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QUARTERLY REPORT

October 1, 1964

DEVELOPMENT OF HYDROCARBON ANALYSES AS A MEANS OF DETECTING LIFE IN SPACE

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SUMMARY

Crude oil, pristane, phytane, sterane-type and optically active alkanes, porphyrins and microfossils have been found in the Nonesuch shale of Precambrian age from Northern Michigan. These sediments are approximately one billion years old. Geologic evidence indicates that they were deposited in a near shore deltaic environment. Porphyrins are found in the shales but not in the crude oils from the Nonesuch formation, which establishes the fact that these chemical fossils are adsorbed or absorbed and immobile. This immobility makes it highly unlikely that the Nonesuch porphyrins could have migrated from younger sediments, and the widely disseminated particulate organic matter and fossils in this Precambrian shale are certainly indigenous. The concatenation of geologic, geochemical, and micropaleontological evidence strongly indicate that the crude oil as well as the other carbonaceous materials in the Nonesuch sediments were deposited concurrently with the mineral matrix. The compositions of the alkanes and the presence of porphyrins in this Precambrian deposit suggests that organisms which apparently existed a billion years ago were metabolically similar to living plants.

INTRODUCTION

Prior to 1950, there was a paucity of evidence for the existence of Precambrian life. Several factors appear to have been responsible for the previous failure of scientists to find paleobiological records in sediments that were greater than 500 million years in age. Among these are: (1) The lack of commercially important fossil fuel deposits in most Precambrian formations; (2) the natural erosion of fossils in many sedimentary rocks destroys the paleontological evidence of former life in most very old sediments; (3) chronological controls, that now are provided by radiogenic dating, were seldom employed before 1950.

Modern dating and analytical techniques, the acquisition of extensive paleontological reference data, and the selection of strata that favor the preservation of fossil structures have resulted in the discovery of concrete evidence of the existence of organisms that lived almost 2000 million years ago[1-4], and the application of paleobiochemical methods are increasing the information that may be acquired by the analyses of carbonaceous materials in ancient rocks. Recently, the presence of biological-type hydrocarbons in the Nonesuch sediments have been independently reported by two research groups[5,6], and microfossils and porphyrins, also, have been isolated from these deposits.[6] In this report, the analyses of the alkanes from the Nonesuch formation are discussed in some detail, and an attempt is made to illustrate how chemical fossils may be used to study the metabolisms of former organisms.

DISCUSSION

Crude oils are believed to be of biological origin, and extensive investigations have established the identities and similarities in the C_{15} and C_{30} alkanes that are common to crude oils, sedimental extracts and biological lipids.[7-11] Data in Table 1 permit a characterization to be made of the crude oil and benzene extract from the Nonesuch siltstone. These data in conjunction with other analyses presented below show that both of the Nonesuch samples contain higher concentrations of alkanes and lower concentrations of aromatic hydrocarbons than an average crude oil. Differences in the results reported for the Nonesuch oil and extracts in Table 1 are not significant, and infrared, ultraviolet and mass spectra of these chromatographic fractions of the Nonesuch samples further confirm their equivalence. Compositionally, the Nonesuch samples resemble a highly paraffinic petroleum of the type frequently produced in Pennsylvania. Oils from older sediments are usually more paraffinic than oils from younger sediments.

Organisms are uniquely capable of synthesizing carbon compounds which possess optical activity.[12] Alkanes from living things[12], recent sediments[8,13], and crude oils[14] rotate polarized light in a clock-wise or positive direction. Values for the optical activities of alkanes from these sources and the Nonesuch crude oil[6] are presented in Table 2. It is apparent from these values that the Nonesuch alkanes have optical rotations comparable in direction and magnitude to other biological and sedimental alkanes.

A gas chromatogram of the alkane fraction from the Nonesuch crude oil is presented in Figure 1. All large peaks in this chromatogram are produced by n-paraffins. In Figure 1, the tops of successive even-carbon number n-C_{18} to n-C_{28} peaks have been joined by dotted lines. It is noteworthy that the odd-carbon number n-paraffin peaks extend above the lines which join their even-carbon number homologs. Biological and sedimental alkanes frequently contain more odd- than even-carbon number n-paraffins[7,10,11,15-18]. Although, this "odd carbon preference" is not pronounced in the alkanes from some organisms[19] and most ancient sediments, the slightly greater abundances of odd- than of even-carbon number n-paraffins in sedimental alkanes has been reported as evidence that these compounds were made either by former life or from biological acids and alcohols[7,11,18]. The "odd carbon preference" of the Nonesuch n-paraffins is greater than that observed in the n-paraffins from some organisms[19,20] and most crude oils, even some oils that are younger than Paleozoic in age[11,17].

Branched-chain alkanes and C_{21} and smaller n-paraffins are concentrated in the first fraction of sedimental alkanes that are eluted by n-heptane from alumina columns[21] and n-paraffins are removed from mixtures of alkanes by molecular sieves[22]. A gas chromatogram of this first n-heptane eluate of the Nonesuch alkanes, obtained after the removal of n-paraffins, is shown in Figure 2. Peaks labeled pristane and phytane in this chromatogram are chromatographically equivalent to pristane and phytane as established in Figure 3. In the latter chromatogram, the sizes of the pristane and phytane peaks were increased by the addition of reference compounds. The chromatographic equivalences of the reference and Nonesuch pristane and phytane were established on five GLC columns, each coated with a different substrate. Retention temperatures for reference pristane and phytane and the pristane and phytane peaks in the Nonesuch alkanes are given in Table 3 for these five columns. Mass spectra of the branched-chain concentrate from the Nonesuch alkanes contained large mass 183 peaks. In the mass spectra of pristane and phytane, large mass 183 peaks are assumed to be caused by 2,2,10-trimethyldecyl ions[23,24].

Polycyclic alkanes and C_{25} and larger n-paraffins are concentrated in carbon tetrachloride eluates of sedimental alkanes from alumina. Mass spectra of the polycyclic alkane concentrates from the Nonesuch alkanes have large mass 372, 218, 217, and 149 peaks which are characteristic of parent sterol hydrocarbons[25]. Similar polycyclic alkanes are apparently found ubiquitously in biological and sedimental alkanes[7,10,18].

Geological evidence, the occurrence of plant fragments within the formation, and the distribution of porphyrins[6,26] clearly indicate that biological materials in the Nonesuch formation were not acquired from younger sediments. Pristane, phytane, optically active alkanes, n-paraffins and steranes in the Nonesuch crude oil and extract, therefore, are apparently indigenous to sediments which were deposited approximately one billion years ago. Identities or similarities in the structures and optical activities of the Nonesuch and of modern biological alkanes suggests that certain hydrocarbons can be preserved for long periods of geologic time in some sediments. Additional support for the view that alkanes may retain their structures for nearly one billion years in some sedimentary environments may be deduced from the distribution of n-paraffins in the Nonesuch formation. Random cleavages of carbon bonds in n-paraffins would produce equal amounts of odd- and even-carbon number molecules. The "odd carbon preference" of the Nonesuch n-paraffins indicates that these compounds are not degradation products.

The pristane, phytane, and porphyrins in the Nonesuch sediments may be derived from chlorophyll. Reaction pathways by which chlorophyll may have been converted into the types of porphyrins which appear widely distributed in ancient sediments have been experimentally defined[27,28], and pristane and phytane contain carbon skeletons that are present in phytol, which appears in an ester substituent in chlorophyll. Phytol has been commonly suggested as precursor of the pristane and phytane in sediments[5,23]. However, pristane is a constitutent of various organisms[29-32], and phytane has been tentatively identified in the bacterium Vibrio ponticus[33].

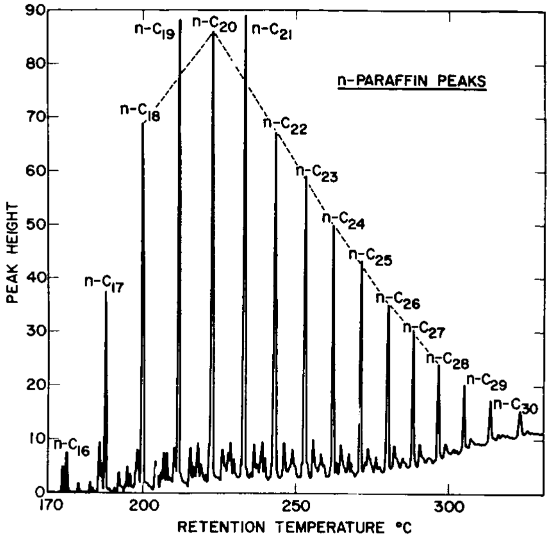
Since living things make pristane and apparently phytane and the production of these compounds from phytol has not been accomplished by abiotic reactions that may feasibly occur in sediments, pristane and phytane are probably less reliable indicators of the pre-existence of photosynthetic organisms that are porphyrins. Nonetheless, considerations of metabolic processes suggest that complementary information about the organisms in Nonesuch-time may be obtained from the presence of porphyrins and alkanes in the Nonesuch sediments.

Chlorophyll absorbs the solar energy that photosynthetically reduces CO_2 to carbohydrates in green plants. Carbohydrates are a source of energy and precursors for the biosynthesis of other biological compounds. Hexoses, derived from carbohydrates, are converted in living cells into pyruvates which in turn participate both in the Krebs' cycle and lipid biosynthesis[34-36]. The biosynthesis of the pyrrole rings in porphyrins have been traced from succinate, a component of the Krebs' cycle[37]. Succinate condenses with glycine, and the glycine carboxyl is then cleaved to form δ-aminolevulinic acid, which cyclizes to form the pyrrole rings of porphyrins[37,38].

Because alkanes are minor and chemically inert products of plants and animals, the metabolism of alkanes has received less attention than most other organic compounds, but alkanes are structurally and isotopically similar to the acid and alcohols in biological fats and waxes[39]. Furthermore, direct metabolic ties between n-paraffins and fatty acids have been established. n-Paraffins are converted by Ω oxidation into fatty acids in the livers of mammals and chickens[40]. Thus, information about the structural[34-37, 40-43] and isotopic selectivities[38,39] of biological processes and limited data on the metabolism of n-paraffins provide substantial reasons for presuming that the alkane constitutents of plant and animal lipids are biosynthesized either from acetates, as are the acids and alcohols, or from the acids and alcohols[7,10,11].

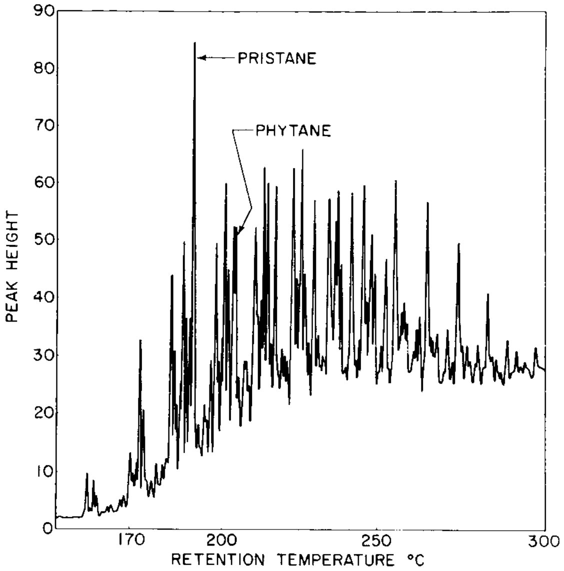
Lipid biosyntheses are defined in even greater detail than is the biosynthesis of porphyrins[36,40-43]. Acetates, made from pyruvates, are the precursors of malonates and mevalonates which serve, respectively, in the production of straight- and branched-chain acids, alcohols, and hydrocarbons. Squalene, a branched-chain C_{30} alkene, is in turn an intermediate in steroid syntheses[36]. Equilibria exist between hexoses pyruvates, acetates, and the tricarboxylates in living cells. Amino acids, building units of proteins,

FIGURE 1



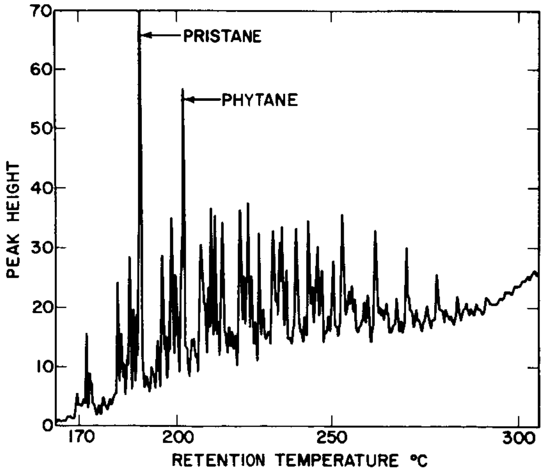
GLC ANALYSIS OF ALKANE n-HEPTANE ELUATE FROM SILICA GEL COLUMNS OF NONESUCH CRUDE OIL.

FIGURE 2



GLC ANALYSIS OF THE n-PARAFFIN FREE, FIRST n-HEPTANE ELUATE OF NONESUCH ALKANES FROM ALUMINA COLUMN. n-PARAFFINS WERE REMOVED FROM THIS ELUATE BY USE OF MOLECULAR SIEVES.

FIGURE 3



GLC ANALYSIS OF THE n-PARAFFIN-FREE, FIRST n-HEPTANE ELUATE OF NONESUCH ALKANES FROM ALUMINA, AFTER THE ADDITION OF REFERENCE PRISTANE AND PHYTANE TO THIS ELUATE.

as well as pyrrole rings are made from the tricarboxylic acids participating in the Krebs' cycle[34,35].

In essence, pyruvates are apparently intimately involved in the production of most biological compounds, and alkanes and porphyrins are probably products of the two major biosynthetic pathways from pyruvates. One pathway leads through acetate to lipids, and the other through the Krebs' cycle to the syntheses of amino acids and the prophyrins. The presence of porphyrins and alkanes in the Nonesuch formation that resemble porphyrins in geogolically young sediments and alkanes in living things may suggest that life which existed in Precambrian times was metabolically similar to existing life[44].

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44. The analyses of the Nonesuch crude oil and shale described in this report were carried out in support of a continuing study of Precambrian life which is being conducted at Harvard University.

Table 1

Silica Gel Chromatographic Analyses

Samples Nonesuch:	Per Cent Eluted By n-Heptane (Alkanes)	Carbon Tetrachloride (Alkanes - Aromatics)	Benzene (Aromatics)	Methanol (Non-Hydrocarbons)
Crude Oil	61.2	22.2	12.7	3.9
Extract	61.5	20.3	15.4	2.8
Ave. 110 Crude Oils	47.2	12.1	32.4	8.3

Table 2

Optical Activities of Alkanes

Sample	Measured Rotation	α_D^{20}	α_{5460}^{35}	α_{5780}^{35}
Biological (38)				
Spirogyra	+1.28°			
Hydrodictyon reticulatum	-1.0°			
Vibrio ponticus	-0.94°			
Recent Sediments:				
Gulf of Mexico (36)		+0.514°		
Atlantic Coast of France(39)		+3.3°		
Crude Oils (40)				
Pennsylvania		+0.25°		
Rodessa		+0.79°		
Mid-Continental #1		+0.93°		
Mid-Continental #2		+1.20°		
California		+2.73°		
Nonesuch Alkanes (11,30)			+0.541°	+1.569°

Table 3

Retention Temperatures ± 1°C. of Reference Pristane and Phytane and of The Pristane and Phytane Peaks in The Nonesuch Alkanes

	Apiezon "L" Column Supplied By Barber-Colman	10% Solution Apiezon "N"	8% Solution n-Heptane Eluate of Apiezon "L"	0.5% Solution Benzene Eluate of Apiezon "L"	0.7% Solution Benzene-Methanol Eluate of Apiezon "L"
Pristane	193	184	186	137	133
Phytane	208	198	199	154	144