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FINAL REPORT

Contracts No. NASw 508 and NASw 1170

28 January 1966

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INDEX

SUMMARY

Alkanes are constituents of organisms and sediments. The structures, distributions, isotopic compositions, and optical properties of C_{15} to C_{30} biological and sedimental alkanes are usually either identical or similar. Pristane and phytane, isoprenoid-type alkanes which are structurally related to the phytol moiety of the chlorophyll molecule, are found in rocks of many geological ages and in organisms. General resemblences in the positions, shapes, and relative sizes of chromatographic peaks are observed in the gas chromatograms of alkanes from most terrestrial rocks and meteorites, and the mass spectrometric cracking patterns of these alkanes share many features. Abiotically produced alkanes do not contain detectable quantities of pristane or phytane, and all definitive analyses of abiotic alkanes differ significantly from equivalent analyses of biological alkanes.

Repeated tests of the sensitivity of our analytical methods show that reproducible characterizations can be obtained of alkanes which comprise 10^{-8} or more parts by weight of samples. Contaminants are the major source of errors in organic geochemical investigations, and the contamination of samples most frequently occurs prior to the receipt of samples in the laboratory. Analyses of extracts from different segments or portions of a sample provide data for assessing the levels and evaluating the effects of contamination. Analyses of alkanes from Paleozoic and Precambrian rocks indicate that life has persisted for more than 3 billion years on earth. Ordinary chondrites, also, contain biological alkanes as well as optically active organic non-hydrocarbons, but our results do not rule out the likelihood that these meteorites were contaminated with terrestrial materials. Analyses of alkanes and aromatic hydrocarbons from fragments of the Orgueil, Type I carbonaceous chondrite, are suggestive of an indigenous and biological origin for some meteoritic carbon compounds. The investigations which were carried out under Contract Numbers NASw508 and 1170 afford additional evidence that alkanes can play an important role in paleobiological and exobiological research.

INTRODUCTION

The major objectives of these investigations have been to test the reliability of alkanes as biological indicators and to develop and maintain reference data on alkanes of various origins.

In dealing with the general problem of recognizing organisms or their remains, one is forced to consider the characteristics that distinguish animate from inanimate things and the variabilities which are displayed by organisms and abiotic carbon compounds. Such considerations necessarily require an appraisal of what constitutes the living state and how it evolved.

The modern theory of the origin of life was initiated by Haldane[1] about 30 years ago, and this theory was significantly augmented by Oparin[2] and other scientists[3]. They assume that organic molecules formed in a reducing atmosphere on primordial earth. Most reactions probably occurred in the upper regions of the atmosphere which received the greatest quantities of solar energy. These molecules accumulated in the oceans and seas. As the concentrations of the organic compounds in terrestrial waters increased, the variety and complexity of the compounds were also increased by interactions, and over long periods of time molecular aggregates

formed. It is believed that some of these aggregates functioned as catalysts to control the reaction rates of other molecules--a beginning of biochemical evolution, and eventually an aggregate formed that functioned as an organism.

Numerous investigators have demonstrated that abiotic processes can produce many biologically significant molecules and structures[2,3,4] and Urey[5] has presented sound theoretical arguments that the primitive atmosphere could provide the reactants for these processes. One of the most difficult problems in the origin of life is to explain the thermodynamics of the molecular polymerizations that converted simple molecules into active aggregates. Major extrapolations are required to bridge the gaps between the molecules and active aggregates and the active aggregates and organisms, but the results and proposals of Calvin, Wald, and Ponnamperuma are closing these gaps[3,6].

Seemingly, the modern theory of the origin of life is sufficiently developed to provide guidance for those who are devising methods of detecting extraterrestrial life. Wald[6] explains why this theory in conjunction with our knowledge of chemistry places severe restrictions on the composition of life and the environments in which it originates. He writes, "The major bioelements therefore present unique properties indispensible for the formation and function of living organisms. They--particularly carbon, hydrogen, nitrogen, and oxygen--form also a number of unique molecules, indispensable or of singular importance for organisms... For these and similar reasons I have become convinced that life everywhere must be based primarily upon carbon, hydrogen, nitrogen, and oxygen, upon an organic chemistry therefore much as on Earth."

In essence, our knowledge of physics and chemistry and our understanding of metabolic processes justify the presumption that the characteristics of organisms on Earth approximately define the characteristics of life which we may find on other celestial bodies.

ANALYTICAL DETECTION OF LIFE

Accepting the Earth as a standard, we may ascertain from considerations of the compositions of the organic materials in a terrestrial sample what analyses may provide the best means of detecting life on other celestial bodies.

A mean sample of the Earth's crust contains 1 to 2 per cent of organic matter and less than 1 per mille of organisms. The preponderance of carbonaceous substances in this sample is composed of the organic residues from former life, but analyses of these residues show that they are for the most part neither chemically nor structurally similar to biological compounds.

The compositional differences between organic remnants and organisms are probably a consequence of the carbon cycle. Organisms are the dynamic force in this cycle. Plants and animals dominate the production and utilization of carbon compounds[7]. The mean compositions of carbonaceous materials on Earth, however, are strongly influenced by slow but continuous accumulations of compounds that have effectively escaped the carbon cycle, and the rate of this escape is inversely related to the catabolic activities of these compounds.

The reverse development of biosynthetic pathways, as suggested by Horowitz[3], and theories of biological evolution favor the efficient utilization of abundant organic compounds. It is not in the best interest of organisms that energy be wasted

and that carbon be squandered in the production of organic materials which are retained in sediments. Thus, we can expect that only the catabolically least active carbon compounds may escape the carbon cycle, and observation and analyses indicate these compounds to be either highly and randomly altered or inherently inactive products of life[8].

It is plausible to assume that the bulk of carbonaceous materials in sediments, which we are unable to relate chemically or structurally to organisms to biological molecules, represent abiotic attritions. Most metabolically active compounds are also chemically reactive. In organisms, the formations and degradations of molecules follow precisely defined and numerically restricted pathways. The rates of biological reactions are necessarily rapid, for otherwise the destinies of active molecules are left partially to abiotic reactions. Stated differently life persists because biological reactions win the race that controls the structures of carbon compounds, but such victories are incomplete.

A slow overlap apparently exists between the slowest metabolic reactions and the fastest abiotic reactions, and a minor portion of biologically important molecules are randomly altered. Once altered by a chance abiotic process a molecule may become metabolically less active, and the probability is increased that this molecule may undergo additional abiotic transformations. Successive abiotic changes occurring alowly but continuously over geologic periods of time may destroy the structurally distinctive features of remnants of former organisms.

Alkanes lack functional groups. These saturated hydrocarbons are non-polar. They are minor constituents of living organisms. C_{15} to C_{30} alkanes are capable of displaying great structural order, and they are probably catabolically and chemically the least active biological molecules. For reasons discussed above, the qualities of these hydrocarbons may permit their partial escape from the carbon cycle as well as insure their preservation in sediments. Investigations indicate that certain C_{15} to C_{30} alkanes are the most easily identified or characterized and best preserved of the widely distributed remnants of former life.[9] Pristane and phytane are examples of specific biological alkanes that can retain their structural integrity for billions of years in sedimentary rocks.[10]

ALKANES AS MOLECULAR FOSSILS

Traditionally, a fossil is defined as a morphological organized entity or remnant of a preexistent organism, irrespective of its size or completeness. This concept of a fossil has been extended in recent decades by increasing the discrimination of the level of observation with the result that at present it is possible to determine biologically produced structures at the molecular or even submolecular level. Isotopic measurements have demonstrated that biological processes are selective at the atomic level, but selectivity is not solely a property of living systems. Abiotic as well as biotic reactions are selective.

The differences between biological and abiotic products are differences of degree. Crystals of minerals are ordered, but the order of these crystals and of most inanimate things may usually be defined by relatively simple equations. Such may also be the case with simple biological molecules. Many amino acids, nucleic acid bases, and other organic compounds may be synthesized abiotically as well as biologically, but the complexities and structures of some biological materials are less easily comprehended and assayed than those of inanimate things. In a gross

sense, we accept as evidence of former life those entities which display an order that is not feasible of abiotic origin and that resembles an order found in an existing organism or its component part. A molecular fossil, therefore, must be of sufficient size to be highly ordered and of sufficient stability to retain a structure that is relatable to the structure of a known biological compound. Research carried out under Contracts NASw508 and 1170 and by other investigators demonstrate that some C_{15} to C_{30} alkanes are reliable and versatile molecular fossils[9,10,11].

To establish that alkanes may be used as fossils, we have shown that alkanes are made by plants and animals, do escape the carbon cycle, and are preserved for geologic periods of times in sediments. We have analyzed a variety of abiotic alkanes, and we have found no evidence that abiotic processes can produce alkanes that are indistinguishable from biologically derived alkanes[8,12]. The experimental results obtained in our laboratory and by other investigators may be summarized as follows:

1. Miller-Urey type syntheses yield alkanes of relatively uniform composition. A typical gas chromatogram of abiotic alkanes, which were made by the irradiation of methane, is shown in Figure 1. The broad and skewed Gaussian-like curve indicates the molecular weight distribution but reveals little about the composition of these complex alkanes. This distribution does not change significantly when the irradiation doses and energies are varied over a wide range.

2. Alkanes produced by Fischer-Tropsch reactions are easily distinguished from biological alkanes[8].

3. Neither equilibria[13] nor non-equilibria abiotic reactions produce alkanes which resemble those in organisms, terrestrial rocks or meteorites.

4. The concentrations of alkanes in fecal lipids are many times the concentrations of alkanes in the lipids of biological tissues[14], and the compositions of alkanes in cow manure are analogous to those of the alkanes in the plants which are consumed by the cows[15]. (These results establish that alkanes can escape the carbon cycle, i.e., biological assimilation.)

5. Pristane and phytane are constituents of biological lipids and terrestrial rocks of many geologic ages[10]. These alkanes are not detectable in abiotic alkanes[8,12].

6. The structures, distributions, isotopic compositions, and optical properties of some C_{17}-C_{30} alkanes in organisms, sedimentary rocks, and meteorites are similar, but certain distributional differences are observed in the alkanes which are isolated either from different samples or from different portions of the same sample.

7. A knowledge of the distributional variations in naturally occurring alkanes provides a means of assessing whether or not a sample is contaminated[16].

ANALYTICAL FACILITIES

Photographs and a floor plan of the organic geochemical laboratory at Esso Research and Engineering Company are presented in Appendix 1. In addition to these facilities, the Analytical Research Division of Esso Research and Engineering Company maintained infrared and ultraviolet spectrometers (Baird Associates Model 4-55 and

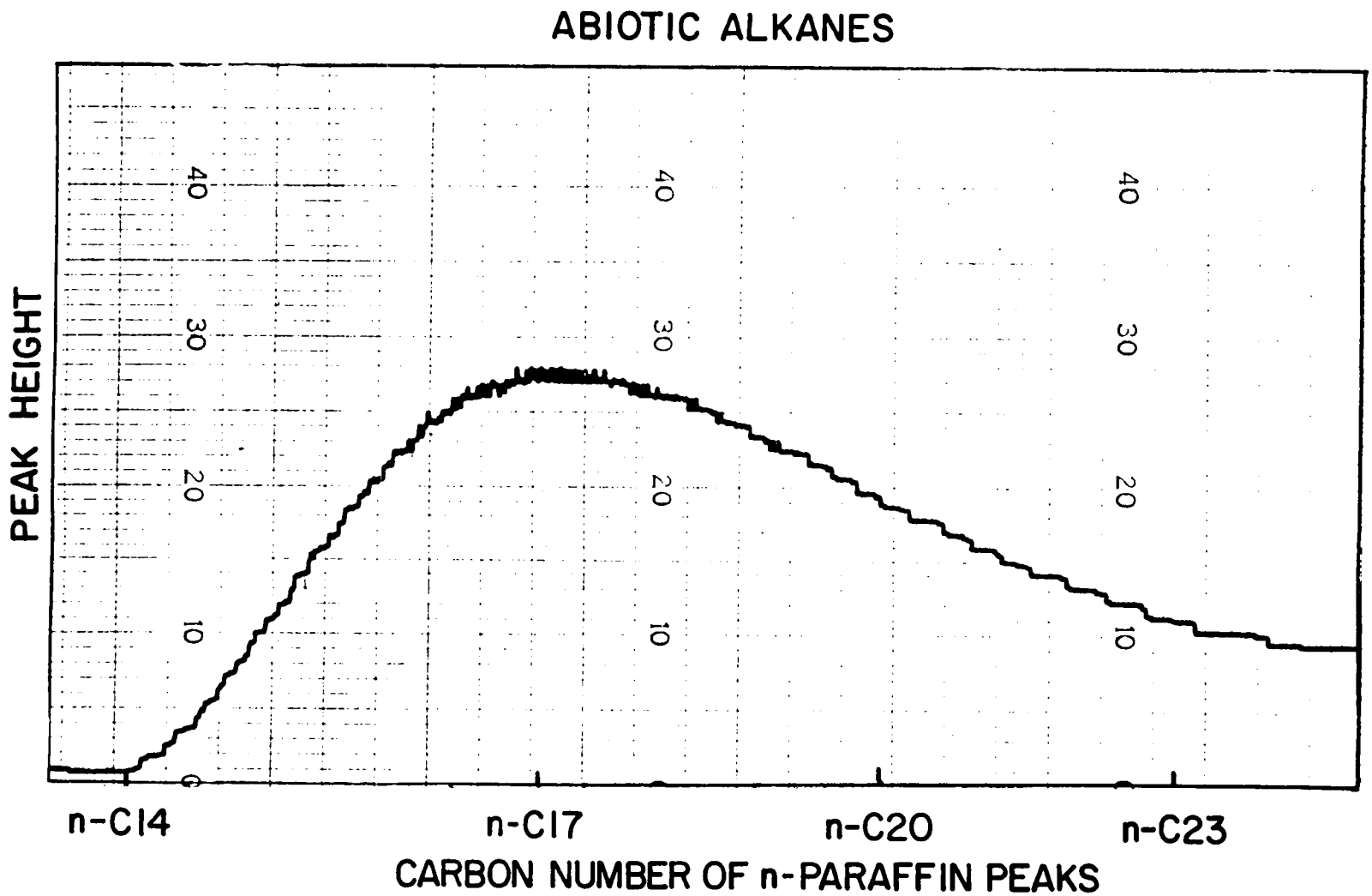

FIGURE 1. GAS-LIQUID CHROMATOGRAM OF ABIOTIC ALKANES MADE BY MILLER-UREY TYPE SYNTHESIS

Cary Model 14, respectively) and a Benson-Lehner Oscar J peak reader (used for measuring mass spectra) which were employed in these investigations. The isotopic corrections and tabulations of mass spectrometric data were done on an IBM 7090 computer by the Mathematics Department at Esso Research and Engineering Company, Florham Park, New Jersey. All facilities and analytical instruments, except for the Barber-Colman Model 10 which was purchased with NASA funds, were supplied by Esso Research and Engineering Company.

The Barber-Colman, Model 10, gas chromatographic instrument was modified to eliminate "coldspots" in the inlet system for the capillary column and to increase the heat capacity of the inlet system for the analytical and preparative scale columns. A valved-split system, also, was added to control the detector and collector flow rates of effluent gases from the preparative scale columns, and the glass chromatographic columns were replaced with stainless steel and copper ones to reduce breakage. Drawings of the modifications which we have made in the Barber-Colman Model 10 instrument are presented in Appendix 2.

Drawings and some photographs of equipment, which we designed specifically for our use in organic geochemical analyses are shown in Appendix 3.

ANALYTICAL PROCEDURES

All samples are processed in the following manner:

1. The sample is extracted.

2. Elemental sulfur, if present, is removed.

3. The extract is fractionated by silica gel chromatography.

4. The alkane fraction and occasionally other fractions are analyzed by infrared and ultraviolet spectroscopy, gas-liquid chromatography and mass spectrometry.

5. Alumina chromatography and gas-liquid chromatography are used to obtain refined fractions of certain alkanes and organic non-hydrocarbons for analyses listed under 4 and for optical rotatory dispersion measurements. The detailed analytical procedures are:

Solvent Purification. Benzene, carbon tetrachloride, n-heptane, and methanol were the principal solvents used in these investigations. All solvents were reagent grade and they were distilled prior to use. Individual distillation apparatuses were maintained for each of the principal solvents. These apparatuses were equipped with 2' glass helices pack columns and reflux heads. The distillation apparatuses are shown in the photographs of the laboratory in Appendix 1. Distilled water was extracted with benzene.

Tubing, Stopcocks, Fittings, Wrappings, and Coverings. Plastic and rubber tubing or fittings and glass stopcocks are sources of contaminants. All tubing, fittings, and stopcocks, which were exposed to solvents in our laboratory, were made of teflon. Reynolds aluminum wraps were used for sample wrappings and covering containers.

Reagents. Repeated tests were run on reagents to insure that they were free of significant contaminants before the reagents were used for analytical purposes.

Glassware and Porcelain and Teflon Utensils. All utensil were cleaned in a 1:1 H_2SO_4-HNO_3 acid bath maintained at 130°C. The acid cleaned utensils were rinsed successively in distilled water, methanol, and benzene.

Analytical Blanks. Complete analytical blanks were run on each step of sample preparations. Acid cleaned glass beads or porcelain chips were used as blanks for rock and meteorite samples.

Extractions. Benzene-methanol (9:1 v/v to 2:1 v/v was most commonly employed as the extraction solvent, but a limited number of samples were extracted with benzene-methanol and then with n-heptane. Rocks and meteorites were crushed and pulverized in mortar with pestle, disc grinders, or ball mills. Samples, which were carefully collected and handled so as to make contamination highly unlikely, were crushed and extracted. Samples, with incompletely controlled collection or storage histories, were extracted in stages. The whole samples were extracted and then they were crushed and reextracted. The crushed rocks or meteorites were finally immersed for 1 day to 7 days in hot (60-80°C.) 48 percent hydrofluoric acid and the HF slurry was extracted[16]. Extractions of whole and crushed samples were accomplished by one of three means. The extractions were done in: (1) a 150 watt ultrasonic extractor at an operating frequency of 20 kcy sec; (2) Soxhlet extractors; or (3) the ball mill-extractor (see Appendix 3). The HF slurries were extracted in 2-liter glass separatory funnels, using benzene as the extraction solvent. The HF treatments were carried out in 1-liter Teflon beakers. The Soxhlet and ball-mill-extractor extractions were performed under a pressure of filtered nitrogen. The nitrogen (5 psig) was passed through a silica gel trap and entered the extraction apparatus by means of an unsecured ball-joint at the top of a condenser. The unsecured joint served as the pressure control for the system.

Sample Recovery. Samples were recovered from solvents intthe sample recovery system shown in Appendix 3. This system is maintained at 40 $\pm$ 1°C. and it has recepticles (48) for 200 ml beaks. Aluminum inserts are available for adapting the recepticles to accommodate either 28 ml (1 oz.) bottles or 8 ml vials. Containers larger than 200 ml volume are placed on top of the recovery system. Nitrogen at 30 psig is filtered through a series of two silica gel columns and a glass wool packed column and it is directed by a manifold into the sample bottles. Solvents and volatile constituents (alkanes and other organic compounds with vapor pressures greater than C_{15} hydrocarbons) are removed in the nitrogen streams.

Sulfur Removal. Many rocks and meteorites contain elemental sulfur. The removal of sulfur from the extracts of rocks or meteorites can be accomplished either by adding colloidal copper to the extracting solvent or by passing an extract in solution through a column of colloidal copper[17].

Elemental sulfur may be separated from the aromatic hydrocarbons and the nitrogen, sulfur and oxygen containing (organic non-hydrocarbons) carbon compounds by silica gel chromatography. Elemental sulfur is eluted from silica gel columns by carbon tetrachloride. Since some organic non-hydrocarbons may react with colloidal copper, it is sometimes desirable to isolate the sulfur and alkanes from an extract by silica gel chromatography; then the sulfur may be removed from the inert alkanes on a colloidal copper column.

Liquid-Solid Chromatography. Silica gel chromatography with n-heptane, carbon tetrachloride, benzene, and methonal as successive eluants is used to fractionate all extracts. The n-heptane and carbon tetrachloride eluants are composed

of alkanes (plus elemental sulfur when it is present). The benzene eluates contain mainly aromatic hydrocarbons plus some esters, alcohols, and other organic non-hydrocarbons. The methanol eluates are composed of organic non-hydrocarbons[18].

Alumina columns are superior to silica gel columns for type separations of either alkanes or aromatic hydrocarbons and for separating hydrocarbons from organic non-hydrocarbons, but silica gel columns are better than alumina columns for separating alkanes from aromatic hydrocarbons. A limited number of alumina chromatographic fractionations were carried out in this investigation. The procedures used in these fractionations have been previously described[19].

Infrared Spectroscopy. Scans were obtained either as smears on sodium chloride windows or in spectral grade carbon bisulfide solutions in matched 0.1 mm length cells equipped with sodium chloride windows[18].

Ultraviolet and Visual Spectroscopy. Ultraviolet and visual spectra were run in matched 1-cm length cells equipped with quartz windows, and spectral grade iso-octane was used as solvent[18].

Mass Spectrometry. Samples were introduced as liquids through gallium covered frits or as solids through the solids-inlet system. Descriptions and discussions of the acquisition, computation, and interpretation of mass spectral data have been published[18].

Gas-Liquid Chromatographic (GLC) Analyses. Major attention in these investigations were paid the alkanes, and the columns used in GLC were selected on the basis of their efficiency in alkane fractionations. Apiezon "L" or the n-heptane eluates of Apiezon "L" from silica gel chromatographic columns provided the best substrates for the GLC analyses of alkanes. Our capillary columns were coated by 10 percent solutions of Apiezon "L" in n-hexane or n-heptane. Analabs 80-90 mesh ABS coated with 10% Apiezon "L" or SE-30 in benzene were commonly employed as packings for analytical and preparative scale columns. The inlet argon pressures for the capillary and for the analytical and preparative scale columns were usually 30 psig and 20-10 psig respectively. A split ratio of 35:1 was normally maintained on the capillary columns, and the effluent splitting on the analytical and prep columns were varied by the valve (see Appendix 2) to obtain a satisfactory detection level. A scavenge rate of 85-100 ml/min. argon was used on the detectors. The analytical and prep columns were temperature programmed at 1-2°C./min. for the ranges of 50-300°C. for Apiezon "L" columns and 100-375°C. for SE 30 columns. An effort was made to vary the chart speeds and temperature programs for different capillary columns so that the chromatograms obtained with these columns had similar appearances. Chart speeds of 7.5 and 15 in./hr. and temperature increases of 2° and 4°C./min. for the 70-300°C. range were most frequently used for the capillary columns.

Inlet temperatures of 400-450°C. were maintained for biological and sedimental alkanes, but abiotic alkanes were severely degraded at temperatures in excess of 300°C. Except for studies of the thermal degradations of abiotic alkanes, the inlet temperatures for these hydrocarbons were in the 250-270°C. range. Previous publications present additional information about the operating conditions[12].

Analytical Capabilities and Limitations. GLC analyses, using capillary columns and the microionization detector (radium sulfate source), can provide definitive chromatograms of 2 micrograms of alkanes. Complete analytical blanks indicate that in our analytical procedures we rarely introduce more than 1 microgram of alkanes into a sample. We have repeatedly obtained reproducible characterizations

of alkanes which comprise as low as 5 parts per 100 million parts by weights of rocks. We have not observed significant variations of the size of our analytical blanks when the quantities of our solvents and reagents are varied over a wide range. Although it is reported that pristane and phytane are present in concentrations of 10^{-7} to 10^{-8} parts by weight in reagent grade and distilled reagent grade solvents[20], blanks on 10 liter portions of our solvents consistently indicate that these solvents contain less than 10^{-9} parts by weight of all C_{15} and larger alkanes. Our results suggest that the inlet system or capillary columns of the Barber-Colman Model 10 instrument, rather than our reagents, solvents, and laboratory facilities, may be the principal sources of analytical blanks. Furthermore, it would appear that our analytical facilities and procedures could permit us to obtain reliable analyses of 1 kilogram samples that contain 5×10^{-9} parts by weight of alkanes.

JOINT RESEARCH PROGRAMS

Several geological, paleontological, and paleobiochemical investigations have been conducted jointly with Dr. E. S. Barghoorn and Mr. J. W. Schopf of Harvard University. These investigations have provided invaluable checks on the reliability of alkanes as molecular fossils. In studies of the rocks from the 1 billion year old Nonesuch formation, alkane analyses provided the initial evidence for Precambrian life, and this evidence was confirmed by paleontological analyses. In the cases of rocks from the 2 billion year old Gunflint and 3 billion year old Figtree formations, the paleontological evidences for life were confirmed by alkane analyses. Detailed discussions of the investigations of the Nonesuch and Gunflint samples have been published[10], and the results of the Figtree studies have been submitted for publication[21].

As a part of an investigation of meteorites carried out with Dr. Clifford Frondel, Harvard University, we conducted an extensive study of the sensitivity of our analytical procedures, the contamination problem, and the compositions of meteoritic alkanes. We analyzed many meteoritic extracts, every direct or indirect souce of alkanes in our laboratory, and 50 to 150 year old teaching specimens of minerals from the Harvard Museum. These analyses may be summarized as follows:

1. Our analytical methods provide reproducible characterizations of alkanes which comprise as low as 10^{-8} parts by weight of rocks and meteorites.

2. We extract an average of more than 10^{-4} parts of organic material by weight from ordinary chondrites.

3. The average concentration of alkanes in these chondritic extracts is in excess of 20 per cent, and the chondritic alkane and organic non-hydrocarbon fractions are optically active.

4. We have not found contaminants in our laboratory which are a feasible source of the chrondritic extracts.

5. We have found evidence that some rocks in the Harvard Museum are contaminated.

6. Although the contaminants of these rocks are more variable in composition than the chondritic extracts, the contaminants in 3 of the 7 rocks analyzed do contain alkanes similar to the chrondritic alkanes, and an organic non-hydrocarbon

fraction from one of the rock samples displays a positive Colton Effect similar to that of the chondritic organic non-hydrocarbons.

7. Extract of human hands contain organic non-hydrocarbons that have optical properties similar to those of the organic non-hydrocarbons from the rock and chondrites.

8. Tectites do not contain detectable quantities of organic matter.

9. Our analyses of thoroughly cleansed octahedrites and hexahedrite show that these iron meteorites are devoided of alkanes. These results disprove the hypothesis[22] that metallic carbides in meteorites may react with water to produce petroleum-type hydrocarbons.

The alkanes isolated from the Bath, Grady #2, Holbrook, Homestead, and Waconda chondrites are almost identical in composition. A gas chromatogram of these alkanes are presented in Figure 2, and partial gas chromatograms of alkanes from the Holbrook chondrite and a Cambrian crude oil are shown in Figure 3. These analyses in Figure 3 permit a direct comparison of the numbers, spacings, relative sizes, and shapes of the peaks that are intermediate to the n-paraffin peaks. For example, compare the peaks between n-C_{20} and n-C_{21}. The n-C_{20} peaks are followed in succession by a small sharp peak, a complex peak, a triplet of sharp but incompletely resolved peaks and a small doublet. Comparisons of the peaks throughout the n-C_{17} to n-C_{24} regions of these chromatograms provide strong evidence that alkanes in some meteorites and terrestrial rocks are similar. Such similarities as are observed in these chromatograms are commonly observed in the chromatograms of alkanes from rocks of all geologic ages. A comparison of Figures 1 and 2 or of Figure 2 with the published chromatogram of Fischer-Tropsch alkanes[12] show that abiotic alkanes may be readily distinguished from sedimental and meteoritic alkanes.

Whereas our analyses of meteorites strongly indicate that ordinary chondrites contain alkanes of biological origin, this joint meteoritic investigation emphasizes that contamination is a major problem in organic geochemical investigations. We believe that our analyses of meteorites and terrestrial rocks have placed important boundary conditions on the contamination problem. Although the marked variations in the molecular weight distributions and the concentrations of alkanes in contaminated mineral samples from the Harvard Museum are in contrast to the uniformities in compositions and concentrations of alkanes from the chondrites, analogies do exist between the analyses of the extracts of the rocks and ordinary chondrites. These extracts contain alkanes similar to those found in broad distillate fractions of petroleum. Thus, the alkanes could have come from petroleum products such as lubricating oil, hair dressings, furniture polish, or floor cleaning compounds, and the compositional variations of the contaminants in the mineral samples could reflect differences in the porosities and adsorptivities of these samples. Furthermore, the similarities in the optical properties of hand extracts and the organic non-hydrocarbons from the chondrites and rock suggest that contaminants may have been introduced into the museum specimens by handling.

Our results do not prove that the ordinary chondrites were contaminated but they indicate this possibility. The difficulties involved in establishing the source of the chondritic extracts are probably atypical. Successive extracts of terrestrial rocks, unlike the successive extracts of chondrites, usually vary in composition. For example, n-paraffins were the principal alkanes in the extracts of whole rocks from the 2.7 billion year old Soudan formation; while branched chain and cycloalkanes were dominant in the extracts obtained from these extracted rocks after crushing and reextracting them. Such compositional variations in different

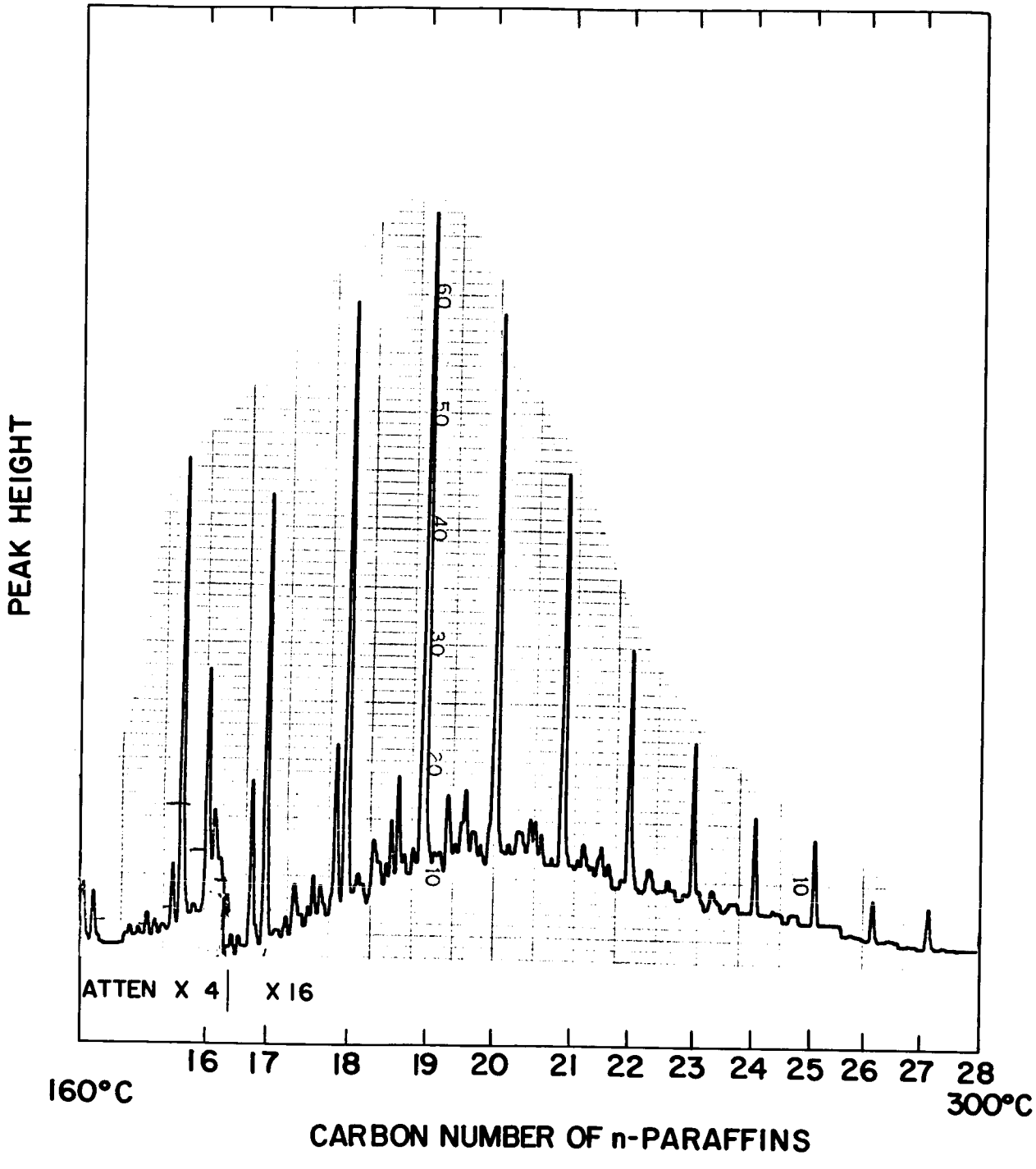

FIGURE 2. GAS-LIQUID CHROMATOGRAM OF ALKANES FROM BATH, GRADY #2, HOLBROOK, HOMESTEAD, AND WACONDA CHONDRITES

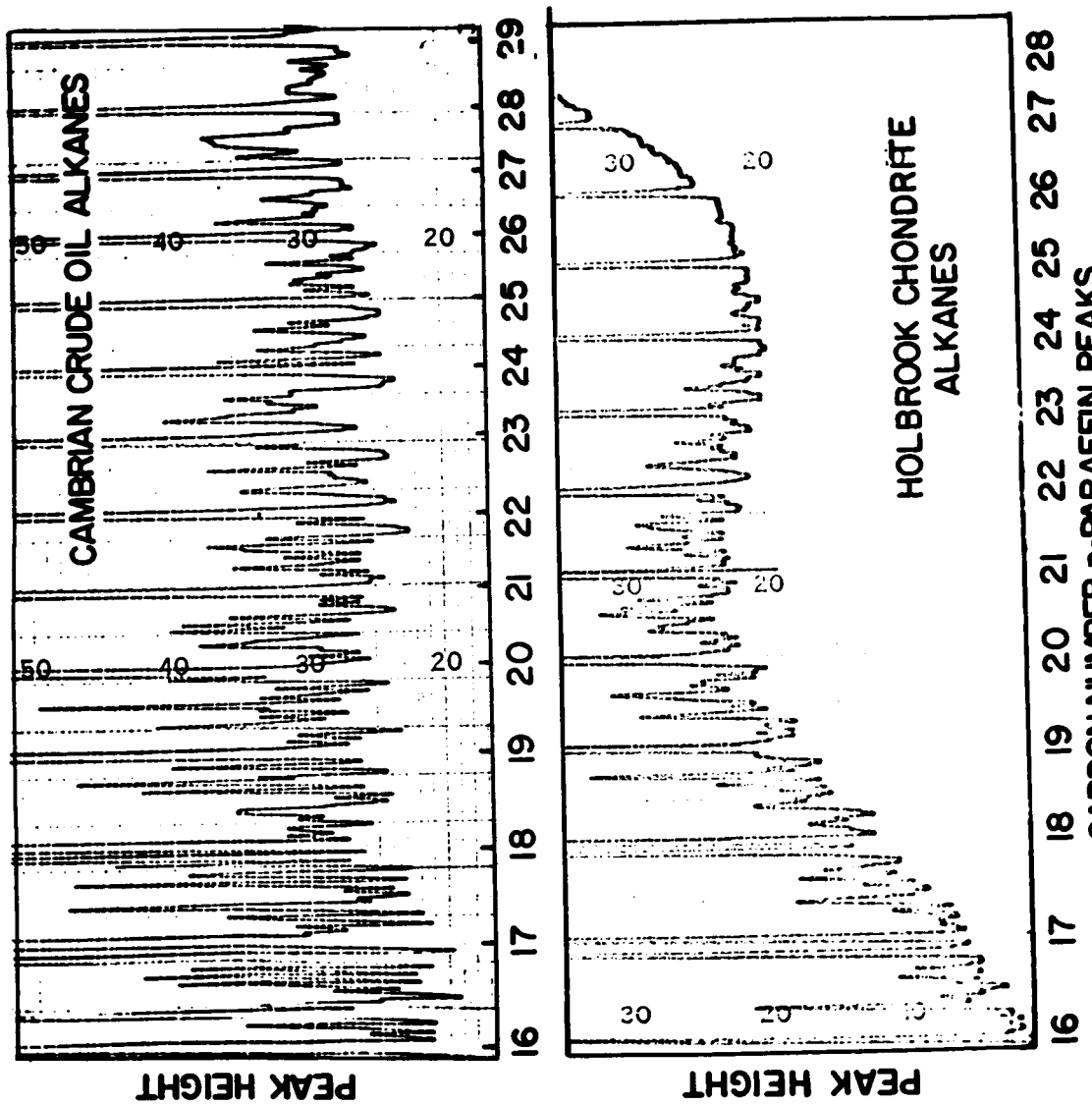

FIGURE 3. PARTIAL GAS-LIQUID CHROMATOGRAMS OF ALKANES FROM HOLBROOK CHONDRITE AND CAMBRIAN CRUDE OIL. PRISTANE, PHYTANE, AND n-PARAFFIN PEAKS ARE OFF-SCALE. THE PRISTANE AND PHYTANE PEAKS APPEAR IMMEDIATELY TO THE LEFT OF THE $n\text{-}C_{17}$ AND $n\text{-}C_{18}$ PEAKS, RESPECTIVELY.

extracts of rocks provide an assessment of the place of origin of the alkanes. The Soudan results[16] clearly indicate that the biological alkanes are indigenous, and the chromatographic fractionations, which separated porphyrins from alkanes, in the 1 billion year old Nonesuch formation also supply strong evidence that living organisms were co-deposited with the Nonesuch sediments[23]. The distributions of hydrocarbons within a specific extract may sometimes indicate whether or not a sample is significantly contaminated. The concentrations of alkanes and aromatic hydrocarbons, the high abundances of odd carbon number C_{21} to C_{29} n-paraffins, the presence of pristane and phytane, the simplicity of the aromatic fractions in our extracts of the Orgueil carbonaceous chondrites can not be readily explained by assuming that meteorite was contaminated either with petroleum products or terrestrial organisms[8,18]. The Orgueil fragments which we have analyzed apparently contained indigenous compounds of biological origin.

We are carrying out a joint research project on the optical properties of alkanes and organic non-hydrocarbons from terrestrial rocks and meteorites with Drs. K. Mislow and P. Laur of Princeton University. The optical measurements discussed above were obtained as a part of this joint project, and a manuscript, describing our research, is being prepared for publication.

CURRENT STATUS

Three of the major objectives of these investigations have apparently been achieved. The widespread use of alkanes in paleobiological research[10,16,20] and the presence of pristane, phytane, and other isoprenoid type hydrocarbons in hydrocarbons in organisms and ancient rocks[10,16,20,24] confirm that alkanes are reliable, detectable and stable biological indicators.

Under Contract Nos. NASw-508 and -1170 analytical methods have been devised and tested that provide means of obtaining definitive analyses of alkanes that are present in samples in the parts per billion by weight range. In excess of 1200 gas chromatograms, 2000 liquid-solid chromatographic analyses, and 500 mass spectra have been recorded for alkanes or other benzene-soluble constituents of organisms, sedimentary rocks, meteorites, and abiotic products, and in collaboration with other investigators spectrophotometric analyses have been run on more than 50 terrestrial and meteoritic samples. Multigram quantities of alkanes have been isolated from seven biological and five sedimental sources. Extensive efforts have been made to identify alkanes from a >1 kilogram fraction of petroleum alkanes, and more than 25 reference C_{15} to C_{30} alkanes have been collected. Phytane has been identified in butter, and a tandem gas chromatographic-mass spectrometric analyses run by Dr. S. R. Lipsky of Yale University confirms this identification. Tentative identifications have been obtained of six isoprenoid-type alkanes in Vibrio ponticus and of dihydrosqualene in petroleum[11].

Three new analytical methods were initally developed in our laboratory. These methods afford means of assaying the distributions of benzene soluble materials in natural samples and of assessing the source of these materials. We believe that sufficient information has been gathered to permit the development of instruments for the analyses of alkanes that may be present on other celestial bodies. Additional information about the compositions and distributions of alkanes in terrestrial samples and meteorites should increase the usefulness of these important biological indicators in exobiological investigations.

ACKNOWLEDGMENTS

We have been partially dependent on other scientists and investigators for samples and analytical facilities or abilities. Dr. T. S. Oakwood, Pennsylvania State University, supplied us multigram quantities of alkanes from Vibrio ponticus and kelp. Dr. H. W. Girard, Rutgers University, carefully processed 3 pounds of uncontaminated butter which we used as a source of biological alkanes. Abiotic alkane samples, made by Miller-Urey type syntheses, used in these investigations were produced under Dr. W. F. Libby's direction at the University of California, Los Angeles, and Dr. Ponnamperuma's direction at Ames Research Center. Dr. Preston Cloud, University of Minnesota, presently at University of California, Los Angeles, furnished samples of rocks from the 2.7 billion year old Soudan formation*. Dr. Dan W. Urry, Harvard University, determined the optical rotatory dispersion curves and Dr. Klaus Biemann and Mr. John Hayes, MIT, obtained high-resolution mass spectrometric analyses of organic non-hydrocarbon fractions which we isolated from ordinary chondrites. Dr. W. H. Johnston, Johnston Laboratories, suggested the need for the ballmill-extractor which we designed. Drs. H. C. Urey and W. F. Libby have discussed various aspects of this research with me, and they have made beneficial suggestions. I appreciate the contributions of these scientist and the analytical assistance that was ably provided by Mr. K. C. Klein and Mr. H. Carrillo, Esso Research and Engineering Company, Linden, New Jersey.

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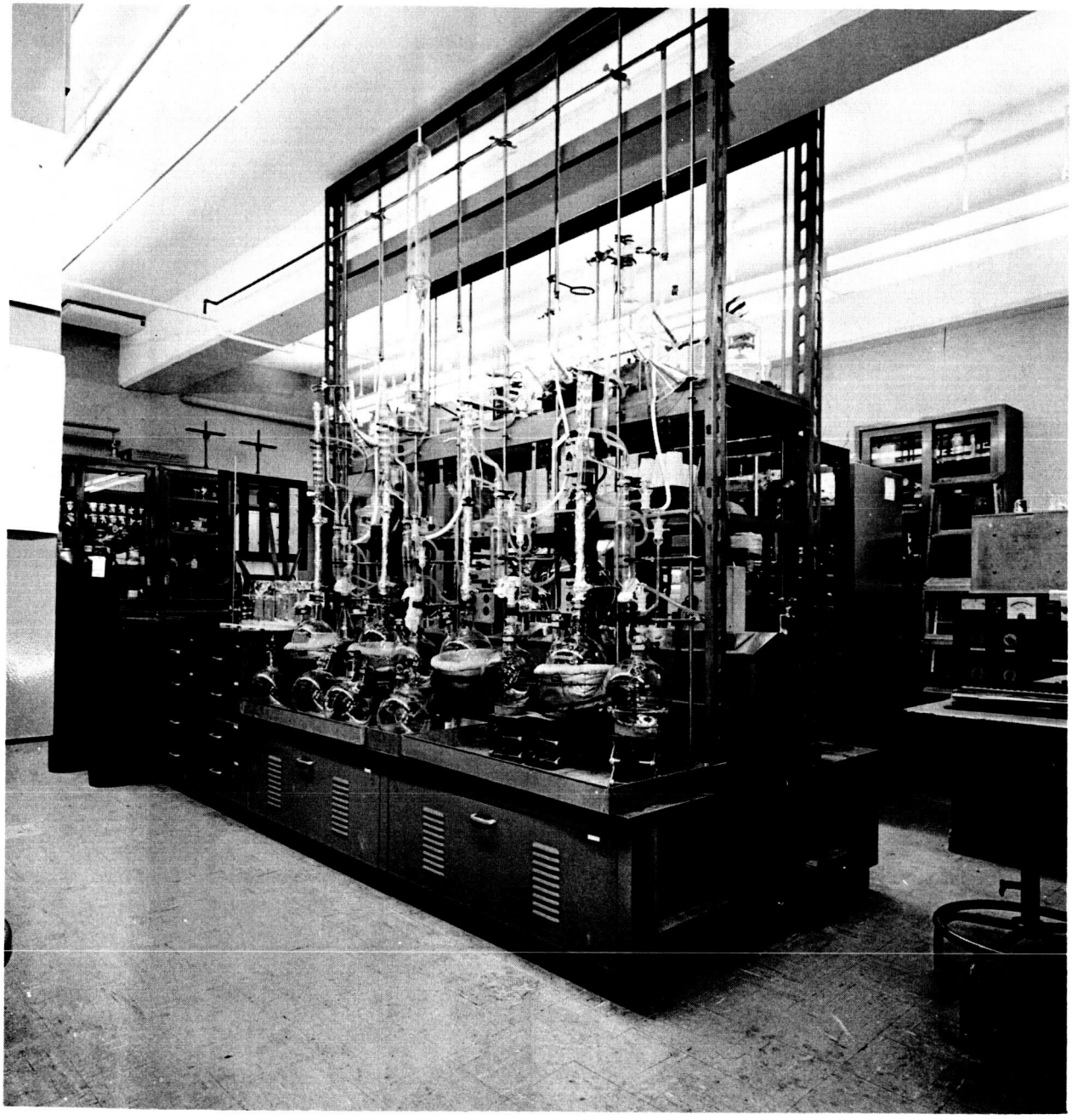
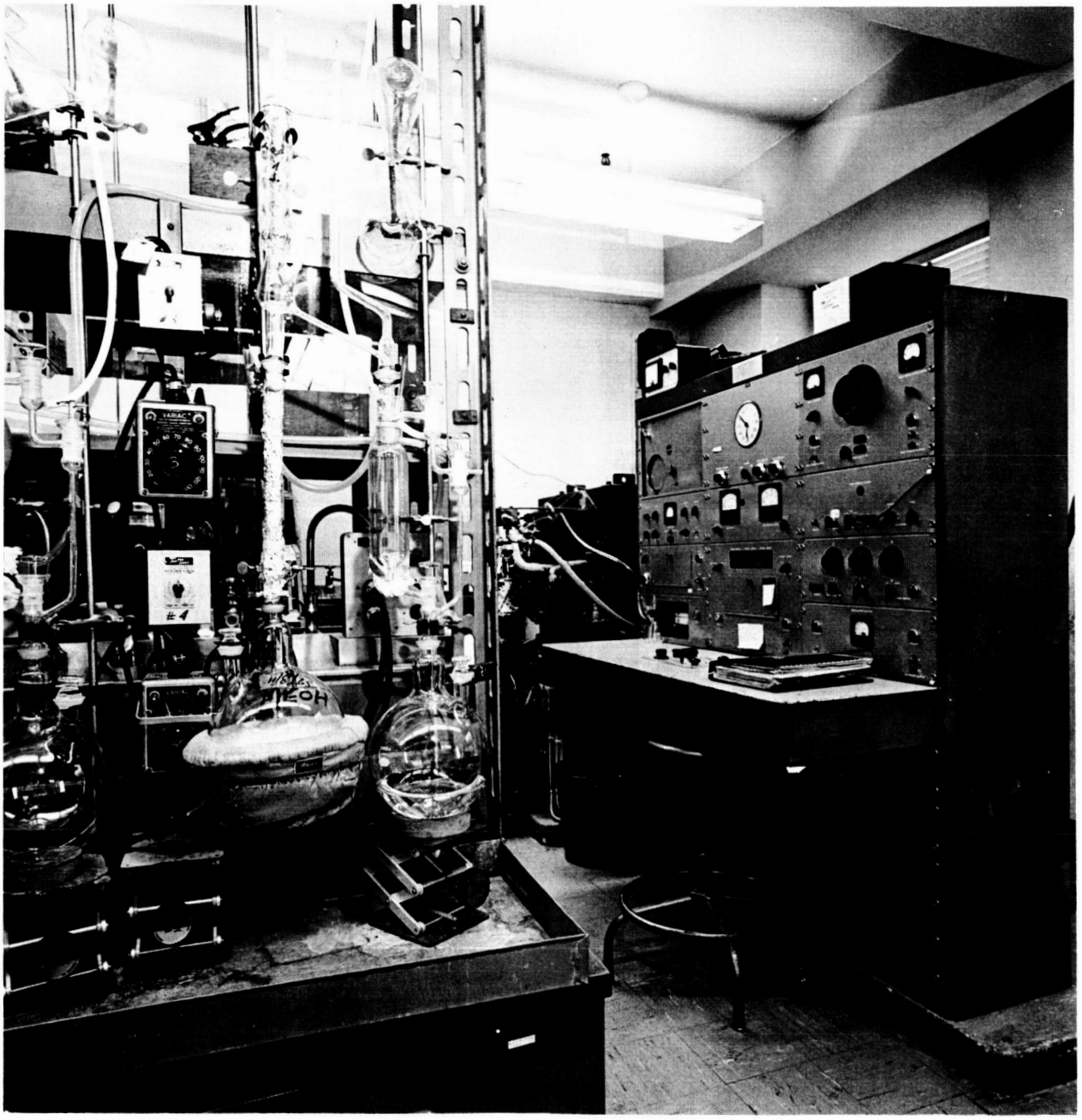
APPENDIX I

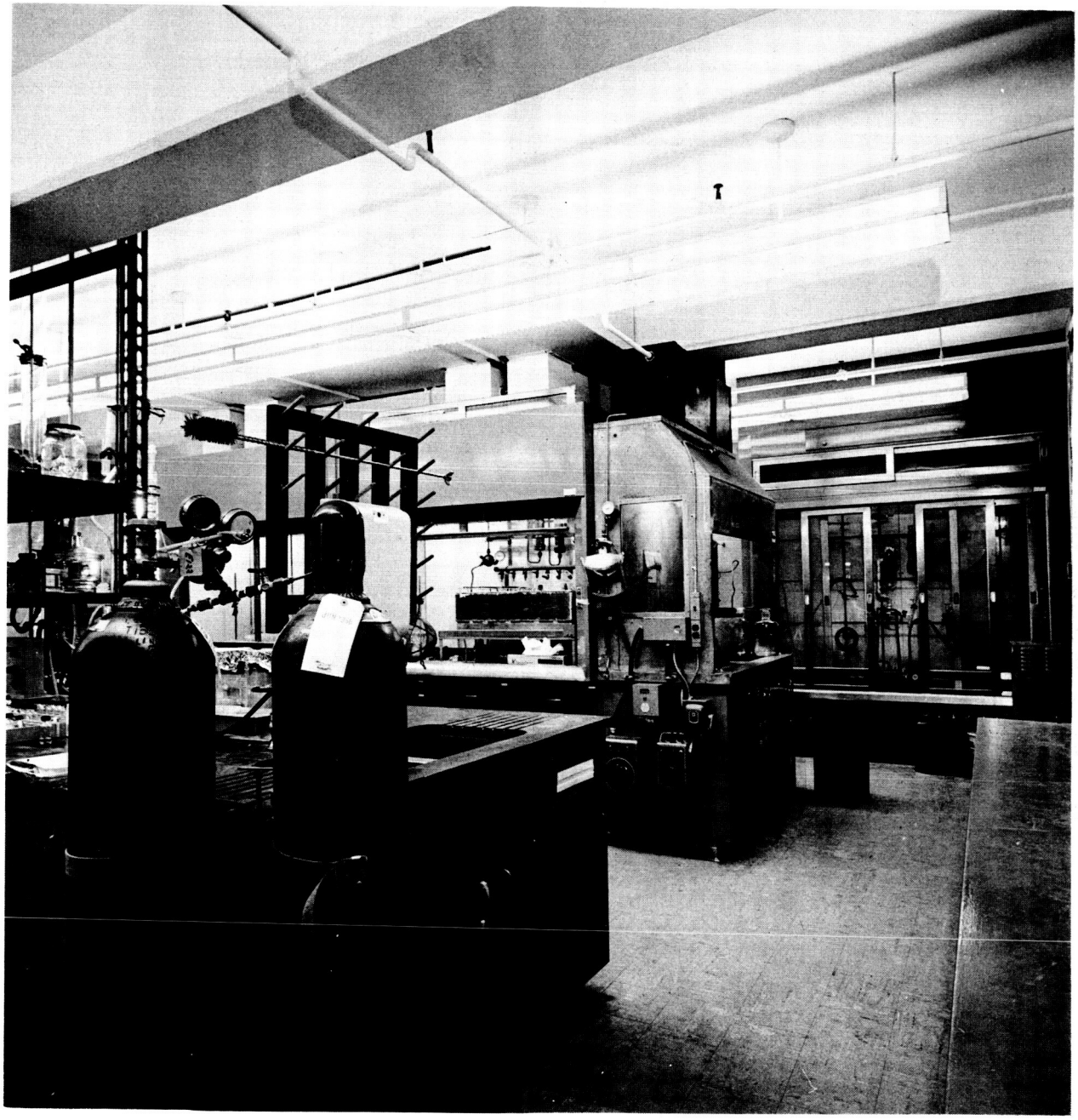
LABORATORY FACILITIES



I. VIEW FROM RIGHT REAR OF LAB SHOWING DISTILLATION APPARATUSES USED FOR SOLVENT PURIFICATION AND HEATED INLET SYSTEM FOR MASS SPECTROMETER



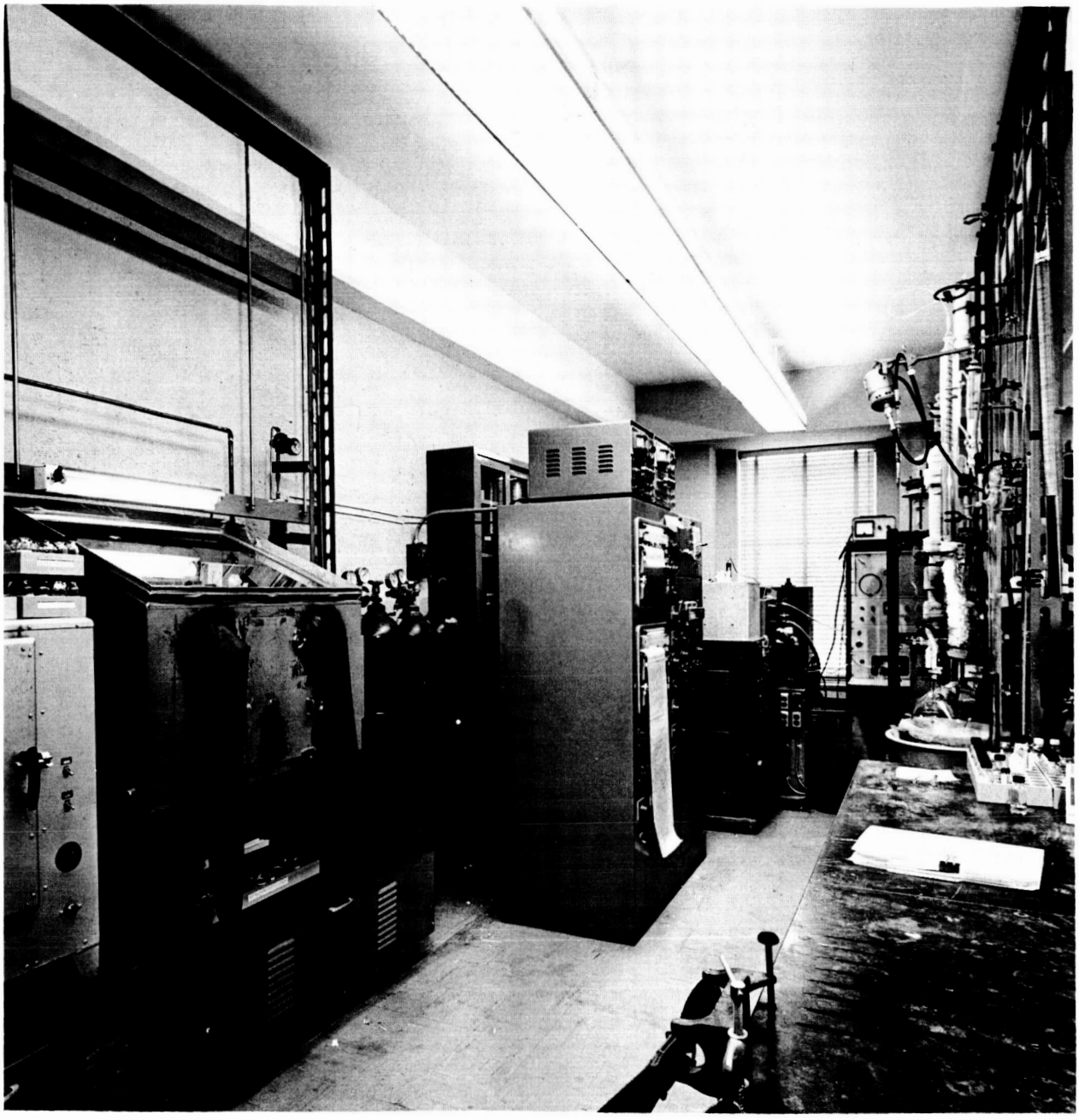
II. VIEW ACROSS RIGHT REAR OF LAB SHOWING MASS SPECTROMETER



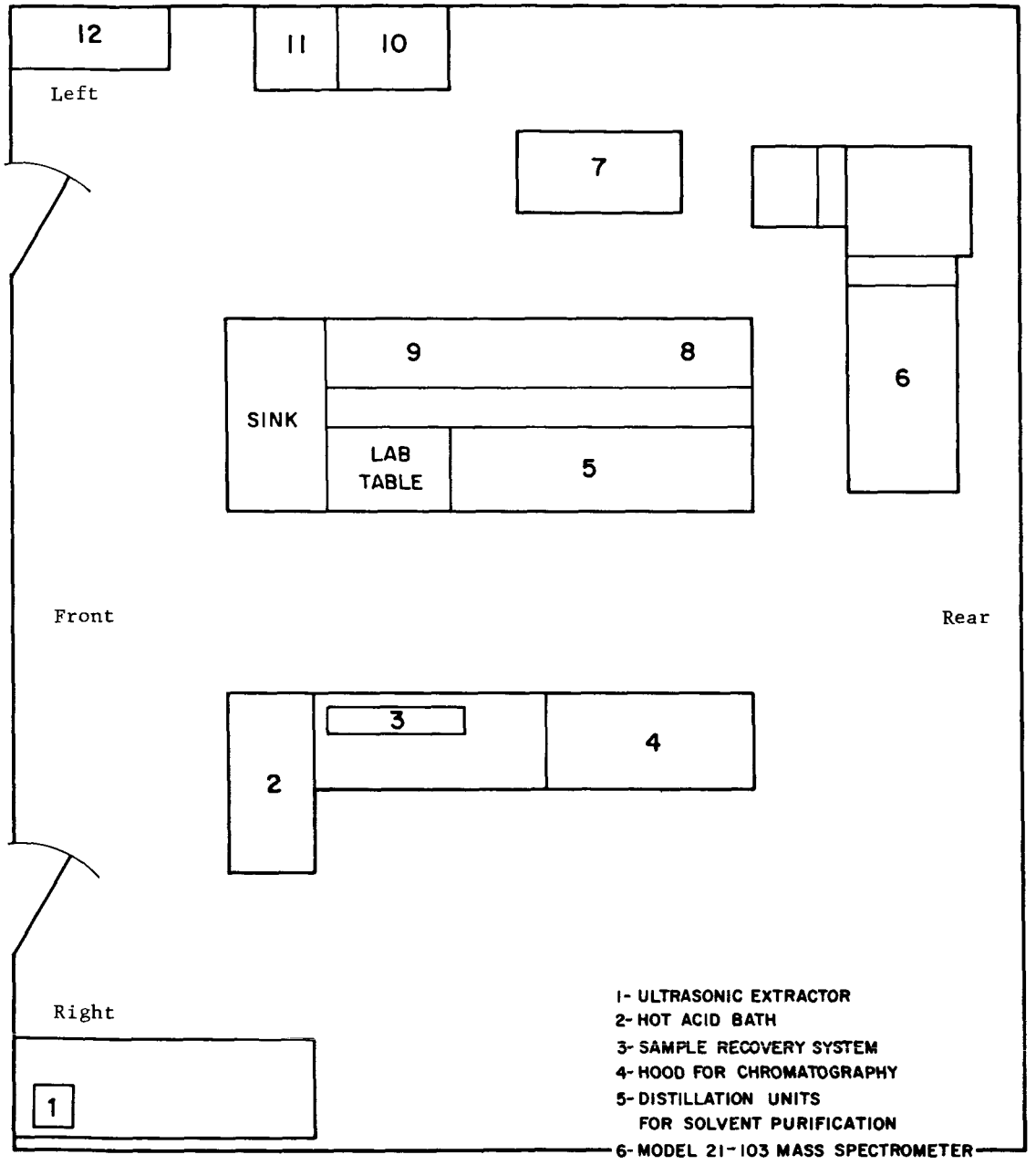
III. VIEW ACROSS LEFT FRONT OF LAB SHOWING FROM RIGHT TO LEFT THE ULTRASONIC EXTRACTOR, ACID HOOD AND ACID BATH FOR CLEANING GLASS-, PORCELAIN-, AND TEFLONWARE, AND SAMPLE RECOVERY SYSTEM

IV. VIEW FROM LEFT FRONT TO REAR OF LAB SHOWING LEFT TO RIGHT THE BARBER-COLMAN GC, MASS SPECTROMETER, LARGE SOXHLET TYPE EXTRACTORS

V. VIEW SHOWING SAMPLE RECOVERY SYSTEM AND HOODED DESK FOR LIQUID-SOLID CHROMATOGRAPHIC ANALYSES



VI. VIEW ALONG LEFT SIDE OF LAB SHOWING SAMPLE STORAGE (DRY BOX), BARBER-COLMAN GC, LARGE EXTRACTORS, AND MASS SPECTROMETER

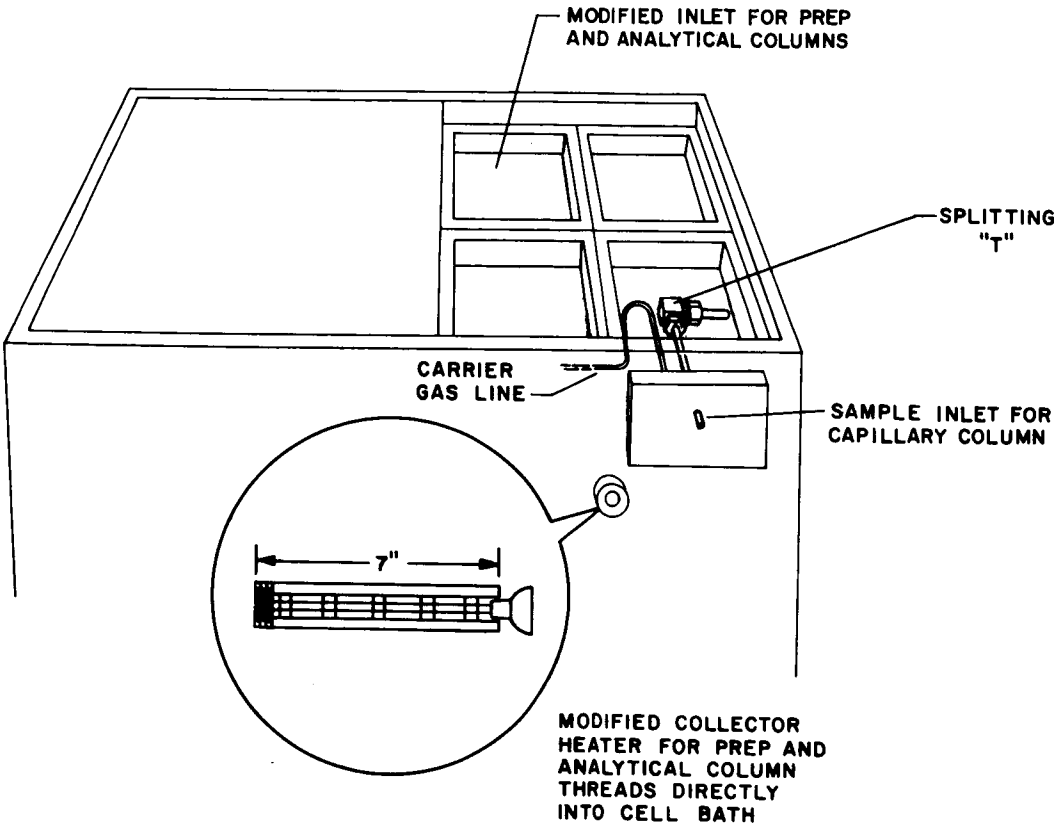


1- ULTRASONIC EXTRACTOR
2- HOT ACID BATH
3- SAMPLE RECOVERY SYSTEM
4- HOOD FOR CHROMATOGRAPHY
5- DISTILLATION UNITS
FOR SOLVENT PURIFICATION
6- MODEL 21-103 MASS SPECTROMETER
CONSOLIDATED ENGINEERING CORP.
PASADENA CALIF.
7- GAS CHROMATOGRAPH
BARBER COLEMEN MODEL 10 MODIFIED
8- LARGE SOXHLET EXTRACTOR
9- STAINLESS STEEL BALL
MILL-EXTRACTOR
10- SAMPLE STORAGE CABINET
NITROGEN ATMOSPHERE
11- OVEN
12- FIREPROOF SOLVENT STORAGE CABINET

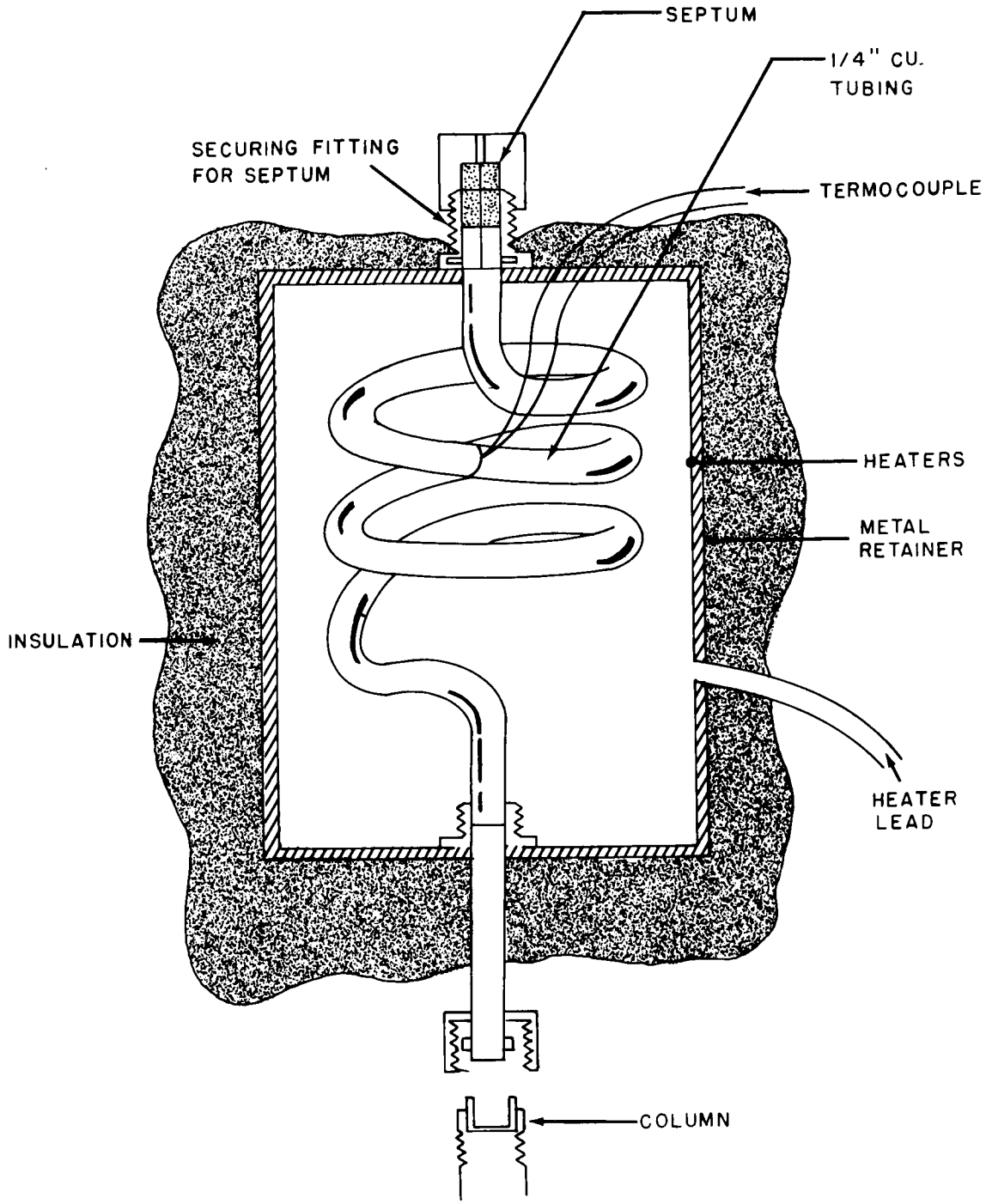
VII. LAB FLOOR PLAN

APPENDIX 2

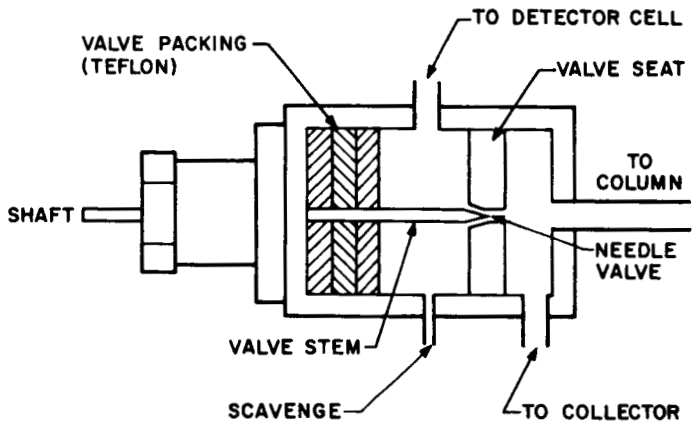
MODIFICATIONS OF BARBER-COLMAN MODEL 10 GAS CHROMATOGRAPHIC INSTRUMENT



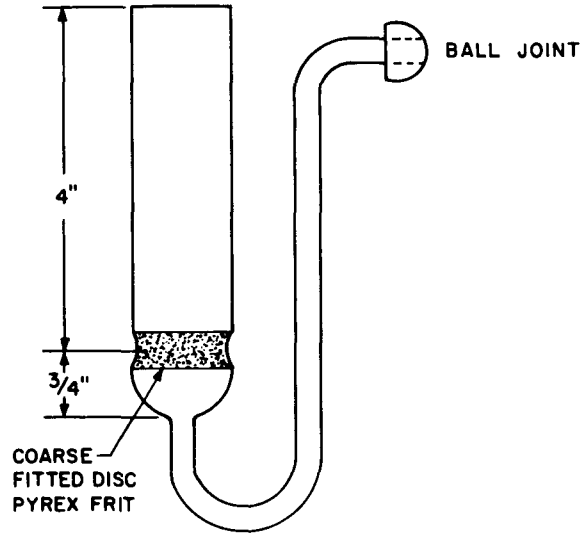
I. MODIFICATIONS OF BARBER-COLMAN MODEL 10 GAS CHROMATOGRAPHIC INSTRUMENT



II. MODIFIED HEATED INLET SYSTEM FOR PREPARATORY SCALE AND ANALYTICAL COLUMNS



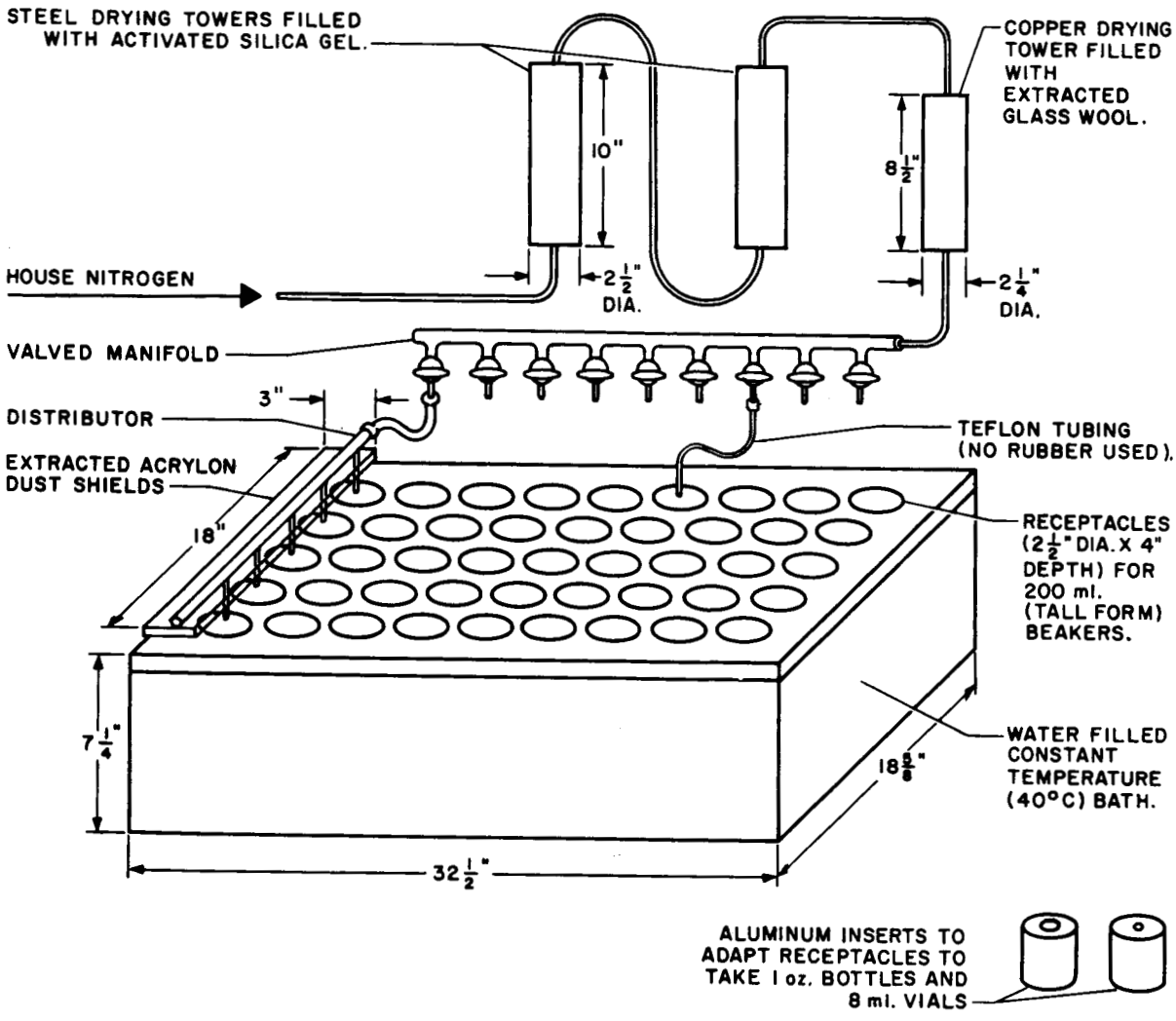
III. VALVE FOR CONTROLLING SPLIT RATIO OF EFFLUENT GASES FROM PREP COLUMN THROUGH DETECTOR AND COLLECTOR



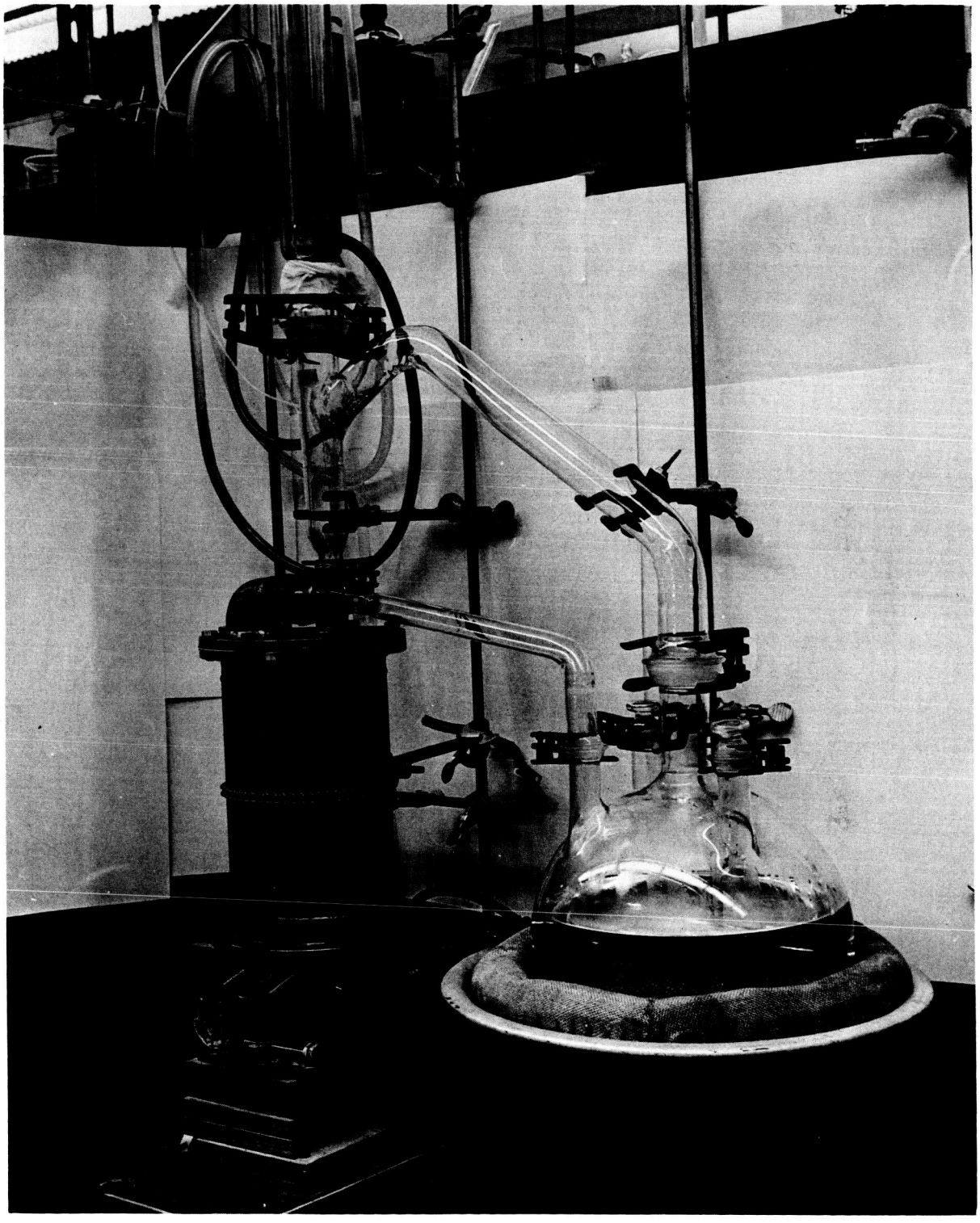
IV. COLLECTOR FOR PREP COLUMN FRACTIONS. SOLVENTS OR COOLING SOLUTION COULD BE ADDED TO INCREASE COLLECTION EFFICIENCY.

APPENDIX 3

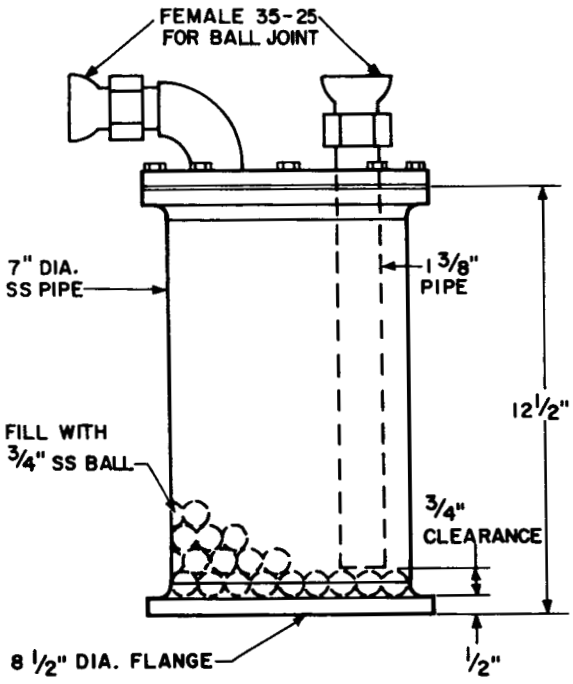
SPECIAL EQUIPMENT



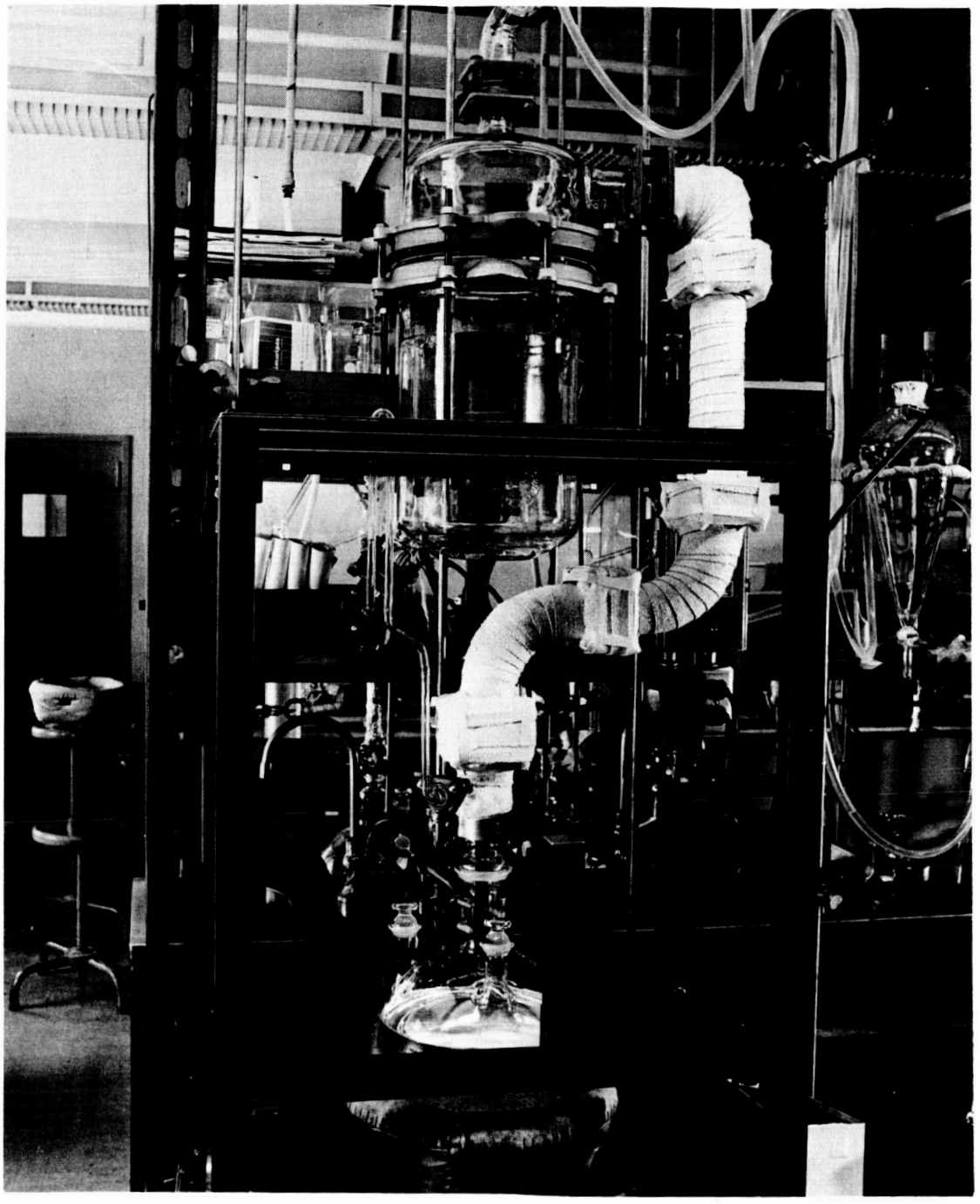
I. SAMPLE RECOVERY SYSTEM



II. BALLMILL-EXTRACTOR



III. SCHEMATIC DRAWING OF BALLMILL EXTRACTOR. AS SHOWN IN PHOTOGRAPH II, THE FEMALE FEMALE CONNECTOR ON LEFT ABOVE IS REVERSED IN THIS DRAWING. THIS CONNECTOR DOES NOT EXTEND BEYOND EDGE OF VESSEL.



IV. LARGE SOXHLET-TYPE EXTRACTOR FOR EXTRACTIONS OF LARGE INTACT ROCK AND METEORITE SAMPLES