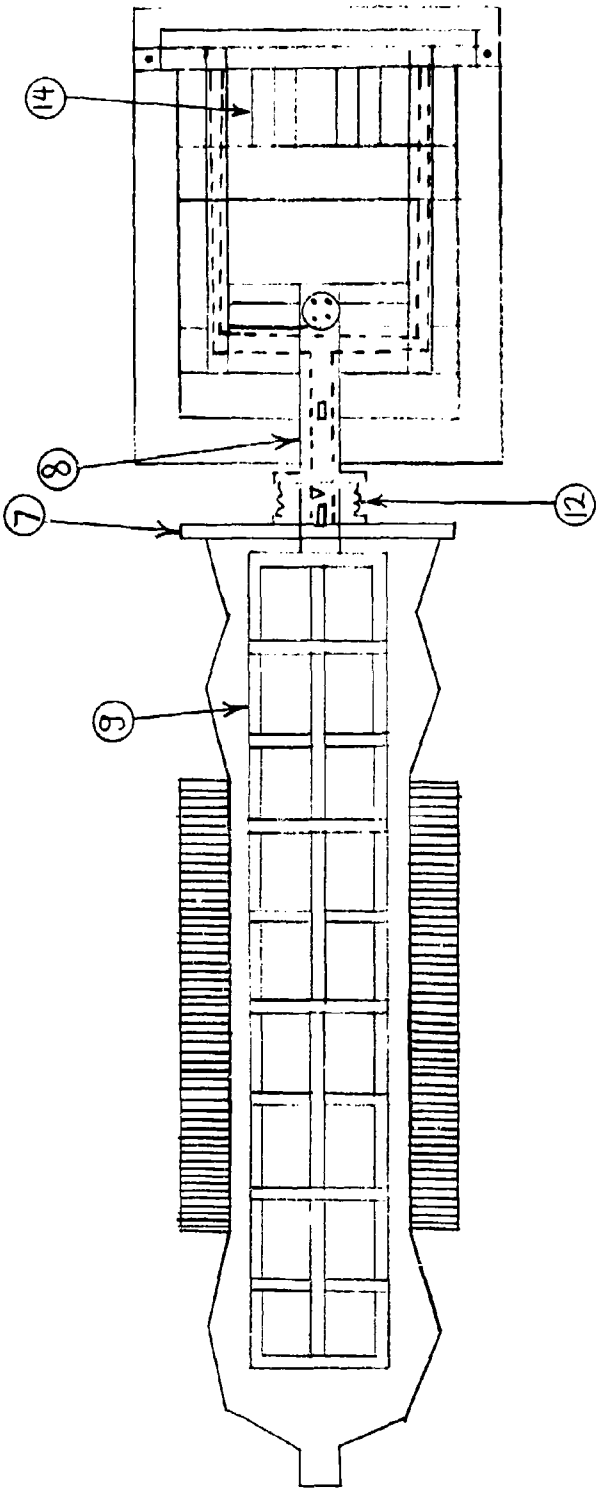

Figure 3
LOADER ASSEMBLY

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of comfort. The stretcher is mounted on the outside supporting frame by means of a 1-1/2 in. bore steel pipe. This joint is made using a 4-in Teflon bellows One metal flange of the bellows is connected to the flange of the joining pipe (Figure 2, No. 15), and the other flange is connected to the cover plate. The Teflon bellows is used to provide flexibility needed for a safe support of the human subject inside the closed glass cell.

The aluminum cover plate, 0.5 in. thick, is provided with an independent counterweight support and is connected to the glass pipe through the metal flange, nuts, and bolts. In addition to the opening for the stretcher-supporting steel pipe, the plate is equipped with two 0.330-in. ID outlets, one leading to the collection system and the other to the safety valve. The entire loader assembly with the man on the stretcher can be moved and guided on tracks mounted on the floor in front of the system inlet.

2.23 Chemical Signature Collectors

The collectors of chemical signatures consist of two spiral Pyrex traps, each 10 in. long and 8-mm ID, and a glass reservoir connected in series with one of the coils. The inner surface of one of the traps is coated with a thin film of Apiezon L grease (Metropolitan-Vickers Electrical Company, Ltd.); the surface of the other trap contains a thin film of diethylene glycol adipate, DEGA (Wilkens Instrument and Research, Inc.).

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The selection of the stationary phases was based on their polar properties, which are intermediate between paraffins and polar liquids. By virtue of these properties and in view of the presence of both polar and nonpolar components in chemical signatures, the use of stationary phases was expected to secure the dissolution of a maximum amount of each of the various components of the original mixture, to store this amount, and to allow its ready transfer into a flash injector for direct chromatographic analysis. During the signature collection experiments, the coated spiral traps are kept at room temperature.

The second unit of the signature collector system comprises a glass condenser and a glass reservoir, 2 liters in volume; both are kept at a temperature close to the feezing point of water during the collection experiment. The objective of the unit is to collect water as a liquid condensed phase together with chemical signatures carried by or soluble in water.

2.24 Sample Collector-Ejector System

To transfer the entire chemical signature sample from the stationary organic phase of the spiral trap onto the chromatographic column and to secure its reliable and reproducible analysis, a special collector-ejector system was designed and constructed. The system was designed to assure a rapid sample vaporization and its delivery to the heated injection port of the chromatograph. The system consists of two essential components, the sample

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collector-ejector, including an aluminum oven for heating the spiral trap, and the collector flash-heating and -cooling assembly.

The collector-ejector is made of a U-shaped capillary stainless steel tubing, 1/16 in. OD and 17.5 in. in overall length. The flash-heating controls include a separate AC circuit that provides current to the entire collector tubing, a power supply control panel, a recorder, and an independent AC circuit that energizes a coil heater. The collector-ejector circuit is equipped with additional resistors balanced so that both parts of the tubing, that immersed in liquid nitrogen and that kept at room temperature, simultaneously attain the same desired final temperature within a few seconds.

The temperature of both parts of the collector is monitored by thermocouples and registered on the recorder. The coil heater is used to vaporize the liquid nitrogen contained in Pyrex tubing, which is open at the bottom and surrounds the collector, and thus to expel the cryogenic liquid before heating the collector. A special solenoid valve closes the exhaust copper tubing at the top of the glass enclosure before the nitrogen is removed.

The actual injection technique involves three steps: removing liquid nitrogen from the glass tubing surrounding the collector, heating the collector proper, and subsequently cooling the collector. The liquid nitrogen is removed by heating the liquid with the coil heater and closing the exhaust solenoid valve. The injector is heated electrically, and it is then cooled to liquid nitrogen temperature by simultaneously turning

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off both heaters and opening the exhaust valve. Figure 4 is a schematic diagram of the entire assembly.

2.3 Procedures

2.31 Cleaning the Glass Cell Assembly

Before each human chemical signature collection experiment, the inner surface of the glass cell and the cover plate as well as the Teflon-coated stretcher are washed with anhydrous methanol, and the solvent is wiped off with cotton gauze. The glass cell assembly is then closed and the residual methanol removed by heating the glass cell walls externally with an electric blanket to about 60°C and flushing the tube with pure dry air.

2.32 Preparation of the Spiral Traps

A weighed amount of the selected stationary phase is dissolved in 5 ml of analytical-grade chloroform and the clear solution introduced into the glass spiral. The solvent is slowly removed with helium zero gas, which flows through the trap heated at 80°C in the aluminum oven. During this procedure, the trap is slowly rotated to ensure a uniform thickness of the coating film. After the solvent has been removed, the heated trap is flushed with helium zero gas for 1 hr.

The resultant effluents are collected in the metal collector-ejector kept at liquid nitrogen temperature. The effluents are then injected onto the chromatographic column and analyzed. The coating purification procedure is repeated until the observed

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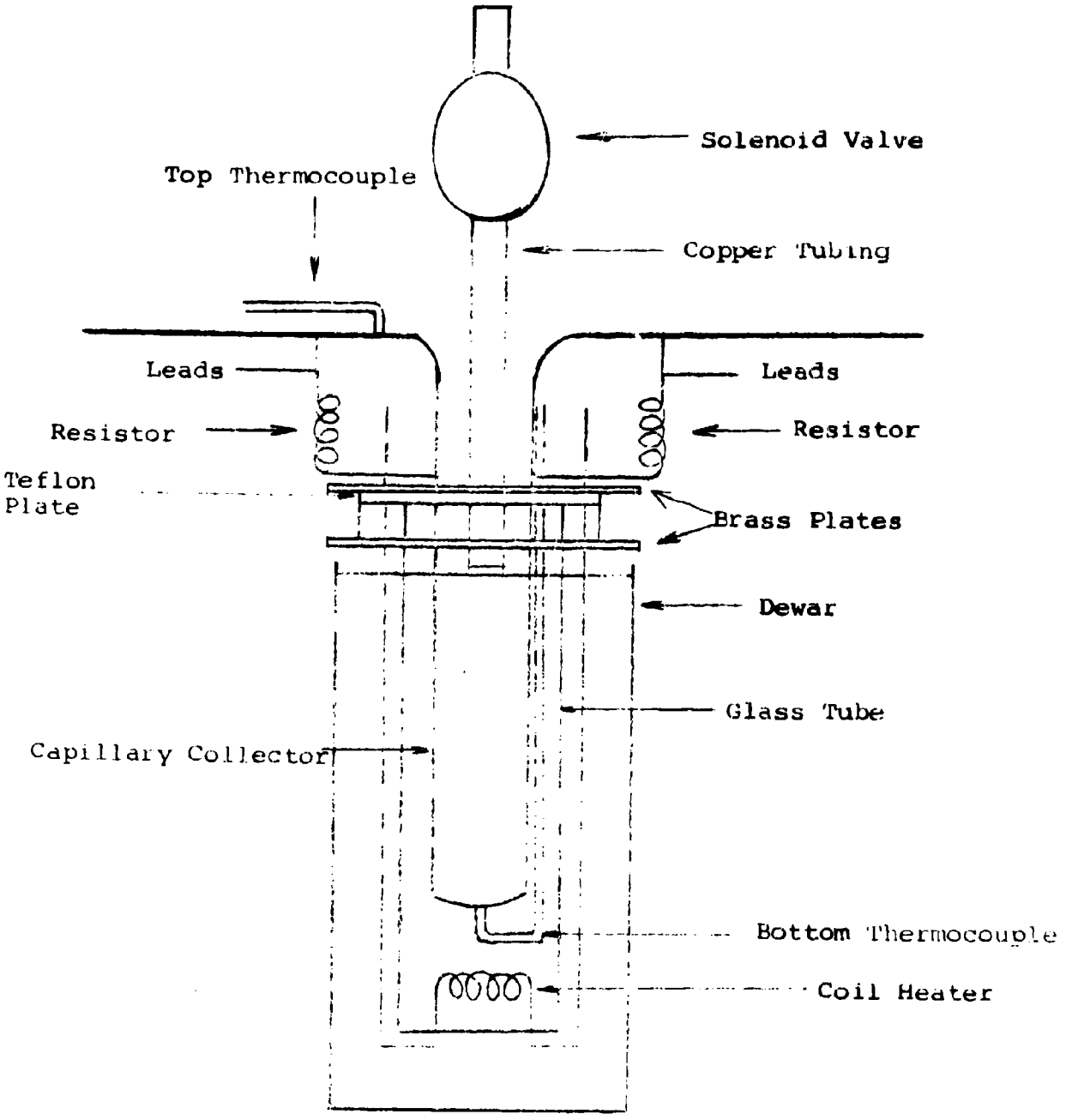

Figure 4

SAMPLE COLLECTOR-EJECTOR SYSTEM

amounts of the contaminants are negligible. These contaminants are removed in order to eliminate their interference with chromatographic peaks of authentic chemical signatures. The purified stationary phase is then immediately employed in the signature collection experiment.

2.33 Establishing the Glass Assembly Chemical Noise

To determine the background contaminants or the chemical noise of the experimental system proper, the prepurified stationary phases residing on the inner surfaces of the spiral traps are exposed to the glass cell assembly under conditions identical to those of the actual experiment on human subjects. The system impurities dissolved in the liquid phases are then transferred into the collector-ejector system, injected onto the chromatographic column, and analyzed. The results of the analysis provide the basis for identifying authentic signatures of human origin. The chemical noise of the system proper is established before each collection experiment.

2.34 Collection of Human Chemical Signatures

After the background determination has been made, the selected human subject, resting on the stretcher in a horizontal position, is closed in the glass cell assembly for 1 hr and provided with air of a measured flow rate. Unless otherwise specified, pure dry air is introduced into the system through an activated carbon filter kept in a cooling bath. The relative

humidity and the oxygen content of the air leaving the system are measured at the outlet of one of the spiral traps, and the temperature inside the system is determined by means of a metal thermometer.

The collection experiments are performed on young healthy human subjects of both sexes and various races. Before their participation in the experiments, the selected subjects are on normal mixed diets. To expose the maximum area of the skin to the airflow, the subjects wear bathing suits during the signature collection period.

2.35 Transfer of Chemical Signatures

At the end of the chemical signature collection experiment, one of the spiral traps is removed from the outlet of the system and closed at both its ends; the other trap (Figure 5, A) is placed into the aluminum oven (B), which is kept at room temperature and which is connected to helium flow system at its inlet (C) and to the collector-ejector (D) at its outlet (E). The spiral trap (F) of the helium system is kept at liquid nitrogen temperature to prevent passage and accumulation of the carrier gas impurities in the collector-ejector during the signature transfer procedure. The carrier gas supplied by the source is independent of the gas flow of the analytical system. Chemical signatures from the stationary phase are transferred into the collector by

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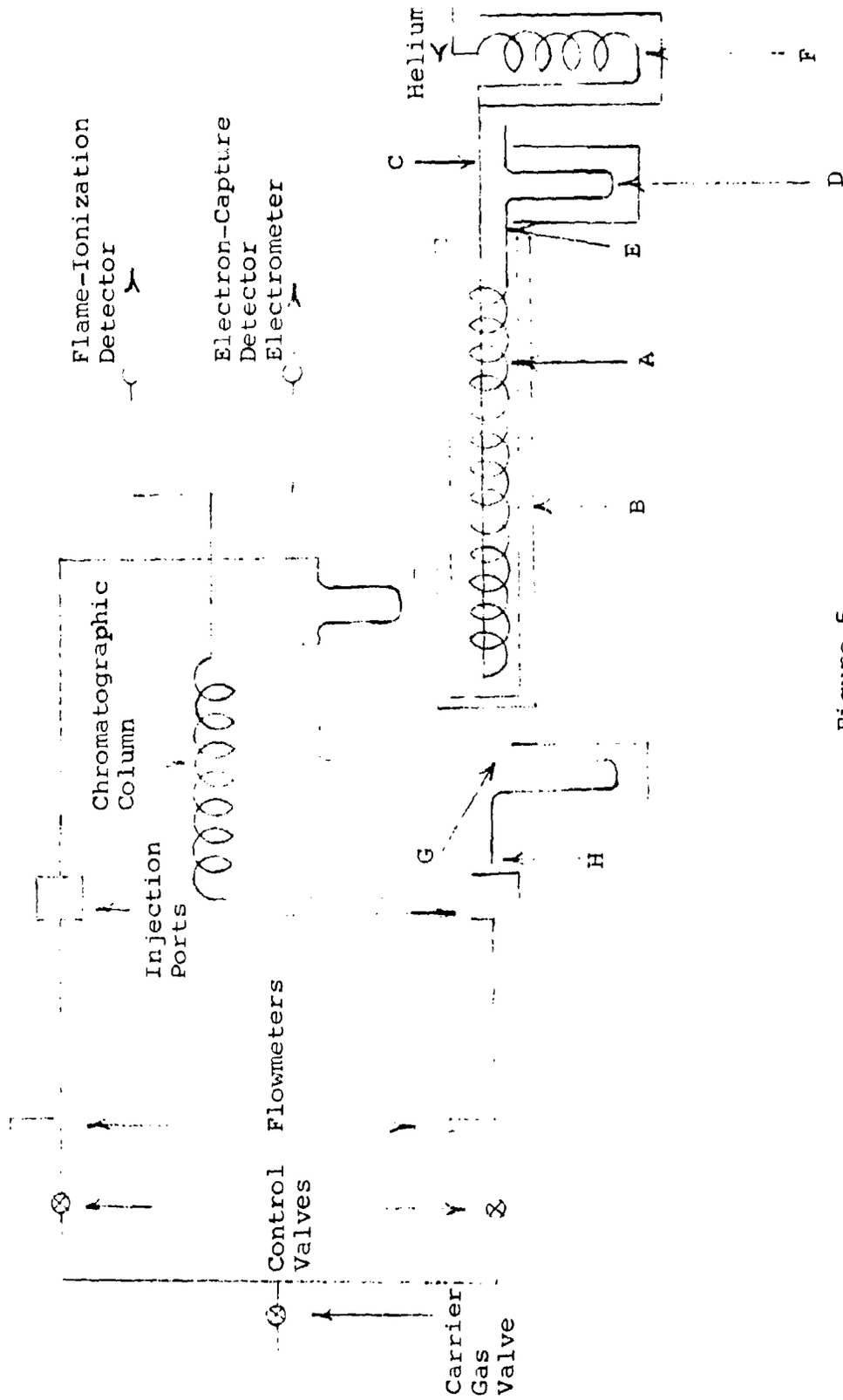

Figure 5

SAMPLE INJECTION AND ANALYTICAL SYSTEM

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heating the glass spiral trap to 80°C and flushing it with helium zero gas. During this procedure, the collector-ejector is kept at liquid nitrogen temperature. The duration of the transfer step is 1 hr.

Before each signature transfer, the collector-ejector system is checked for residual contamination. For this purpose, the system is cooled to liquid nitrogen temperature, and the impurities are discarded into the air by five consecutive thermal ejections. Subsequently, the open end of the system is introduced into the injection port of the chromatograph, and a series of consecutive injections is made until no impurities are detected. The injections are made onto the column kept at 200°C to rapidly elute the materials. The duration of each ejection is 15 sec.

The same transfer and analytical procedures are applied to the contents of the second liquid phase.

2.36 Analysis of Chemical Signatures

At the end of the signature collection period, the collector-ejector, kept at liquid nitrogen temperature, is connected to the helium flow of the analytical system at its inlet (G) and to the injection port of the chromatograph at its outlet (H). The carrier gas flow rate is then established and the signature sample flash-injected onto the chromatographic column.

Unless otherwise noted, the samples were analyzed on a 10 ft, 5 in. x 1/8 in. stainless steel column packed with 5% SE-30 liquid

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phase coated on 60/80 Chromasorb W. The column temperature is programmed at 6°C/min over a range of 24 to 200°C, and the helium zero gas flow rate is 40 ml/min.

The detector oven temperature is kept at 195°C; injection temperature is 240°C; hydrogen pressure is 20 lb; helium 60 lb; and air 5 lb. An Aerograph model 204 two-channel system with hydrogen-flame and electron-capture detectors was used in all the experiments reported.

The contents of the ice-water trap are transferred to a 50-ml glass-stoppered Erlenmeyer flask and their yields determined gravimetrically. An aliquot of an 1 μliter liquid solution of the signatures collected in this trap is directly injected onto the column and analyzed under the same conditions used for the signatures dissolved in the stationary phases.

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3.0 EXPERIMENTAL CONDITIONS

3.1 Water-Saturated and Dry-Air Environment

Initial experimental efforts were directed toward gaining information on the production pattern of chemical signatures from a human subject exposed to two extreme conditions of relative humidity. To this end, a young white healthy male was first exposed to water-saturated breathing air for 40 min (experiment 1). The chemical signatures produced by the subject were collected on a 3.3 mg/cm^2 thick film of Apiezon L and analyzed on a 5-ft SE-30 column heated at the rate of 10°C/min over the range from 40 to 200°C. The airflow rate was 0.75 liter/sec. Its temperature inside the system opposite the chest of the human subject was 31°C, and its relative humidity estimated inside the experimental system was 75%. During the experiment, water condensed on all inner surfaces of the system and the spiral trap. At the end of the experimental period, the human subject showed signs of discomfort.

Experiments 2 and 3, involving the same human subject, were performed in the system supplied with dry air at the flow rate of 0.75 liter/sec. Before entering the system, the air was passed only through activated carbon filter cooled to -12°C. In both cases, the air temperature inside the system was 31°C, and its relative humidity at the outlet of the Apiezon L trap ranged from 52 to 62% and at the outlet of the ice-water-cooled

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reservoir from 40 to 44%. The observed humidity originates from the metabolism of the human subject.

The chemical signatures were collected on two coated spiral traps and in the ice-water-cooled reservoir. The stationary organic phases comprised Apiezon L and diethylene glycol adipate (DEGA). The thicknesses of the Apiezon L and DEGA films employed in the first experiment were 1.0 and 1.2 mg/cm^2, and the respective values in the second experiment were 0.5 and 0.7 mg/cm^2. The duration of each signature collection experiment was 1 hr. The analysis of the signature samples was performed by using a 10-ft SE-30 column and programming its temperature at the rate of 6°C/min over the range from 45 to 200°C.

3.2 Experiments on Sex-Related Chemical Signatures

Experiments 4 and 5 were conducted to select sex-related human chemical signatures. For this purpose, the chemical signatures were collected from two young white healthy female subjects. In each experiment, the collection period was 1 hr and a precooled dry breathing air was used at the flow rate of 1 liter/sec.

In experiment 4, the signature collection assembly included both coated spiral traps and the ice-water-cooled reservoir. The thickness of the Apiezon L coating used was 0.5 mg/cm^2 and that of the DEGA film was 0.6 mg/cm^2. The air temperature

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measured inside the system was 31°C; its relative humidity determined at the outlet of the DEGA trap ranged from 43 to 52%; and the oxygen content of the air leaving the system was 18%.

In experiment 5, the chemical signatures were collected on a 0.8 mg/cm^2 thick film of Apiezon L and in the ice-water-cooled reservoir. The relative humidity of the air measured at the outlet of the Apiezon L trap ranged from 42 to 48%; its oxygen content was 18%, and its temperature inside the system was 31°C. The chemical signatures collected in both experiments were analyzed on a 10-ft SE-30 column. In each analysis the column temperature was programmed at the rate of 6°C/min over the range from 45 to 200°C.

3.3 Experiments on Race-Related Chemical Signatures

To evaluate chemical signatures of various races, the following experiments were conducted on a young healthy American Negro male subject (experiment 6) and a young Indian male (experiment 7) who was born in India but presently is residing in the USA. Both signature collection experiments were performed under dry-air conditions with the airflow rate of 1 liter/sec. The duration of each experiment was 1 hr.

In the experiment involving the Negro subject, the signatures were collected on a 0.8 mg/cm^2 thick Apiezon L film and in the ice-water-cooled reservoir. The air relative humidity measured at the outlet of the Apiezon L trap ranged from 28 to 47% and the

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air temperature inside the system from 28 to 29°C.

The signature collection system used in the experiment on the Indian subject included the Apiezon L film, 0.8 mg/cm^2 thick. The temperature of the air inside the system was 31°C, and its relative humidity, measured at the outlet of the Apiezon L trap, ranged from 40 to 43%.

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4.0 EXPERIMENTAL RESULTS AND DISCUSSION

4.1 Treatment of Experimental Data

The experimental findings are expressed in terms of the retention times of the consecutive chromatographic peaks in the chemical signatures of both the experimental system proper and the system in the presence of the human subject. The peaks of the residual impurities in the stationary phases used in the experiments are not reported since the final conditioning of the materials resulted in a few impurities with negligible peak areas.

Chemical signatures of human origin are selected by eliminating all chromatographic peaks common to the system proper and the system with human subject. The peaks are considered common when their retention times differ no more than $\pm$ 2%. Such a simplified treatment of data reveals only the minimum number of human signature components. The actual number of the components is expected to be much higher than the latter number. This expectation is based on the fact that generally the signature peaks from the system in the presence of a human subject are significantly larger than the respective background peaks. In addition, several of the human signature peaks are only partially resolved. The minimum number of human signature components is used to evaluate the differences in signatures from the human subjects studied.

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4.2 Chemical Signatures of White Male Subject

4.21 Wet-Air Conditions

The results of the signature collection experiment on a white human male subject exposed to a water-saturated airflow are illustrated in Table 1. Because of the large number of chromatographic peaks obtained under these conditions, only the retention times of the major signature components are reported here. The largest peaks in size detected on the hydrogen-flame detector represent the high boiling components with retention times of 13.2, 13.9, and 14.3 min. All these components are electronegative, as evidenced by their large respective signals on the electron-capture detector. The lower boiling region of the chromatogram comprises a group of medium size peaks, some of which exhibit electron-capture properties. The characteristic feature of the signature is reflected in the large number of electronegative components.

The partial resolution of the majority of the peaks and the complexity of the signature sample collected under these conditions prompted us to simplify the experimental conditions. For this purpose, the following experiments were conducted in the system provided with dry breathing air cooled before its use.

4.22 Dry-Air Conditions

The results of two signature collection experiments on the same white male human subject, performed under dry-air conditions,

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Table 1

CHEMICAL SIGNATURES COLLECTED FROM APIEZON L EXPOSED TO THE SYSTEM PROPER AND TO THE SYSTEM IN THE PRESENCE OF A HUMAN MALE SUBJECT[a]

Source of Chemical Signatures	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min[b] Hydrogen Flame	Electron Capture	Observations[c]
Experimental system	30	0.48; 0.66; 1.14; 1.86; 6.84; 7.32; 8.22; 13.8; 14.6	0.48; 0.78	Peaks 0.48-1.14 partially resolved (hydrogen flame).
Experimental system with male subject (experiment 1)	112	0.30; 0.36; 0.60; 0.66; 1.10; 1.50; 1.86; 2.10; 2.46; 2.94; 3.30; 5.70; 6.36; 6.90; 7.86; 9.00; 9.66; 10.1; 10.8; 11.2; 11.6; 12.0; 12.8; 13.2; 13.9; 14.3; 15.3	0.36; 0.48; 1.14; 2.10; 3.00; 4.44; 6.12; 7.26; 8.64; 9.90; 10.7; 13.2; 13.9; 14.3	Peaks 0.30-0.60 partially resolved (hydrogen flame); peaks 13.2-14.3 very large and partially resolved.

[a]Experiment performed under 100% relative humidity inside the system.

[b]Only the retention times of major consecutive peaks are reported here.

[c]Apiezon L analyzed immediately before each signature collection experiment displayed a small number of impurities with negligible peak areas.

are summarized in Tables 2 through 5. Comparison of the chemical signatures collected on the Apiezon L traps show similarities in their production pattern. The essential difference is the magnitude of the respective components, as evidenced by the respective chromatograms. In the first experiment of this series, the relative concentrations of the low boiling components exceed that of the respective compounds from the second experiment by several orders of magnitude. The high boiling components, however, are found to be much larger in the second experiment. In both experiments, the general production pattern of the human chemical signatures collected on the DEGA coating and detected on the hydrogen-flame detector is preserved.

The differences are evident in the number and magnitude of the electronegative components. In the first experiment (Table 3), the minimum number of such components is 22; the respective value obtained in the second experiment is only 6 (Table 4). In addition, the areas of peaks observed in the first experiment are significantly larger than those of the second experiment. The water solution of the chemical signatures collected in the ice-water-cooled reservoir in the first experiment amounted to 9.5 g; in the second experiment, 5.9 g.

4.3 Chemical Signatures of White Female Subjects

The retention times of the components of chemical signatures collected on the Apiezon L and DEGA coatings from the

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Table 2

CHEMICAL SIGNATURES COLLECTED FROM APIEZON L EXPOSED TO THE SYSTEM PROPER AND TO THE SYSTEM IN THE PRESENCE OF A HUMAN MALE SUBJECT[a]

Source of Chemical Signatures	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min		Observations[b]
		Hydrogen Flame	Electron Capture	
Experimental system	31	0.36; 0.60; 1.42; 2.40; 3.50; 3.80; 4.40; 4.80; 5.16; 6.24; 6.96; 8.70; 9.20; 10.8; 11.6; 12.1; 12.8; 13.8; 14.2; 15.9; 16.5; 17.9; 20.1; 22.4; 23.4; 27.6; 34.2	0.36; 1.42; 3.80; 5.16	
Experimental system with male subject (experiment 2)	70	0.36; 0.45; 0.66; 0.93; 1.14; 1.50; 1.86; 2.70; 3.00; 3.24; 3.42; 4.10; 4.50; 4.86; 5.40; 5.61; 6.12; 6.48; 7.10; 7.40; 7.95 8.82; 9.30; 9.45; 10.4; 10.8; 11.6, 11.8; 12.8; 13.5; 13.9; 14.3; 15.1; 15.6; 16.5; 17.2; 17.4; 18.4; 18.9; 19.5; 20.4; 20.9; 21.6; 22.0; 22.9; 23.6; 23.9; 24.8 25.9; 27.4; 28.9; 20.9; 33.6; 38.7	0.36; 1.38; 1.56; 1.92; 2.22; 15.2; 19.3; 20.4; 22.5 22.9; 24.0; 24.6 25.7; 26.9; 30.8; 33.9	Peaks 0.36, 0.45, 0.66, 1.50 and 1.86 prominent in size, partially resolved (hydrogen flame); peaks 3.42, 5.61, 7.95, 10.4, 12.8, 15.1, 23.9 and 25.9 prominent in size and resolved (hydrogen flame); peak 15.2 prominent in size (electron capture).

[a] One hour collection in both experiments.

[b] Apiezon L analyzed before each signature collection experiment displayed a small number of impurities with negligible peak areas.

Table 3

CHEMICAL SIGNATURES COLLECTED FROM DEGA EXPOSED TO THE SYSTEM PROPER AND TO THE SYSTEM IN THE PRESENCE OF A HUMAN MALE SUBJECT[a]

Source of Chemical Signatures	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min		Observations[b]
		Hydrogen Flame	Electron Capture	
Experimental system	35	0.36; 0.66; 0.96; 1.20; 1.38; 1.74; 4.50; 6.00; 7.32; 7.62; 7.98; 8.46; 10.2; 10.4; 11.2; 12.2; 12.5; 13.1; 13.6; 14.5; 14.8; 15.3; 17.5; 18.4; 19.6; 19.9; 21.7; 22.0; 24.0; 25.7	0.36; 0.66; 1.20; 1.80; 7.90	Peaks 0.66 and 1.38 prominent in size (hydrogen flame detector).
Experimental system with male subject (experiment 2)	76	0.36; 0.48; 0.54; 0.66; 1.38; 1.98; 2.16; 2.34; 2.52; 2.94; 3.84; 3.96; 4.14; 4.44; 4.52; 5.22; 5.94; 6.54; 7.56; 8.94; 9.36; 9.90; 10.5; 11.4; 11.9; 12.4; 12.7; 13.0; 14.4; 14.6; 15.3; 15.6; 16.6; 17.5; 17.8; 18.2; 18.5; 19.8; 20.2; 20.6; 21.6; 21.8; 22.7; 23.0; 23.7; 24.4; 24.9; 25.7; 30.6; 47.0	0.36; 0.48; 0.54; 0.66; 2.40; 2.64; 3.00; 3.90; 7.60; 8.52; 9.10; 10.9; 11.6; 12.0; 13.1; 14.5; 20.8; 21.4; 23.3; 24.5; 25.9; 30.7; 33.7; 38.4	Peaks 0.36-0.54 and 2.34-5.94 large and partially resolved; peaks 8.94-10.5 and 14.4-15.6 large and partially resolved; peaks prominent in size and resolved: 17.5, 18.2, 20.6, 22.1, 23.7, 25.7 and 47.0 (flame detector); large peaks: 8.50, 9.1, 14.5, 20.8, 23.3, 33.7 and 38.4 (electron capture detector).

[a] One hour collection in each experiment.

[b] The DEGA coating analyzed before its exposure to the system proper and to the system in the presence of human subject exhibited impurities with negligible peak areas.

Table 4

CHEMICAL SIGNATURES COLLECTED FROM APIEZON L EXPOSED TO THE SYSTEM PROPER AND TO THE SYSTEM IN THE PRESENCE OF A HUMAN MALE SUBJECT[a]

Source of Chemical Signatures	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min		Observations[b]
		Hydrogen Flame	Electron Capture	
Experimental system	27	0.33; 0.36; 0.45; 0.48; 0.70; 0.90; 1.25; 1.32; 1.80; 2.04; 2.88; 3.60; 4.20; 4.50; 5.76; 6.48; 7.26; 8.52; 9.84; 12.1; 15.8; 19.3; 22.4; 26.3;	0.36; 1.32; 1.80	Peaks 0.33-0.48 partially resolved, low boiling components; peaks with retention times 1.32 and 4.50 prominent in size (hydrogen flame).
Experimental system with male subject (experiment 3)	67	0.33; 0.36; 0.45; 0.48; 0.66; 0.90; 1.20; 1.32; 1.92; 2.10; 2.40; 2.70; 3.48; 3.66; 3.90; 4.02; 4.50; 5.40; 5.88; 6.12; 6.78; 7.32; 7.50; 8.40; 9.00; 9.18; 9.66; 9.84; 10.1; 10.5; 11.1, 11.6; 12.0; 12.7; 13.1; 14.1; 14.6; 14.8; 15.1; 15.6; 16.7; 17.6; 17.9; 18.6; 19.0; 19.5; 19.9; 20.6; 21.3; 22.2; 22.8; 23.4; 24.0; 24.9; 25.6; 26.2; 27.0; 28.0	0.36; 1.32; 5.50; 16.5; 17.2; 17.8; 19.8; 26.4	Peaks 0.33-0.48 and 1.32 common to both chromatograms; peaks 1.8, 2.04 and 4.20, 4.50 partially resolved; peak with retention time 4.50 common to both chromatograms and prominent in size; peaks 7.32 and 7.50 partially resolved; peak 8.40 prominent in size; peaks 9.00, 9.18, 9.66 and 9.84 partially resolved; prominent peaks: 20.6, 22.2, 24.0. 24.9; the largest peak: 23.4.

[a]One hour collection in each experiment.

[b]Hexane analyzed immediately before its use in both experiments displayed a few low and high boiling impurities with negligible peak areas.

Table 5

CHEMICAL SIGNATURES COLLECTED FROM DEGA EXPOSED TO THE SYSTEM PROPER AND TO THE SYSTEM IN THE PRESENCE OF A HUMAN MALE SUBJECT[a]

Source of Chemical Signatures	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min		Observations[b]
		Hydrogen Flame	Electron Capture	
Experimental system	19	0.30; 0.42; 1.38 4.00; 4.44; 7.56; 7.98; 8.04; 9.84; 11.8; 15.5; 18.9; 20.8; 21.8; 24.8;	0.30; 0.42; 1.38; 21.2	Prominent peaks in size: 0.42, 1.38, and 4.44; electron capture detector; all peaks small in size.
Experimental system with male subject (experiment 3)	52	0.30; 0.42; 0.48; 1.20; 1.86; 3.42; 3.80; 4.32; 4.80; 5.76; 7.20; 7.80; 8.22; 8.52; 9.36; 9.90; 10.5; 11.5; 11.7; 12.2; 12.5; 13.6; 14.0; 14.3; 15.2; 15.6; 16.2; 16.6; 16.9; 17.6; 18.1; 18.6; 19.2; 19.4; 20.8; 21.1; 21.6; 22.6; 23.3; 23.6; 24.2; 24.9; 25.8; 27.7; 33.0	0.48; 1.14; 2.64; 17.6; 19.9; 22.9; 25.9	Prominent peaks in size: 4.80, 5.76, 11.5, 11.7, 12.2, 14.3, 15.2, 16.2, 16.6, 18.1, 19.4, and 22.6; electron capture detector, major peak 2.64.

[a] 30-Minute collection in both experiments.

[b] The DEGA coating analyzed immediately before its expos e to the system proper and to the system with a human subject displayed a few low and high boiling impurities with neglibible peak areas.

first white human female subject are presented in Tables 6 and 7. The data on signatures collected on the Apiezon L coating in the experiment on the second white female subject are summarized in Table 8. Comparison of the results of both experiments shows that the essential difference is not in the total number but in the distribution of the major components in the respective signatures. In the first experiment (Table 6) the major components appear in the range of the retention times from 3.66 to 11.6 min; in the second experiment (Table 8), the prominent components are distributed over a wider range of retention times, including the high boiling region of the signature sample.

A significant difference is observed in the number of the electronegative components of the signatures. The signature collected in the first experiment exhibits only 4; that from the second experiment, 22 components. The experimental data on the chemical signature collected on the DEGA coating from the first female subject indicate that the major part of the sample is expressed by the high boiling components, with retention times ranging from 14.2 to 16.8 min, and includes the prominent peak with a retention time of 13.2 min (Table 7, hydrogen-flame detector). The chemical signature collected under these conditions exhibit 8 electronegative components. The water solution of the chemical signatures obtained in the first experiment amounted to 9.7 g; in the second experiment, 4.7 g.

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Table 6

CHEMICAL SIGNATURES COLLECTED FROM APIEZON L EXPOSED TO THE SYSTEM PROPER AND TO THE SYSTEM IN THE PRESENCE OF A HUMAN FEMALE SUBJECT[a]

Source of Chemical Signatures	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min: Hydrogen Flame	Retention Times of Consecutive Peaks, min: Electron Capture	Observations[b]
Experimental system	39	0.30; 0.36; 0.42; 0.51; 0.66; 0.78; 1.10; 1.20; 1.38; 1.92; 2.16; 2.46; 2.70; 2.94; 3.12; 3.60; 4.08; 4.44; 5.04; 5.46; 6.06; 7.02; 7.44; 8.16; 8.84; 9.48; 10.4; 10.9; 11.3; 11.6; 13.1; 15.2; 18.6; 20.2; 21.5	0.30; 0.48; 0.66; 1.38	Peaks 0.30-0.51, relatively large. partially resolved. low boiling components (hydrogen flame detector).
Experimental system with female subject (experiment 4)	71	0.30; 0.36; 0.42; 0.45; 0.54; 0.66; 0.72; 1.14; 1.20; 1.44; 1.74; 1.92; 2.28; 2.64; 2.68; 3.66; 3.84; 4.04; 4.38; 4.56; 5.04; 5.52; 5.94; 6.30; 6.60; 6.84; 7.50; 7.74; 8.04; 8.22; 8.64; 9.12; 9.18; 9.44; 9.66; 9.90; 10.1; 10.4; 10.9; 11.2; 11.6; 12.6; 14.2; 14.4; 14.7; 15.2; 16.4; 16.6; 16.8; 16.9; 17.0; 17.2; 17.4; 18.1; 19.9; 20.2; 20.7; 21.2; 23.2; 23.6; 24.0; 24.9; 25.9; 26.6; 30.7; 36.2	0.30; 0.36; 0.54; 1.44; 21.5	Peaks 0.30-0.54 partially resolved; peaks 3.66-11.6 large and partially resolved.

[a] One hour collection in both experiments.

[b] Apiezon L analyzed before its exposure to the system proper and to the system with human subject exhibited a small number of impurities of negligible peak areas.

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Table 7

CHEMICAL SIGNATURES COLLECTED FROM DEGA EXPOSED TO THE SYSTEM PROPER AND TO THE SYSTEM IN THE PRESENCE OF A HUMAN FEMALE SUBJECT[a]

Source of Chemical Signatures	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min		Observations[b]
		Hydrogen Flame	Electron Capture	
Experimental system	20	0.24; 0.30; 0.33; 0.36; 0.43; 1.32; 4.38; 6.78; 7.26; 8.04; 8.82; 11.4; 11.9; 18/2; 21.2; 23.7;	0.24; 0.36; 0.66; 1.32	Peaks 0.24-0.42 very small in size, partially resolved; peak 1.32 large in size; peaks 6.78-14.7 small (hydrogen flame detector); peaks 0.24-1.32 all very small (electron capture detector).
Experimental system with female subject (experiment 4)	60	1.74; 1.80; 1.86; 1.92; 1.98; 2.04; 2.28; 2.70; 3.18; 3.66; 4.02; 4.44; 4.56; 5.64; 6.06; 6.78; 7.32; 7.68; 8.10; 8.78; 8.82; 9.84; 10.4; 11.2; 11.6; 12.1; 13.0; 13.3; 13.4; 14.2; 15.0; 15.8; 16.8; 18.2; 18.8; 19.1; 19.3; 19.9; 20.3; 21.1; 22.1; 22.7; 23.2; 23.7; 24.3; 24.8; 25.2; 25.9; 26.0; 26.2; 27.5 28.4;	1.56; 1.70; 1.74; 1.80; 2.10; 2.34; 17.4; 19.9	Peaks 1.74-3.66 large. partially resolved; peak 4.56 large in size; peaks 14.2-16.8 the most prominent in size; peak 18.2 very large.

[a] One hour collection in each experiment.

[b] The DEGA coating analyzed before its exposure in each experiment displayed a small number of impurities of negligible peak areas.

Table 8

CHEMICAL SIGNATURES COLLECTED FROM APIEZON L EXPOSED TO THE SYSTEM PROPER AND TO THE SYSTEM IN THE PRESENCE OF A HUMAN FEMALE SUBJECT[a]

Source of Chemical Signatures	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min: Hydrogen Flame	Retention Times of Consecutive Peaks, min: Electron Capture	Observations[b]
Experimental system	14	0.30; 0.36; 0.48; 0.59; 3.84; 7.57; 12.6; 15.5; 17.2; 18.5; 20.2; 23.2; 23.6	0.30	Peaks not measurable: 0.30-0.54; the remaining peaks significantly smaller in size than the respective peaks from the system with female subject
Experimental system with female subject (experiment 5)	71	0.30; 0.36; 0.48; 0.54; 0.66; 1.12; 1.46; 1.92; 2.16; 3.48; 3.84; 4.80; 5.28; 5.90; 6.00; 6.30; 6.62; 6.84; 7.57; 7.78; 8.60; 10.7; 10.9; 11.6; 12.6; 13.4; 13.8; 14.2; 14.7; 15.2; 15.5; 15.9; 16.4; 16.8; 17.2; 18.1; 18.5; 19.6; 20.2; 21.2; 21.8; 22.7; 23.2; 23.6; 24.0; 24.7; 24.9; 26.6	0.30; 0.54; 0.66; 1.56; 1.86; 2.88; 4.50; 5.82; 6.30; 8.76; 9.54; 13.5; 15.0; 15.9; 16.5; 16.9; 17.9; 18.5; 19.5; 21.2; 22.2; 23.1; 23.8	Peaks prominent in size: 0.66; 3.84; 5.90; 14.2; 15.5; 16.8; 17.2; 20.2; 23.6 (hydrogen flame). Peaks partially resolved: 16.8 and 17.2. Peaks prominent in size: resolved: 2.88; 13.5; 15.0; 21.2; partially resolved: 1.56; 1.86; 8.76; 9.54; 15.9; 1.65; 16.9; 17.9; 18.5; 19.5; 23.1; 23.8 (electron capture).

[a] One hour collection in each experiment.

[b] Apiezon L analyzed before its exposure to the system proper and to the system with female subject exhibited a small number of impurities of negligible peak areas.

4.4 Chemical Signature of Negro Male Subject

The retention times of the signature components collected on an Apiezon L coating from a Negro male subject are given in Table 9. Consideration of these findings reveals that the total number of the signature components detected on a hydrogen-flame detector is only 25, and the respective number of the electronegative components is 6.

Evaluation of the relative concentrations of the signature components observed on the flame detector reveals their characteristic distribution. The major part of the signature sample is represented by medium boiling components. The retention times of these components range from 11.3 to 13.5 min. The latter value corresponds to the largest component. Note that signature components with retention times of 4.10, 5.28, 7.90, and 8.22 min appear as chromatographic peaks with flat tops. The interesting feature of the signature is reflected by the large electronegative component with a retention time of 21.0 min.

4.5 Chemical Signatures of Indian Male Subject

The experimental data on chemical signatures collected on Apiezon L coating from an Indian male subject are summarized in Table 10. The characteristic feature of the signature is the relatively small number of components detected by the hydrogen-flame detector and the absence of electronegative components.

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Table 9

CHEMICAL SIGNATURES COLLECTED FROM APIEZON L EXPOSED TO THE SYSTEM PROPER AND TO THE SYSTEM IN THE PRESENCE OF A NEGRO MALE SUBJECT[a]

Source of Chemical Signatures	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min		Observations[b]
		Hydrogen Flame	Electron Capture	
Experimental system	16	0.30; 0.45; 0.66; 0.84; 1.32; 1.74; 2.22; 8.22; 10.5; 15.6; 17.5; 19.4; 20.9	0.30; 0.66; 2.22;	Peaks with measurable areas: 0.84 and 1.32 (hydrogen flame) and 0.30 and 0.66 (electron capture). The areas of the remaining peaks are insignificant in size.
Experimental system with Negro male subject (experiment 6)	46	0.30; 0.45; 0.54; 0.60; 0.66; 0.72; 0.84; 1.14; 1.32; 1.74; 2.22; 3.24; 3.40; 4.10; 5.28; 7.90; 8.22; 9.46; 10.5; 11.3; 11.8; 12.5; 13.5; 14.3; 14.8; 15.6; 16.5; 17.5; 18.4; 18.9; 19.4; 19.9; 20.4; 20.9; 21.5; 21.9; 22.4; 22.9	0.30; 0.42; 0.66; 1.14; 17.1; 18.6; 21.0; 21.7	Peak 13.5 is the most prominent in size; large and partially resolved peaks: 113, 11.8, and 12.5: large peaks with flat tops: 4.10, 5.28, 7.90, and 8.22(hydrogen flame). Peak 21.0 is the largest on electron capture tracing.

[a]One hour collection in each experiment.

[b]Apiezon L analyzed before each signature collection experiment displayed a small number of impurities with negligible peak areas.

Table 10

CHEMICAL SIGNATURES COLLECTED FROM APIEZON L EXPOSED TO THE SYSTEM PROPER AND TO THE SYSTEM IN THE PRESENCE OF INDIAN MALE SUBJECT[a]

Source of Chemical Signatures	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min		Observations[b]
		Hydrogen Flame	Electron Capture	
Experimental system	19	0.30; 0.42; 0.60; 0.66; 3.12; 8.58; 10.4; 11.2; 12.8; 14.6; 15.6; 16.4; 17.2; 17.4; 18.2; 19.0; 21.5; 22.9	0.30	Peaks with measurable areas: 14.6-22.9; the areas of the remaining peaks are insignificant (hydrogen flame). Peak 0.30 on electron capture of insignificant area.
Experimental system with Indian male subject (experiment 7)	43	0.30; 0.42; 0.60; 0.66; 0.93; 1.20; 2.40; 3.12; 5.16; 5.66; 6.12; 6.36; 7.10; 7.20; 7.40; 7.96; 8.10; 8.34; 8.38; 9.30; 10.4; 11.2; 11.6; 12.0; 12.4; 12.8; 13.5; 13.9; 14.1; 14.6; 15.6; 16.4; 17.2; 17.4; 18.2; 19.0; 19.3; 19.9; 20.4; 21.5; 22.0; 22.9	0.30	Peaks prominent in size: resolved, 0.93 and 1.20; partially resolved, 5.66-8.58 (hydrogen flame). Peak 0.30 on electron capture of measurable area.

[a]One hour collection in each experiment.

[b]Apiezon L analyzed before each signature collection experiment displayed a small number of impurities with negligible peak areas.

The major part of the signature sample centers on medium boiling components, represented by partially resolved peaks with retention times extending from 5.66 to 8.50 min. The low boiling region of the signature is characterized by two large components with retention times of 0.93 and 1.20 min.

4.6 Chemical Signatures Collected in Water

Experimental results on water solutions of chemical signatures collected from a white male subject (experiments 2 and 3) and a white female subject (experiment 4) are given in Table 11. These results exhibit several interesting and important features. Comparison of the retention times of chromatographic peaks obtained with the flame detector in experiment 2 with the respective data from experiment 3 shows that 91% of the peaks are common to both signatures. Since these experiments involved the same human subject, the large number of common peaks points to the excellent reproducibility of the experimental results. A similar comparison made between results of experiments 2 and 4 indicates that only 58% of the chromatographic peaks are common to both signatures. Since the experiments were performed on male and female subjects, the decreased number of common peaks might reflect the sex-related difference.

The differences in compositions of signatures collected in water and those from the Apiezon L coating are similarly

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Table 11

COMPOSITION OF WATER SOLUTION COLLECTED
FROM THE SYSTEM IN THE PRESENCE OF HUMAN SUBJECTS

Source of Chemical Signatures[a]	Total Number of Chromatographic Peaks	Retention Times of Consecutive Peaks, min		Observations
		Flame Ionization	Electron Capture	
Experimental system with male subject (experiment 2)	41	0.30; 0.84; 1.20; 3.48; 6.90; 7.14; 7.38; 7.74; 9.96; 10.7; 11.3; 12.1; 13.4; 14.0; 15.0; 16.3; 17.3; 17.9; 18.4; 19.1; 19.9; 20.5; 21.4; 22.4; 23.2; 23.5; 23.9; 24.4; 25.3; 25.9; 26.8; 31.8; 35.6	0.30; 0.84; 1.20; 4.38; 17.3; 25.3; 26.2	Peaks 0.84 and 1.20 very large and partially resolved; peak 4.38 very large with flat top probably water; peaks 9.96-12.1 very large, partially resolved.
Experimental system with male subject (experiment 3)	39	0.30; 0.84; 1.20; 3.48; 6.90; 7.14; 7.40; 7.70; 9.90; 10.7; 11.3; 12.0; 13.3; 14.0; 15.0; 16.2; 17.3; 17.9; 18.3; 19.0; 19.8; 20.5; 21.4; 22.4; 23.5; 24.4; 25.3; 25.9; 28.7; 30.6; 31.8; 35.7	0.30; 0.84; 1.20; 4.38; 17.3; 19.8; 25.9	Peaks 0.84 and 1.20 prominent in size, partially resolved; peak 4.38 very large with flat top, probably water; peaks 9.90-12.0 very large, partially resolved.
Experimental system with female subject (experiment 4)	38	0.30; 0.90; 1.74; 3.24; 7.38; 10.9; 11.6; 12.9; 13.7; 14.3; 15.5; 16.6; 17.2; 17.6; 18.1; 18.4; 18.9; 19.7; 19.9; 20.6; 21.3; 21.5; 21.9; 22.1; 22.6; 22.9; 23.4; 23.7; 25.3; 28.9; 33.0; 34.1; 37.5; 42.7		Peaks 0.90 and 1.74 prominent in size, partially resolved; peaks 18.1-23.7 major components, partially resolved.

[a]The same male subject was used in both experiments.

evaluated in terms of the numbers of peaks in common. In experiment 2, 45% of peaks are common to the respective signatures, in experiment 3, 50%, and in experiment 4, only 27%. The essential differences are the contents of the low boiling signature components.

In general, the signatures from Apiezon L trap contain a larger number of low boiling components than the water solutions. The majority of peaks of the water signatures that differ in retention times from those of the Apiezon L signatures are high boiling and large components. Thus these components may provide additional information on the composition of human signatures. To make this information more useful, it is necessary to select a preconcentration technique capable of showing the presence of other sample components and of achieving better resolutions of all chromatographic peaks.

A very promising preconcentration technique that was evaluated in our laboratories is the headspace-gas-sampling technique of Bassette et al (ref. 7). Basically, this technique consists of salting out the headspace gas from dilute aqueous solution of organic compounds by adding anhydrous sodium sulfate or other suitable salts. According to our results, the increased concentration of organics of the headspace gas makes possible their direct chromatographic analysis.

In our experiments, the relative salting-out efficiency of sodium sulfate and lithium chloride was investigated. For this

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purpose, an average of 1.2 g of each salt was transferred into a 5-ml vial, and 2 ml of the solution to be analyzed was added. The vial was sealed with a serum cap and its contents shaken for 5 min. It was then heated for 3 min at 60°C in a water bath. A 1-ml sample of the headspace gas was finally withdrawn through the rubber serum cap with a gas-tight syringe and injected onto the chromatographic column.

The analysis of each sample was carried out on SE-30 column of the Kromotog K-7 chromatograph, with both the hydrogen-flame and electron-capture detectors and the dual-channel recorder. The column temperature was kept at 125°C throughout the analysis. The salting-out experiments were performed on samples collected at ice-water temperatures from the system alone and in the presence of a human subject. The same analytical procedure was applied to a sample of water before its use in the air humidification procedure.

The results of sodium sulfate salting-out experiments on water solution from human subjects and from the system are illustrated in Figures 6 and 7, respectively. Both detection systems reflect a prominent difference between the two chromatogram traces shown. The flame-ionization detector indicates five peaks in the human subject. Such peaks are absent in the chromatogram of the system proper. The electron-capture detector reveals several large concentration components in the human subject, compared with a few small concentration components of the system sample.

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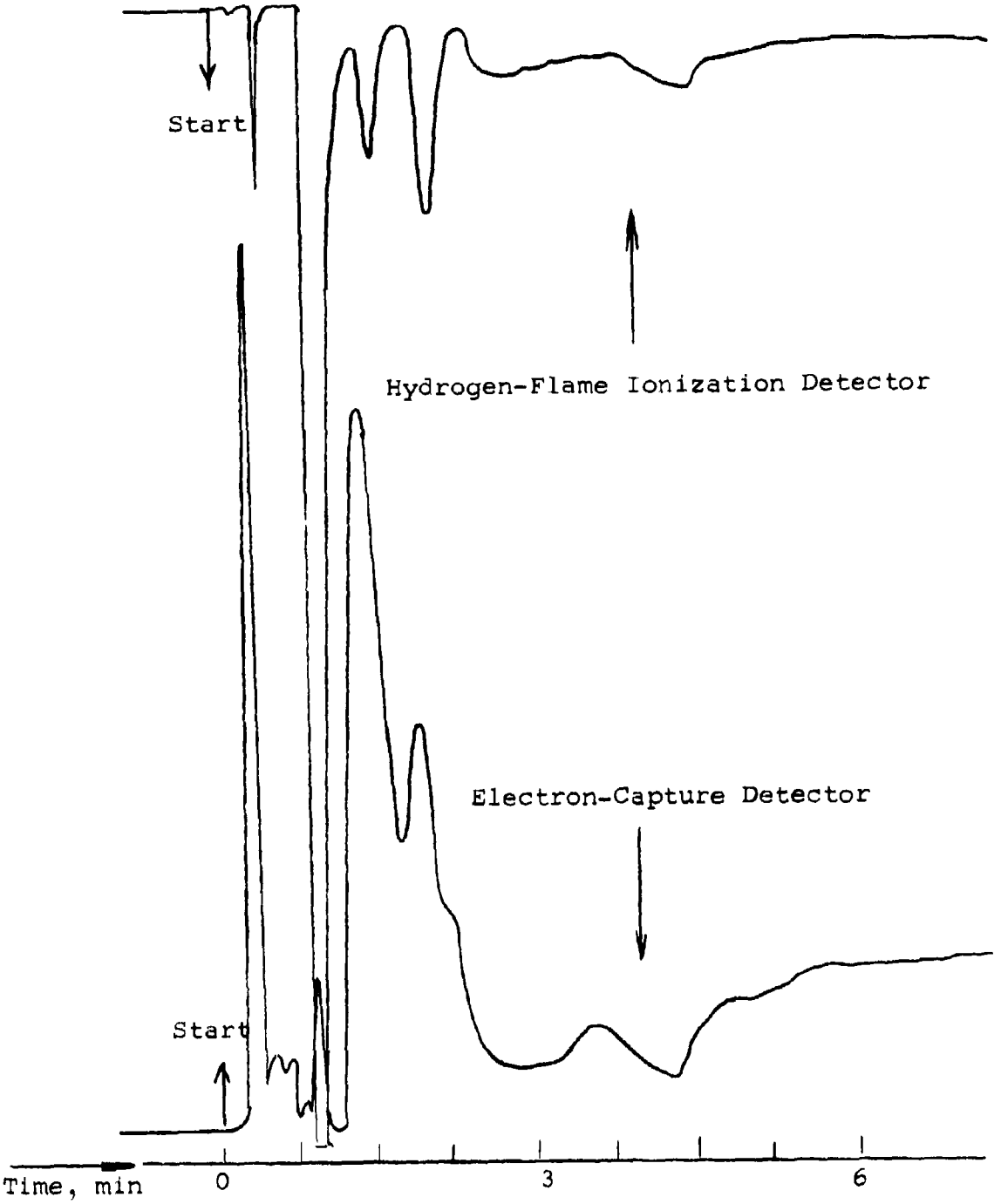

Figure 6

CHROMATOGRAM TRACE OF HEADSPACE GAS FROM HUMAN SUBJECT
(SALTING AGENT: SODIUM SULFATE)

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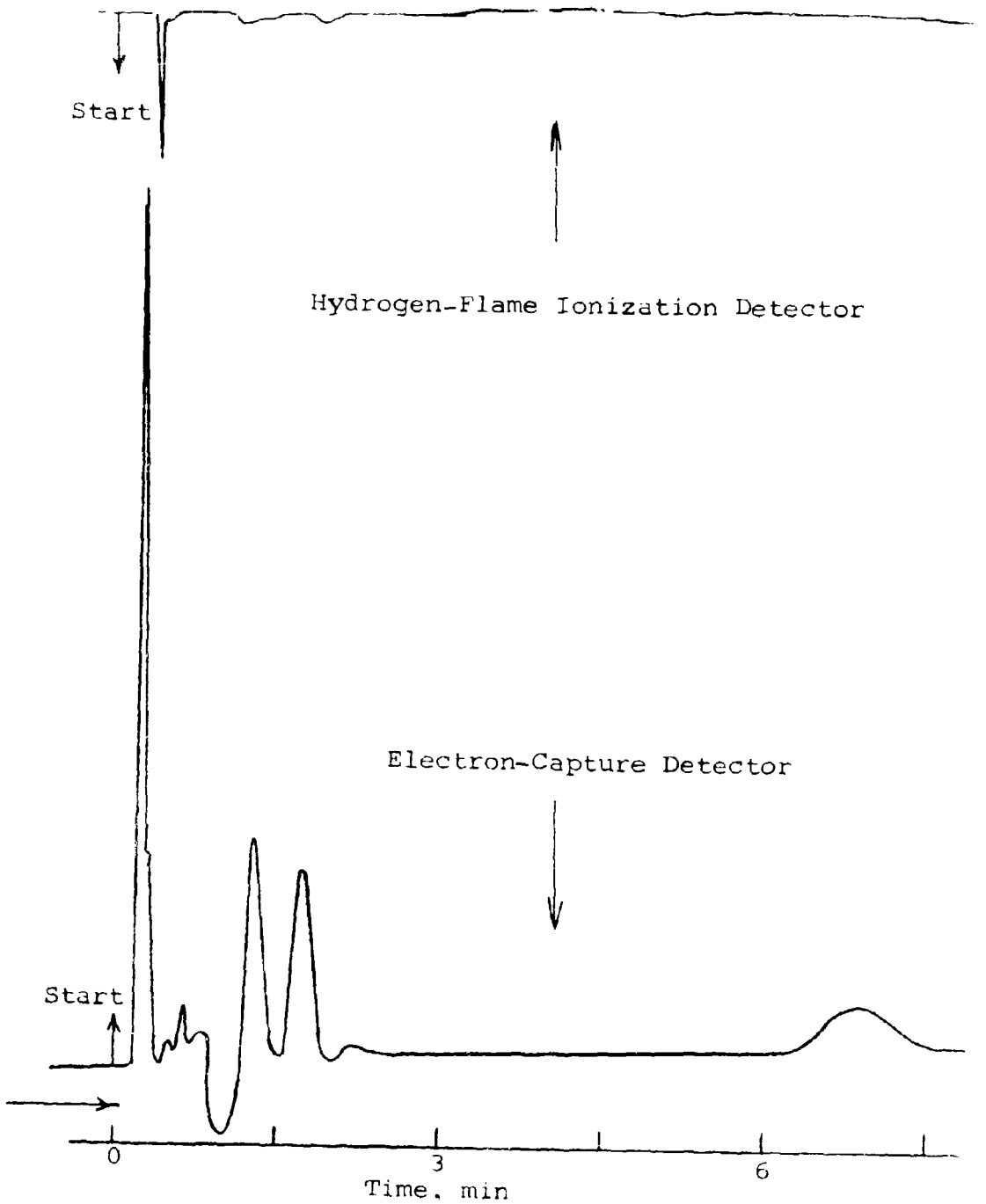

Figure 7

CHROMATOGRAM TRACE OF HEADSPACE GAS FROM THE SYSTEM
(SALTING AGENT: SODIUM SULFATE)

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The salting-out efficiency of lithium chloride is illustrated in Figures 8 and 9. Note that this efficiency is smaller than that of sodium sulfate for the respective samples of human origin. The authenticity of the peaks attributed to the human subject was supported by the results of the analysis of the pure water sample, similarly treated with sodium sulfate.

In order to more efficiently apply the headspace-gas-sampling technique to the analysis of water samples from human subjects, the technique can be combined with the preconcentration method developed in our laboratories, which utilizes a stationary organic phase to trap the components of the signature. For this purpose, the entire headspace gas sample and organics dissolved in water can be readily passed over the stationary phase by bubbling helium zero gas through the liquid sample after each salting-out procedure. The sample components collected on the stationary phase can be then quantitatively transferred into the collector-ejector assembly and analyzed.

Another approach of potential use for the analysis of water samples from human subjects is the zone melting technique. Although the technique and the commercial equipment have not as yet been applied to water solutions, they can be modified and adapted for this purpose.

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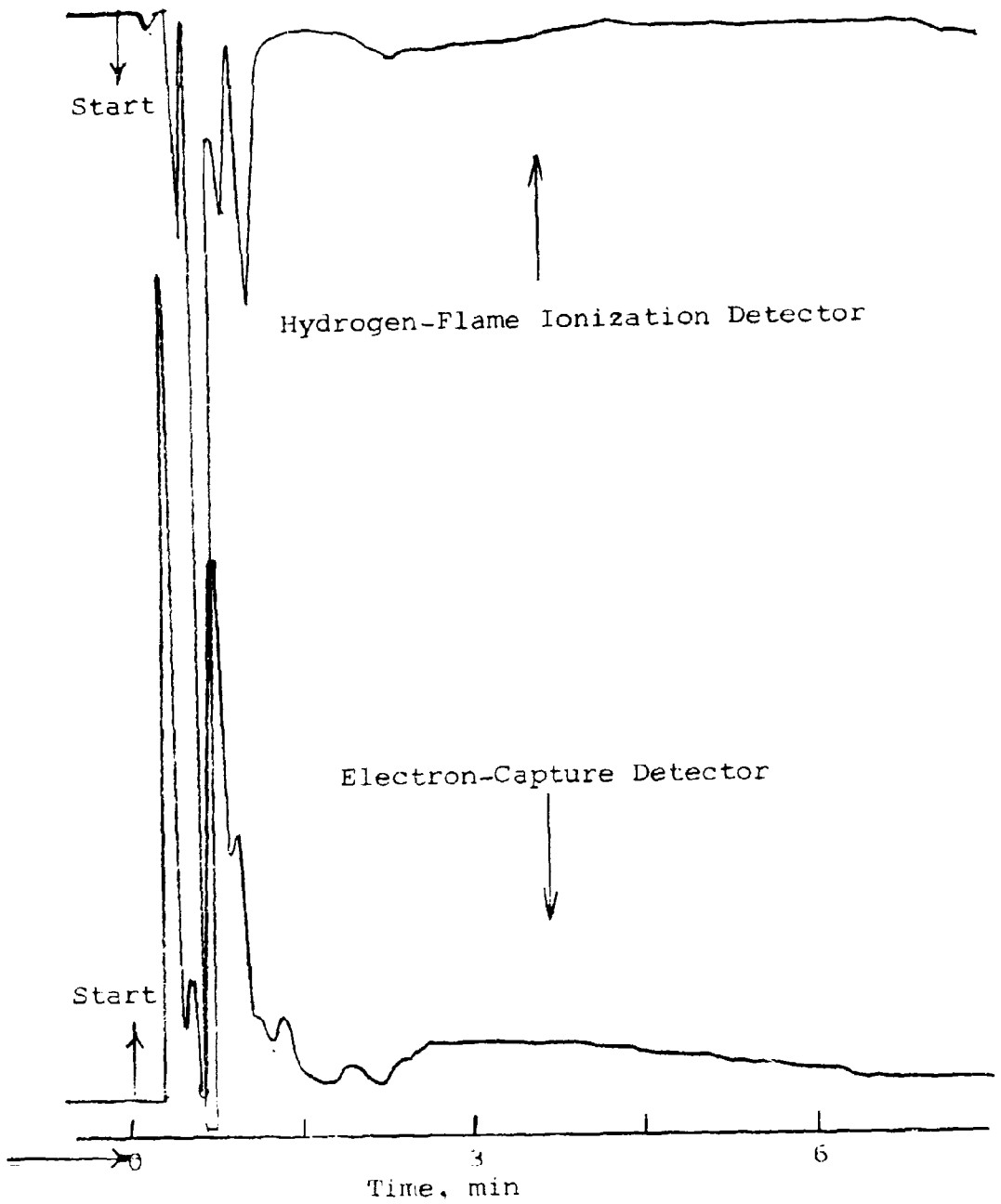

Figure 8

CHROMATOGRAM TRACE OF HEADSPACE GAS FROM HUMAN SUBJECT
(SALTING AGENT: LITHIUM CHLORIDE)

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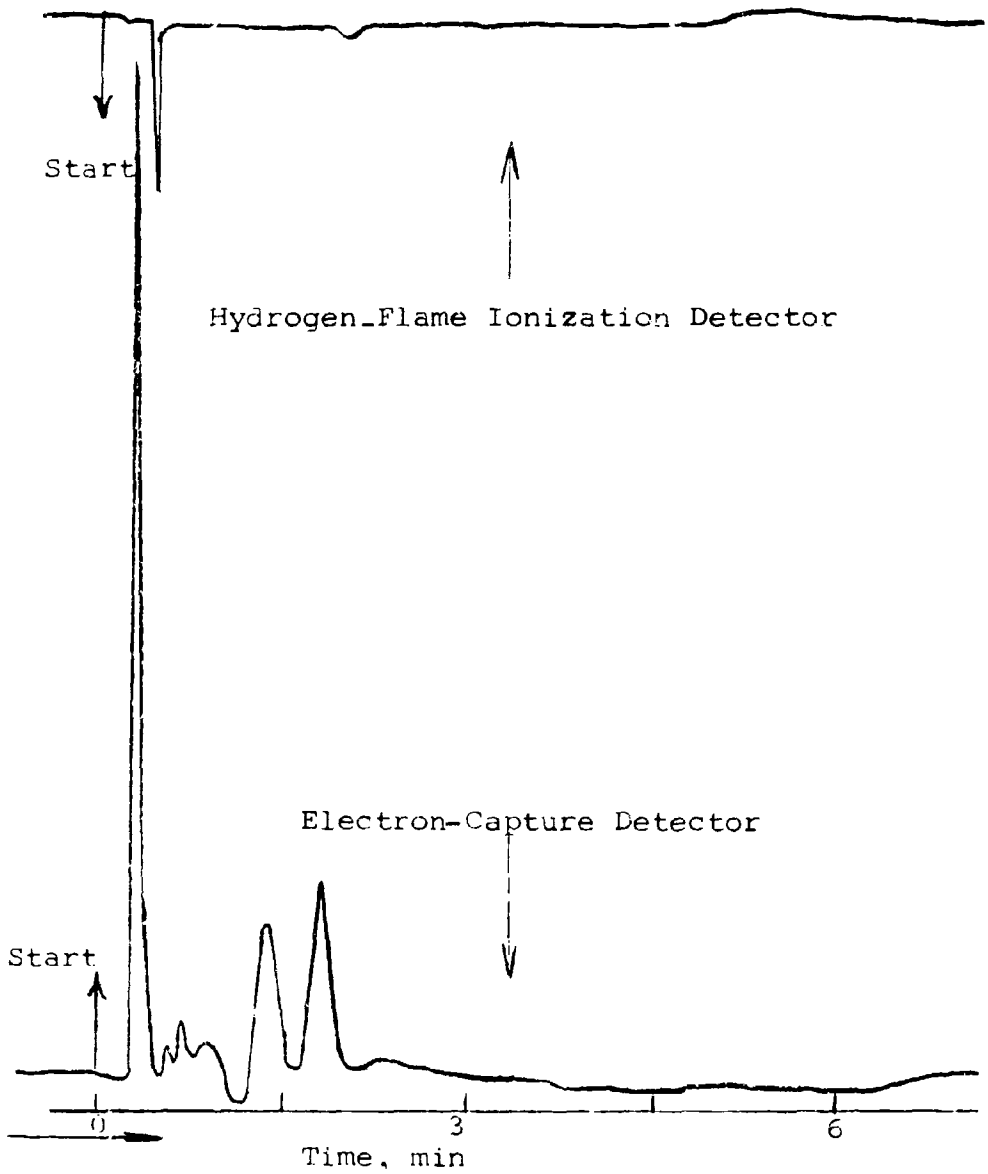

Figure 9

CHROMATOGRAM TRACE OF HEADSPACE GAS FROM THE SYSTEM
(SALTING AGENT: LITHIUM CHLORIDE)

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4.7 Quantitative Aspects of Experimental Results

4.71 Minimum Number of Human Signature Components

The minimum numbers of components of chemical signatures from male and female subjects studied are summarized in Table 12. These numbers are of the same order of magnitude as those for signatures collected on Apiezon L and DEGA coatings in experiments 2 and 3 on a white male subject and in experiments 4 and 5 on a white female subject. Such numbers are significantly smaller for both the Negro and the Indian subject.

The numbers of electronegative signature components are less reproducible. Even in experiments 2 and 3, which were performed on the same subjects, the minimum numbers differ significantly. The lack of reproducibility can be partially attributed to the known detrimental effects of water on the detection sensitivity of the electron-capture detector. The signature samples obtained in our experiments contained a small amount of water even when collected on an Apiezon L coating.

4.72 Comparison of Human Chemical Signatures

The results of comparison of human chemical signatures from various subjects studied are presented in Table 13 and illustrated in Figure 10. The comparisons are made by selecting components common to the signatures compared; the components are considered to be common when their retention times are identical or differ no more than $\pm 2\%$. The percent of common

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Table 12

CHEMICAL SIGNATURES OF HUMAN ORIGIN

Source of Chemical Signatures	Exp. No.	Minimum Number of Components in Chemical Signatures			
		Apiezon L		DEGA	
		Hydrogen Flame	Electron Capture	Hydrogen Flame	Electron Capture
Male Subject					
White	1[a]	44	39	-	-
White	2	38	14	33	22
White	3	40	6	35	6
Negro	6[a]	25	6	-	-
Indian	7[a]	24	-	-	-
Female Subject					
White	4	40	4	42	8
White	5[a]	35	22	-	-

[a]The DEGA trap was not used in these experiments.

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Table 13

COMPARISON OF HUMAN CHEMICAL SIGNATURES FROM MALE AND FEMALE SUBJECTS

Sources of Chemical Signatures	Exp. No.	Components Common to Signatures Compared: Apiezon L — Hydrogen Flame Number	Apiezon L — Hydrogen Flame Percent[a]	Apiezon L — Electron Capture Number	Apiezon L — Electron Capture Percent[a]	DEGA — Hydrogen Flame Number	DEGA — Hydrogen Flame Percent[a]	DEGA — Electron Capture Number	DEGA — Electron Capture Percent[a]
White male	2 3	25	66	2	14	21	64	2	10
White male White female	2 4	15	40	2	33	20	57	3	14
White male White female	3 4	19	47	3	75	17	40	4	66
White male White female	2 5[b]	12	32	6	43	-	-	-	-
White male White female	3 5[b]	16	40	3	50	-	-	-	-
White male Negro male	2 6[b,c]	10	26	-	-	-	-	-	-
White male Indian male	2 7[b,c]	11	29	-	-	-	-	-	-

[a] Percent based on the minimum numbers of signature components from white male subjects. experiments 2 and 3.

[b] The DEGA trap was not used in these experiments.

[c] No common peaks on the electron-capture detector.

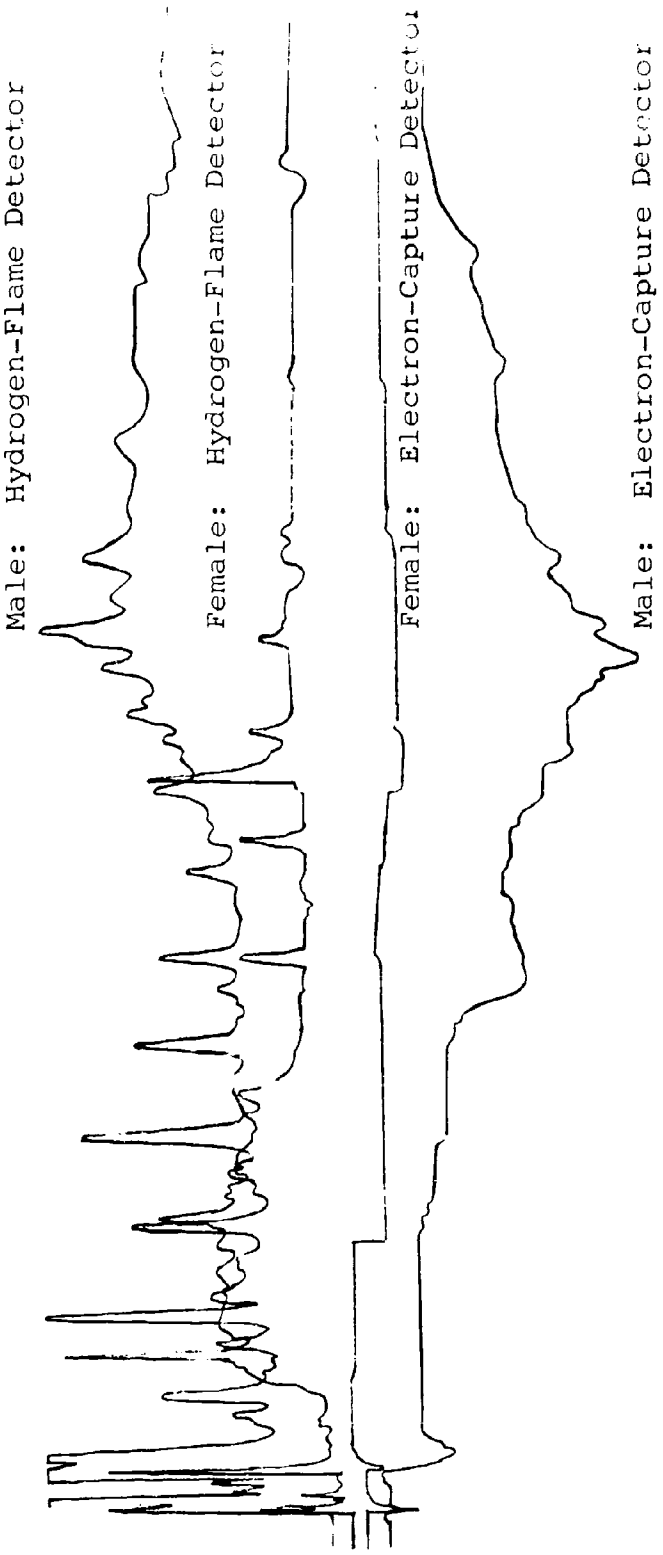

Figure 10

CHEMICAL SIGNATURES OF HUMAN SUBJECTS

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components is based on the minimum number of signature components from the white male subject (experiments 2 and 3).

The first comparison involved the results of two experiments performed on the same human subject (white male, experiments 2 and 3). The data in Table 13 show that the results are practically reproducible in terms of the signature components detectable on the hydrogen-flame and electron-capture detectors and collected on both coating materials.

The second comparison was aimed at the selection of sex-related differences. For this purpose, the number of signature components common to white male and white female subjects were determined and the respective percent values computed. The data obtained on the hydrogen-flame detector show that the sex-related differences reflect themselves in small numbers of peaks common to signatures compared. No trend of any kind, however, is shown by the electronegative signature components.

The next comparison was concerned with a selection of race-related differences. To this end, the percent of signature components common to white and Negro males and white and Indian males were determined; they were significantly smaller than the respective values from other comparisons. Thus the race-related differences revealed by this comparison are more evident than the sex-related differences.

Intercomparison of signatures from the four human subjects studied is illustrated in Figure 11. The signature components

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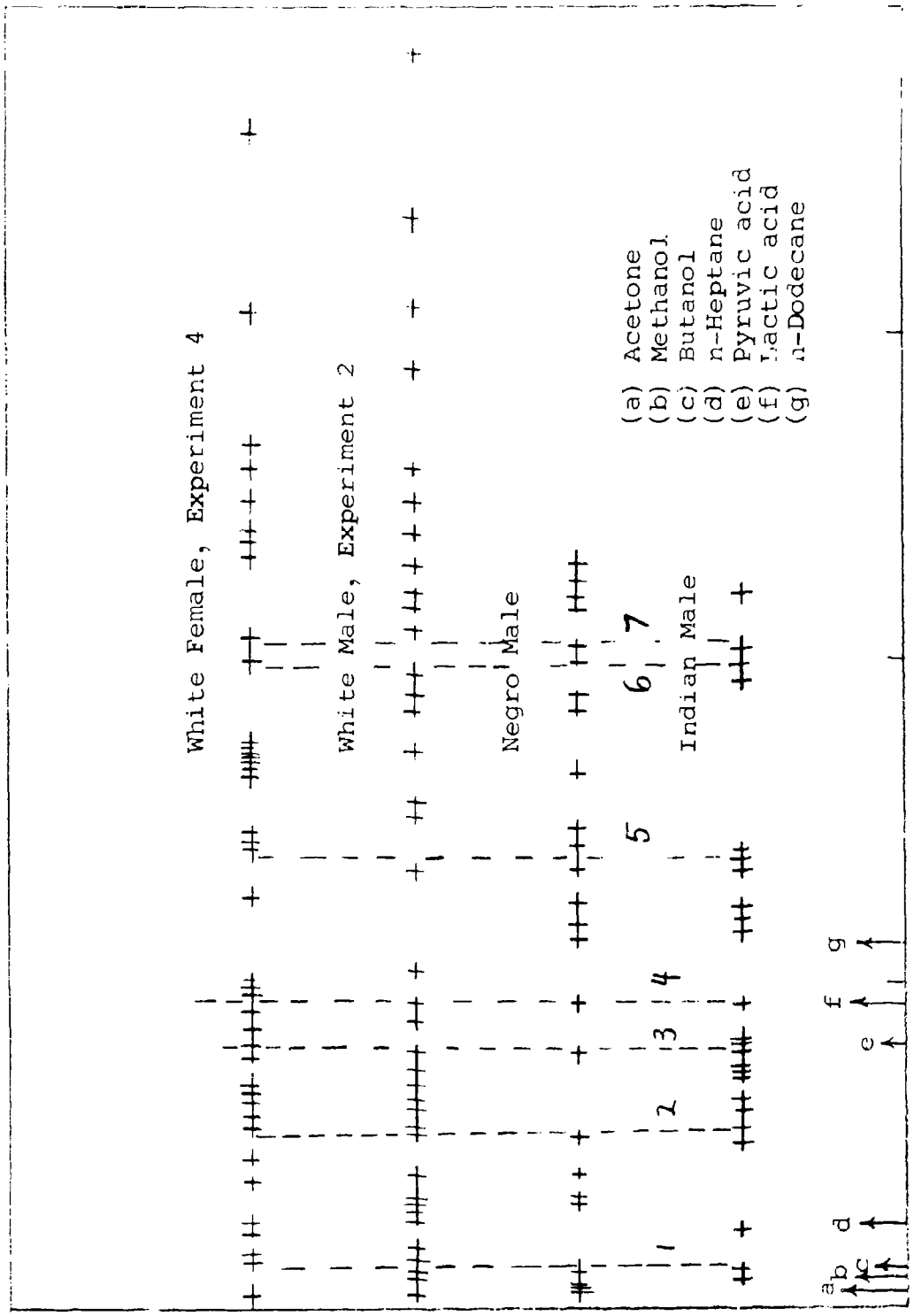

Figure 11

COMPARISON OF SIGNATURES FROM VARIOUS HUMAN SUBJECTS

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common to all four subjects are indicated by dotted lines and respective numbers. The retention times of several authentic compounds determined under the established analytical conditions are shown by arrows.

The data in Figure 11 reveal seven components that are common to all four human subjects studied. The first component falls within the low boiling region of the signature and exhibits an average retention time of 1.23 ± 0.10 min. The 8% deviation from the mean value is considered acceptable in view of the inherent difficulty in reproducing the retention times of low boiling compounds. The following four components, with average retention times of 5.46 ± 0.12, 7.96 ± 0.05, 9.47 ± 0.09, and 13.8 ± 0.03, represent the medium boiling point region of the signatures. The last two components appear in the high boiling region of the signature, as indicated by their average retention times of 19.8 ± 0.15 and 20.6 ± 0.20 min. It is of interest to note at this point that butanol, pyruvic acid, and lactic acid, with retention times of 1.26, 8.10, and 9.30 min, respectively, appear to be components common to signatures of all human subjects considered.

4.73 Identification of Human Signature Components

The inherent complexity and richness of the human chemical signatures observed in our experiments and the partial resolution of several chromatographic peaks made it difficult to fully

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identify all components at this stage of the investigation. In attempting to select the most probable signature components, we determined the retention times of several pure compounds under standardized analytical conditions. In accordance with this determination, methanol (0.96 min), ethanol (0.96 min), butanol (1.26 min), acetone (0.66 min), pyruvic acid (8.10 min), and lactic acid (9.30 min) can be considered constituents of human chemical signatures. Except for lactic acid, this finding agrees with the results of experiments performed by scientists of the Beckman Instruments, Inc., in which the skin bioeffluents of man were monitored.

However, to achieve more reliable use of chemical signatures in the detection and characterization of humans, emphasis should be placed on a larger number of components, especially those with higher boiling points, because of the uncertain origin of the low boiling components. Butanol originates from the action of microorganisms, for instance, yeast, residing on various surfaces in the human environment. The higher boiling signature components are expected to be more characteristic of man. In addition, because of their lower vapor pressures, they are readily collected in measurable amounts on selected stationary phases, as evidenced by our experiments.

4.74 Total Organics Collected from a Human Subject

In order to estimate the total yield of organic compounds collected from a human subject in a typical experiment of 1 hr,

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the contents of both the DEGA coating and the water solution obtained in experiment 4 were examined. The chromatographic peak areas of the respective samples were determined and expressed in terms of n-decane, which was used for their calibration. The yields of organics estimated under these conditions ranged from 3.6×10^{-5} to 5.4×10^{-5} g for experiments in which both spiral traps were used and from 3.4×10^{-1} to 5.1×10^{-1} g for water solutions. Comparison of the yields from the two trapping systems used indicates that the amounts of organics collected on stationary phases correspond to equilibrium conditions.

From the volumes of the air passed through the large glass cell with a human subject, from the volume of the water condensed, and from the collected amounts of organics in the Apiezon film and in water, the following gross partition coefficients for organics can be estimated: Apiezon/air, 5×10^2 to 10^3 and water/air, 10^5. Of course, the partition coefficients for the individual compounds will differ widely from these values.

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5.0 CONCLUSIONS

The following conclusions have been drawn from the evaluation of the airborne chemical signatures collected separately from a limited number of human subjects at 27 to 31°C and 45 to 100% relative humidity.

(1) Total emission of organic compounds from a resting, partially nude human body, with the effluents in breath included, is of the order of 0.5 g/hr.

(2) The lowest chromatographically indicated number of organic components emitted by an individual was 24, and the highest was 44.

(3) Since the chromatographically indicated number of components depends on the resolution of the apparatus used, the actual number of the components in the chemical signatures may be considerably higher.

(4) The volatilities of the majority of the components correspond to those of organic compounds with up to sixteen carbon atoms.

(5) Approximately seven of the components were common to effluents from a white, a Negro, and an Indian (Asian) male, and a white female.

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(6) It is probable that ethanol, butanol, acetone, and pyruvic and lactic acids are among the above common components; this agrees with the findings of scientists at Beckman Instruments, Inc.

(7) In addition to the seven components found common for the compared subjects, other common components were found when selected pairs of subjects were compared.

(8) Some components appear characteristic only for a certain individual among those studied, and hence they either do not occur in others or occur in others below the detection limit.

(9) The similarity in the chemical signatures of the same white male taken 14 days apart is larger than the similarity between his and other signatures.

(10) From the initial data, prospects exist that chemical signatures can be useful not only in the detection of humans but also in the identification of types of humans and perhaps even individuals.

(11) The cylindrical glass and Teflon cell designed for this study is well suited for obtaining airborne chemical signatures of humans.

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(12) Through the equilibration technique of sample collection developed in the study, characteristic features of the chemical signature can be obtained without interference from the large excess of water vapor, although less than 0.01% of the total organic effluents is contained in the sample.

(13) The major fraction of the components of the chemical signature is found in the collected water condensate.

(14) Many components found in the water condensate are also represented in the equilibration trap.

(15) More experimental data on more subjects and on the chemical signatures of nonhuman origin must be obtained before generalizations can be made on the similarities and dissimilarities of the chemical signatures and on applications to military tasks.

(16) To narrow the gap between the findings on the nature of the chemical signatures and the future devices for their utilization in military tasks, further improvements in the techniques are desired, especially in acceleration of the tests, processing of the water condensate, and data analysis.

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(17) The investigation indicates that detection and identification of humans probably should be based on detection and recognition of several components (rather than one) of the chemical signature, preferably those possessing lower volatilities.

(18) The objectives of the project work have been accomplished, and recommendations for the most needed aspects of the future work have been made. The latter are detailed in the following section.

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6.0 RECOMMENDATIONS FOR FUTURE WORK

The results of this study demonstrated that objectively detectable similarities as well as differences occur in the airborne chemical signatures of individuals of different sexes and races. However, the amount of the data obtained is as yet insufficient to justify generalizations and to separate those parts of signatures that are influenced by diet or that could be obscured by the chemical signatures of vegetation, soil, and other indigeneous sources of organic vapor.

More knowledge and a better understanding of the chemical signatures are needed before conclusions can be reached on the technological requirements for sensing and identifying human subjects through airborne chemical signatures. To advance toward this goal, work in the following directions is recommended.

(1) More experiments must be conducted on different individuals, on the degree of constancy of the chemical signature of the same individual as influenced by diet, and on the nature of the compounds represented, especially those found characteristic of humans or certain human types.

(2) Chemical signatures of other sources such as vegetation and soils must be studied to assess the magnitude of the problems in discriminating the sets of man-related and environment-related components.

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(3) Additional advances in the experimental techniques must be made to accelerate the chemical signature collection and analysis processes, to improve the analytical procedures, especially for the condensed water effluent, and to refine the equilibrium technique of effluent collection.

(4) Procedures must be developed to permit a rational comparison of the chemical signatures in terms of some similarity index and a rapid computerized estimate of the probability that a certain complex signature, e.g., man and environment, contains the chemical components in amounts and ratios characteristic of a man.

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13. ABSTRACT

This investigation was initiated to study human airborne chemical signatures. A novel apparatus was designed and constructed with inner surfaces of glass and Teflon to permit close control of experimental environment and to monitor humidity and oxygen content. An efficient signature collection system was developed, and techniques of sample acquisition and gas chromatographic analysis were established.

The results of signature collection experiments performed on selected human subjects revealed several important features. Seven signature components were found to be common to white, Negro, and Indian males and a white female. It is probable that ethanol, butanol, acetone, and pyruvic and lactic acids are among the common components. Other compounds were found to be common to selected pairs of subjects compared. Some components were characteristic only of a certain individual among those studied and either do not occur in others or exist below the detection limits. Chemical signatures from the same white male individual obtained 14 days apart exhibited large similarities. Less significant similarities were observed between the latter signatures and those from other subjects.

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