Paleobiology of a Precambrian Shale

Geology, organic geochemistry, and paleontology are applied to the problem of detection of ancient life.

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Little more than a decade ago serious effort to invade the paleobiological "desert" of the Precambrian was limited to a few devoted, hopeful, and persistent paleontologists employing with ingenuity the traditional techniques of field observation and laboratory microscopy. The cumulative efforts of nearly a century of study of rocks underlying the Cambrian fossiliferous "boundary" have resulted in a small but impressive body of evidence indicating that life had originated and diversified at a time far more remote in geologic history than the lowermost Paleozoic. Unfortunately much of this earlier evidence was equivocal and based on alleged metazoan megafossils or stromatolitic structures secured from partially or highly metamorphosed sediments. It is understandable that an attitude of scepticism has traditionally prevailed in the assessment of Precambrian fossils and their acceptance as bona fide evidence of organisms. On the other hand, geologists and biologists have, somewhat inconsistently, long accepted the view, on grounds of sheer logic, that life must have existed long before the appearance of the diversified "parade" of metazoans and multicellular algae in the lower Paleozoic.

Several factors appear to have been responsible for retarding progress in the understanding of the Precambrian record of life. Among these are: (i) preoccupation with the problem of discovering megascopic invertebrate fossil organisms and a concomitant lack of attention to, or interest in, microfossils or the fine structure of potentially fossiliferous sedimentary rocks, such as cherts; (ii) lack or paucity of chronological control in the Precambrian, such as is now provided by radiogenic dating; and (iii) lack or inaccessibility of corroborative evidence such as is now available through application of paleobiochemical techniques and the sensitive instruments of organic chemical analysis.

It might be postulated that organisms early in the history of living things would be unicellular, or at least simply organized multicellular aggregates, and of microscopic size. Concrete evidence for this assumption has been obtained in recent years through the discovery of a varied assemblage of microorganisms showing definitive and three-dimensional morphology in sediments almost 2000 million years old (*1*). The lithologic environment of preservation of these organisms is a cryptocrystalline chalcedonic chert (Gunflint chert), the incompressibility of which permitted retention of three-dimensional form. Present evidence and continuing study of the paleochemistry of the Gunflint rocks indicate the presence of relatively complex organic molecules directly associated with the structurally preserved organic remains (*1*). The Gunflint assemblage of organisms establishes, at least temporarily, a minimal time for the duration of multicellular life. Between Gunflint time and the beginning of the lower Cambrian, however, there is an extreme paucity of coherent evidence of organic activity and of fossils (*2*). This article deals with various aspects of the geology, organic geochemistry, and paleontology of one of the more interesting intermediate links in the continuity of life—with evidence from rocks approximately 1000 million years old.

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Geology

The genesis of the economically important copper ore present in the basal portion of the Precambrian Nonesuch shale formation, one of the several cupriferous deposits of northern Michigan, has been the subject of considerable controversy (*3–8*). In part because of this controversy, and in part because of the economic importance of the formation, many of the petrological, structural, and inorganic geochemical aspects of the basal portion of this ancient deposit have been investigated (*3, 6, 7, 9, 10*). In a preliminary report we stated that the Nonesuch shale appears to be the oldest formation in which crude oil, optically active alkanes, porphyrins, and the isoprenoid hydrocarbons pristane and phytane are known to occur, and, in addition, that what appear to be fossil microorganisms of several types occur in the formation (*11*). The preservation of this varied and unique assemblage of paleontological and organic geochemical evidence of early life must be directly related to the depositional, diagenetic, tectonic, and thermal history of the formation, a history which would include the period when copper minerals were emplaced in this deposit.

Geologic setting. The White Pine area, located in the northeast half of Township 50 N, Range 42 W, Ontonagon County, northern Michigan, is situated just southeast of the Porcupine Mountains (see Fig. 1). The Nonesuch formation, as exposed at White Pine, is a dominantly gray, well-bedded siltstone and shale, easily differentiated from the red-to-brown coarser-grained rocks of the formations above and below. The cupriferous zone comprises the basal 7½ to 9 meters of the Nonesuch formation and includes also the uppermost 1½ meters of the underlying middle Keweenawan Copper Harbor conglomerate (*3*). The biological remnants present at White Pine occur in the cupriferous zone. Both the Nonesuch shale and the overlying Freda sandstone are of late Keweenawan age. The radiogenic age of the Nonesuch formation, based on rubidium-strontium ratios in whole-rock samples from the White Pine area, has recently been determined as 1046 ± 46 million years, by S. Chaudhuri and G. Faure (*12*). Figure 2 represents a generalized columnar section of these upper Precambrian formations as they occur in the White Pine area.

The Keweenawan strata of northern

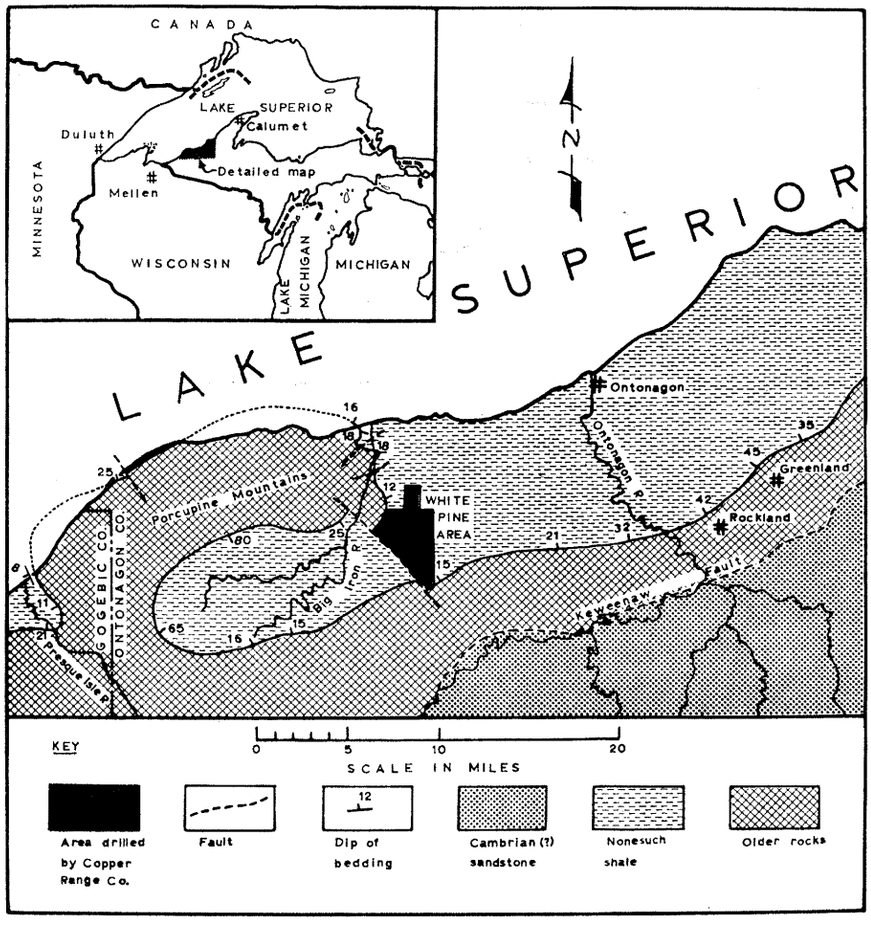


Fig. 1. Index map showing location of White Pine area, altitude and location of the base of the Nonesuch shale, and presumed subsurface areal extent of the formation. [After White and Wright (*3*)]

Michigan and northern Wisconsin lie on the southern flank of the Lake Superior basin and dip gently northwest and north toward the center of the basin at most places (*3*). The structure of the Nonesuch shale in the vicinity of White Pine is dominated by the White Pine fault (see Fig. 1). In addition to this major fault, which has a horizontal displacement of several hundred meters (*3*), many small-scale, steeply dipping strike-slip faults disrupt the strata in the vicinity of White Pine.

Distribution of organic material. The cupriferous zone in the White Pine area has been divided, from oldest to youngest, as follows: Lower sandstone, Parting shale, Upper sandstone, and Upper shale (*3*). Mineralogically, with the exception of the copper minerals which were probably emplaced after burial but prior to lithification (*3, 6*), the shales and sandstones of the cupriferous zone appear to be similar to most other shales, silts, and sands. Figure 3 represents a detailed columnar section of this zone as it occurs near White Pine.

The Parting shale and the Upper shale units of the cupriferous zone contain sporadically distributed vugular structures which often contain small amounts of crude oil; liquid hydrocarbons also occur, associated with fractures in these strata. The organization of asphaltic material present within the interstices of the Parting shale unit, as observed in thin section in transmitted light, is typified by alternating, sharply delineated layers and spheroids of reddish and light amber material interpreted as residual petroleum exhibiting varying degrees of devolatilization (Fig. 4, parts 1, 2, and 4). This organic material is frequently arranged in an undulating, concentric manner about a mineral center, often chalcocite. The significance of these structures with regard to the paleontological differentiation between biogenic morphological organization at or above the microscopic level ("fossils"), abiogenic morphological organization ("pseudofossils"), and biogenic indicators at the molecular level ("chemical fossils") is discussed in a later section.

Finely disseminated carbonaceous material, locally but scantily present in the two sandy units (*3, 13*), is relatively abundant and found throughout the shale units of the cupriferous zone. In general, the vertical and horizontal distribution of copper minerals within the zone, predominantly chalcocite and native copper, appears to vary in direct proportion to the amount of organic matter present (*3, 9*). In the case of the Lower sandstone unit this spatial relationship appears to be specific at a microscopic level—that is, particles of native copper are commonly surrounded by, and in intimate contact with, anthraxolitic organic material (*14*).

The direct association of copper minerals with finely disseminated carbonaceous matter, anthraxolitic organic material, and partially or wholly devolatilized asphaltic organic residues within the cupriferous zone tends to substantiate the hypothesis, presented by other investigators (*4, 6*), that the precipitation of copper minerals in the basal Nonesuch shale was facilitated by the presence of organic material. Inasmuch as lateral and vertical distribution of the copper minerals strongly suggests their diagenetic emplacement (*3, 6, 9*), a syngenetic or early diagenetic origin for the organic material may be inferred. The possibility of petroleum migration from younger rocks is negated by consideration of the great thickness of coarse-grained, permeable sediments devoid of organic matter directly overlying the Nonesuch formation, and by the nearly complete absence of crude oil in the 180 meters of essentially similar silts and sands directly overlying the basal oil-bearing cupriferous zone.

Summary of geologic history. The geologic history of the cupriferous zone of the Nonesuch shale in the White Pine area may be briefly summarized as follows:

1) Pre-Keweenawan, basic and acidic, volcanic and plutonic igneous rocks to the south, with a minor contribution from sedimentary and metamorphic strata, produced particles which were carried north from their terrain of rather low relief to a proximate coarse-sand delta by a fluvial system. The gradual aqueous inundation of the delta and associated sand plain and the simultaneous decrease in the average particle size of the detritus deposited at White Pine resulted in the deposition of highly organic, laminated silts and shales in shallow-water, peri-

odically reducing environments present in apparently disconnected depressions of the delta. Further inundation resulted in the deposition of plant remains within a laterally continuous, fairly homogeneous siltstone unit. During the deposition of this unit the local basin reached a state of maximum submergence; this was followed by a gradual subsidence of water level, culminating in exposure of the strata to subaerial weathering and the end of the first sedimentary cycle.

2) Following this diastem and an associated readjustment of drainage patterns in the delta, the depositional cycle was repeated. Minor authigenic alteration of the less stable minerals of the sediment took place (*9*), followed by burial, partial degradation of the organic material, and the accumulation of petroleum. Prior to lithification, copper was introduced and probably distributed by connate water (*6*). Subsequently the sediment compacted and the synchronous chromatographic segregation of organic molecular types took place. At some time following lithification the formation was locally faulted and folded. This was apparently associated with both the uplift of the anticlinal Porcupine Mountains and the genesis of the White Pine fault. Some copper minerals were probably redistributed during this tectonic activity (*3–8*). Nevertheless, metamorphism has been minimal, and the formation has had a mild thermal history. Further deposition, uplift, and the subsequent erosion of much of the overburden has exposed the present formation.

Paleobiology

Ranges of variabilities of animate and inanimate materials have not been completely defined. In dealing with the general problem of recognizing evidence of ancient life, one is forced to consider what similarities between biological remnants and existing organisms are required to ensure that abiogenic entities may not be mistaken for biologically produced substances. Although an absolute evaluation of the reliability of "fossil evidence" cannot be made, historical and theoretical considerations provide a basis for limited appraisals of the accuracies and potentials of various methods used for detecting the remains of former living things.

Traditionally, a fossil is defined as a morphologically organized entity or remnant of a preexistent organism, ir-

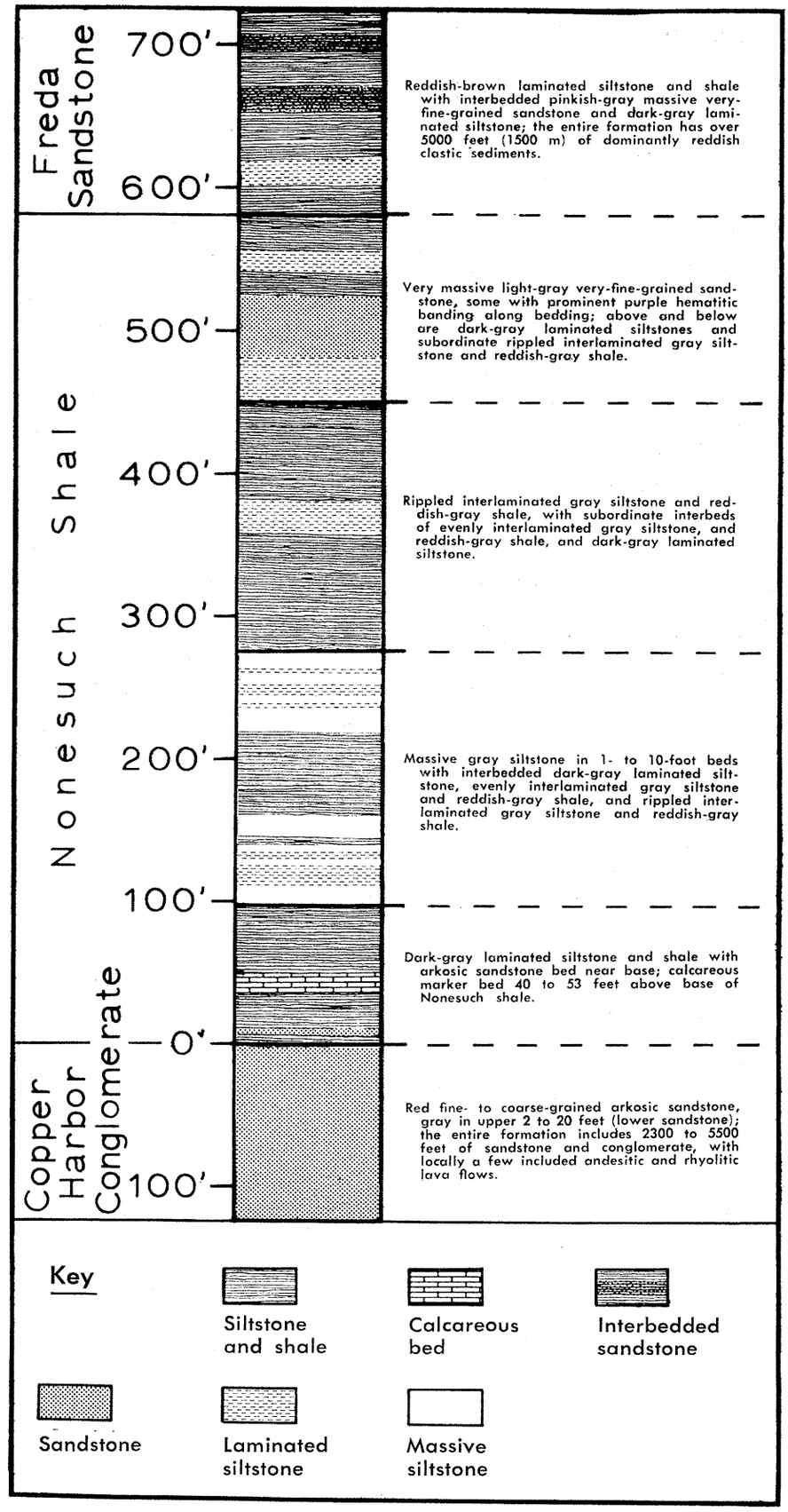


Fig. 2. Columnar section of Nonesuch shale formation in the White Pine area, Michigan. [After White and Wright (*3*)]

respective 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 in many cases to determine biologically produced structures in organic residues at the molecular or even the submolecular level. Isotopic measurements have demonstrated that biological processes are selective at the atomic level, and purely inorganic manifestations of biological activity, such as certain carbonates, phosphates, and free sulfur, for example, are known. Thus, the term *fossil* in its broadest sense may embrace *any* evidence of ancient life, and the reliability of identifications of biological remnants, as well as the information that can be gained from their study, may be increased by the use of a variety of analytical and observational methods. It is for this reason that the results of organic chemical and isotopic analyses, in addition to classical geological and traditional micropaleontological observations, have been used in this investigation of biological remnants in the Nonesuch formation.

Paleobiochemical Experiments

Samples. Siltstone and crude oils were obtained from the Parting shale subdivision of the Nonesuch shale formation at White Pine, Michigan (*15*).

Solvents. The solvents used were of reagent grade, and the solvents used in the alkane analyses were distilled and tested prior to use, as described elsewhere (*16*).

Porphyrin isolation and characterization. Soxhlet extractions were carried out on three batches of siltstone pulverized to pass 200 mesh. The total weight of sample was 215 grams, and a solution of 2 volumes of benzene in 1 volume of methanol was used as the extracting solvent. Each batch of siltstone was extracted for 24 hours. The solvent was removed by evaporation under vacuum at 65°C. The residue (0.2 g) was redissolved in a minimum volume of chloroform (about 5 ml), and asphaltenes were precipitated by the addition of 50 milliliters of hot isooctane. The asphaltene precipitate was removed by vacuum filtration. Additional asphaltenes were separated from the filtrate by repeating these asphaltene removal steps, and the asphaltene-free residue was dissolved in a minimum volume of isooctane for subsequent chromatographic fractionation.

A column (2 by 30 cm) was filled with 125 grams of activated Merck alumina (reagent grade) in isooctane. The asphaltene-free residue (0.18 g) was placed on this column, and elution was accomplished by successive additions of 200 milliliters of each of the various solvents and solvent solutions in ratios (by volume) as follows: isooctane; isooctane and benzene (1:1); benzene; benzene and diethyl ether (3:1; 1:1; 1:3); diethyl ether; diethyl ether and methanol (1:1); and methanol.

Separate eluates were collected as each solvent solution passed onto the top of the column. Visual spectra, obtained with a Perkin-Elmer 202 visible ultraviolet spectrophotometer, indicated that, although some vanadyl porphyrins were present in the ether-methanol eluate, most of these porphyrins were concentrated in the benzene-ether eluates. Ultraviolet spectra were also obtained at this time. Infrared absorption spectra of these eluates were measured on a Perkin-Elmer 137 Infracord spectrophotometer.

Petroleum from the Nonesuch formation was also investigated for evidence of the presence of porphyrins. Three grams of crude oil were dissolved in an excess of benzene, and the suspended rock particles were removed by vacuum filtration. Benzene was removed by evaporation under vacuum at 65°C. Asphaltene precipitation was accomplished by means of the chloroform-isooctane method described above; this separation was carried out three times. The asphaltene-free oil was placed on a chromatography column prepared as described. Elution and collection of eluates was accomplished by the successive addition of 200-milliliter volumes of solvent solutions of the kinds and in the ratios specified above. Visible spectra obtained by the method utilized in identification of vanadyl porphyrins from the siltstone unit of the formation yielded no evidence of the presence of porphyrins in this crude oil. This isolation procedure was repeated with a petroleum sample weighing 14 grams, and again no evidence of the presence of porphyrins was detected.

To complete the study, the asphaltene portion of the crude oil was investigated for evidence of the presence of porphyrins. The asphaltene precipitates from the two oil samples were com-

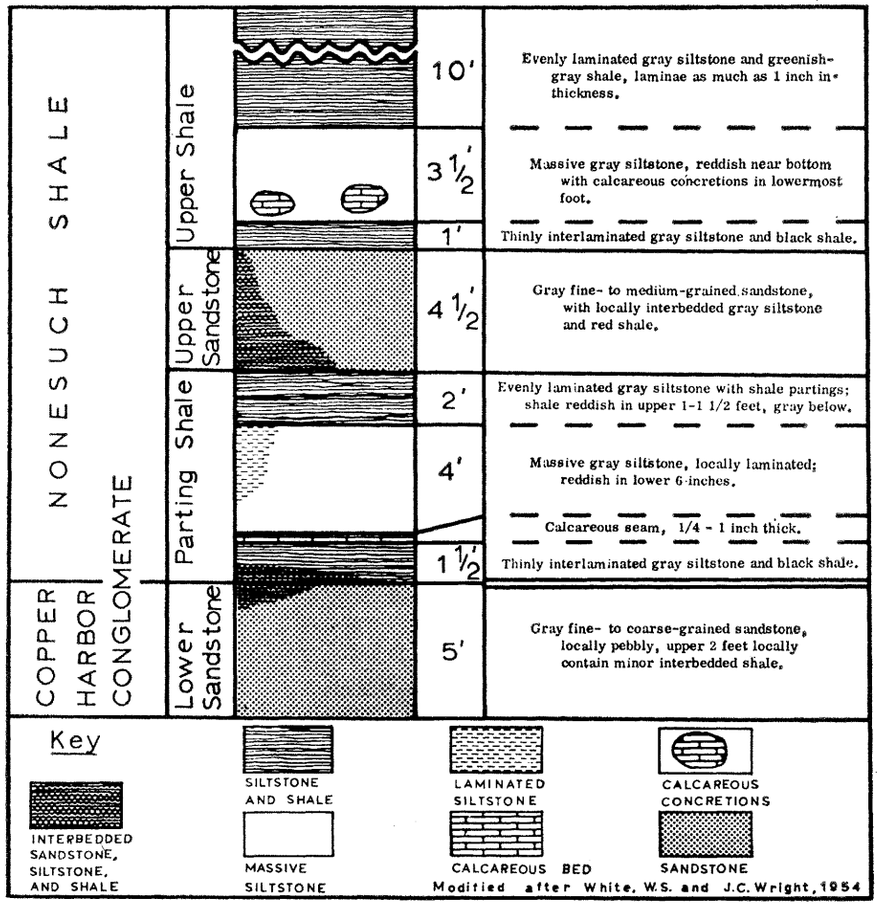


Fig. 3. Detailed columnar section of cupriferous zone of the Nonesuch shale in the White pine area. [After White and Wright (*3*)]

bined, redissolved in benzene, and placed on an alumina chromatography column (2 by 30 cm; Merck activated alumina, reagent grade) packed in benzene. Except for the fact that no isooctane-containing solvent solutions were used, elution, collection, and spectral analysis were accomplished as described above. As in the case of the asphaltene-free portion of the oil from the Nonesuch formation, no porphyrins were detected in the asphaltene constituents.

Alkane analysis. Descriptions of the procedures employed in extraction, removal of solvents or sample recovery (*17*), and liquid-solid phase chromatography, in alkane analysis, have appeared elsewhere (*16–18*). A 10-gram sample of siltstone and 10 grams of crude oil were separately analyzed. Mass spectrometric, ultraviolet, and infrared spectroscopic analyses were made of alkanes from the siltstone and the crude oil (*18*).

The optical activities of the alkanes from the crude oil were measured on an O. C. Rudolph and Sons instrument [model 80 CSPZA (Calcite Optics)] with a No. 600 mercury light source, a 3-millimeter micro device, and a simm angle of about 2 to 3 degrees. Measurements were made (in a 100-mm tube with 6-mm bore) on a solution (0.396 g/ml) of the alkanes in spectral-grade isooctane (*19*).

Gas-liquid chromatography analyses of the alkanes were made by means of five capillary columns (J. Bishop and Company type 316 stainless steel, 0.025 by 3000 cm). One of these columns was coated with 10-percent solution of Apiezon L; this column was prepared by the Barber-Colman Company. Another column was coated with a 10-percent solution of Apiezon N, and the remaining three columns were coated with one or another of three different liquid-solid chromatographic fractions of Apiezon L. These fractions were separated on silica gel (ratio for gel to Apiezon L, 100:1); *n*-heptane, benzene, and a solution of benzene and methanol (1:1, by volume) were used successively as eluants. In each case the ratio of eluant to silica gel was 1.5:1 by volume. The concentrations of the various Apiezon-L fractions in the benzene used for coating the columns are shown in Table 1.

Gas-liquid chromatography analyses of reference hydrocarbons and of the Nonesuch alkanes were made with the five capillary columns described above, and the analyses presented in Figs. 5, 6, and 7 were made with the column supplied by the Barber-Colman Company. These chromatograms were obtained with a Barber-Colman model 10 instrument equipped with a temperature programmer and a modified inlet system. The modification consisted of mounting the inlet against the detection cell block and placing additional insulation about the inlet system. Temperature was controlled to increase 1°C per minute (from 25° to 300°C) for conditioning the columns and 5°C per minute (from 70° to 300°C or 320°C) for the analyses. The argon-inlet pressure on the columns was 30 pounds per square inch (2.04 atm). The split flow was 35 milliliters per minute.

Table 1. Identical retention temperatures (in degrees ± 1°C) of the peaks for reference pristane and phytane and for pristane and phytane in the Nonesuch alkanes.

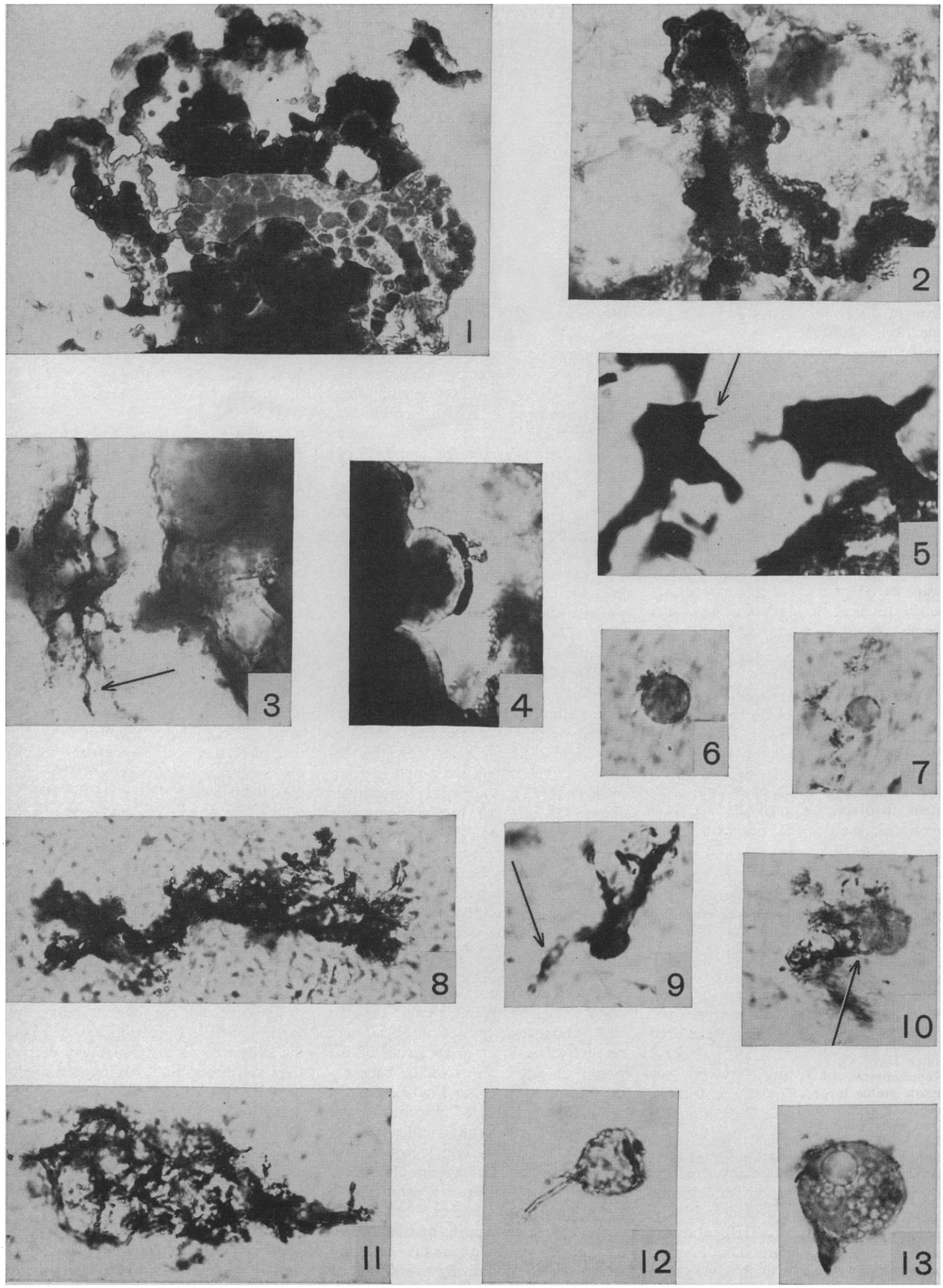
Compound	Apiezon L column supplied by Barber-Colman	10% Solution of Apiezon N	8% Solution of *n*-heptane eluate of Apiezon L	0.5% Solution of benzene eluate of Apiezon L	0.7% Solution of benzene-methanol eluate of Apiezon L
Pristane	193	184	186	137	133
Phytane	208	198	199	154	144

Operation conditions were as follows: inlet temperature, 450°C; microionization detector (radium sulfate source), 1000 volts; amplifier sensitivity setting, 10; attenuation, 2 to 16; range, 1×10^7; chart speed, 38 centimeters per minute.

Results and discussion: Porphyrins. The visible spectrum (in chloroform) of the porphyrin aggregate isolated in the benzene-ether eluates from the siltstone of the Parting shale unit is given in Fig. 8. This aggregate constitutes about 45 parts of the original rock sample per million. The peak configuration and their locations (411, 534, and 573 μ) are typical of vanadyl porphyrins isolated from crude oil and bituminous strata (*20*). Although most of the porphyrins were isolated in the benzene-ether eluates, the presence of minor amounts (about 5 parts per million) of vanadyl porphyrins in the more polar ether-methanol eluate suggests that at least two types of porphyrins are present. The infrared spectrum of the porphyrin aggregate from the benzene-ether eluates, given in Fig. 9, shows a well-defined bifurcated absorption band at about 5.8 microns, indicating the presence of two carbonyl groups. Although careful chromatographic techniques seldom produce a sample with porphyrin content higher than 50 percent (*21*), the intensity of these peaks and their presence in the fractions eluted by the relatively weakly polar benzene-ether solvent solutions suggests that they are related to the dominant species of the aggregate and that they contribute polarity to a rather large and otherwise nonpolar molecule. Analyses of the visible and ultraviolet spectra indicate that vanadium-porphyrin complexes are the dominant members of this aggregate. The chromatographic characteristics of these rather large and otherwise nonpolar molecules are probably the result of polarity contributed by attached carbonyl groups.

The limit of porphyrin detection in the procedure used in this investigation is about 0.05 part per million. For this reason it might be suggested that the apparent absence of porphyrins in the crude oil of the Nonesuch formation is due to insufficient detection sensitivity. However, the two portions of asphaltene-free oil placed on the alumina column were 14 and 65 times, respectively, the volumes of analogous material obtained from the extracted siltstone. The evidence therefore suggests that there has been a very nearly complete segregation of porphyrins between the siltstone and the petroleum phases of the formation. The presence of vanadyl porphyrins within the fine-grained strata, together with their absence in the petroleum present in the associated vugular structures of the Parting shale unit, establishes the fact that the porphyrins have had limited mobility within the formation and indicates that they are indigenous to the siltstone and not secondarily emplaced.

The vanadyl porphyrins indigenous to this ancient formation appear to be the oldest respiratory pigment derivatives yet discovered. Although it might be postulated that they represent degradation products of hemoglobin or other animal pigments, any such animals must have ultimately relied on plants as a source of oxygen and as a food supply. This consideration and the presence of fragments of plant tissue within the strata indicate that the vanadyl porphyrins of the Nonesuch formation constitute the oldest presently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known direct evidence of the photosynthetic mechanism.

The presence of porphyrins within the cupriferous zone of the Nonesuch shale has important bearing on the origin of the economically important copper minerals of the deposit. Sales (*4*) and Joralemon (*5*) have suggested that all copper minerals of the basal Nonesuch were emplaced by magmatic hydrothermal solutions emanating from the White Pine fault at a late period in the history of the formation. White and Wright (*3*) and White (*6*) have suggested that most of the copper minerals of the zone were deposited through diagenetic processes, involving relatively low temperatures, prior to lithification. Thus, the time and temperature of copper emplacement are important factors in evaluating these two conflicting theories.

As early as 1934 it was recognized that preservation of porphyrins is dependent upon mild geothermal conditions (*22*). Calculations based on the activation energy required for the thermal degradation of porphyrins indicate that these chemical fossils could not have persisted longer than about 100 years in a lithologic environment subjected to temperatures of 250°C, for longer than about 11.5 days (10^6 seconds) at temperatures of 350°C, or longer than about 100 seconds at temperatures of 500°C (*23*). The presence of porphyrins within the basal Nonesuch is consistent with a low-temperature origin for the copper minerals of the deposit and renders highly unlikely any proposed mechanism of emplacement involving widespread high temperatures, such as the epigenic hydrothermal theory would probably require.

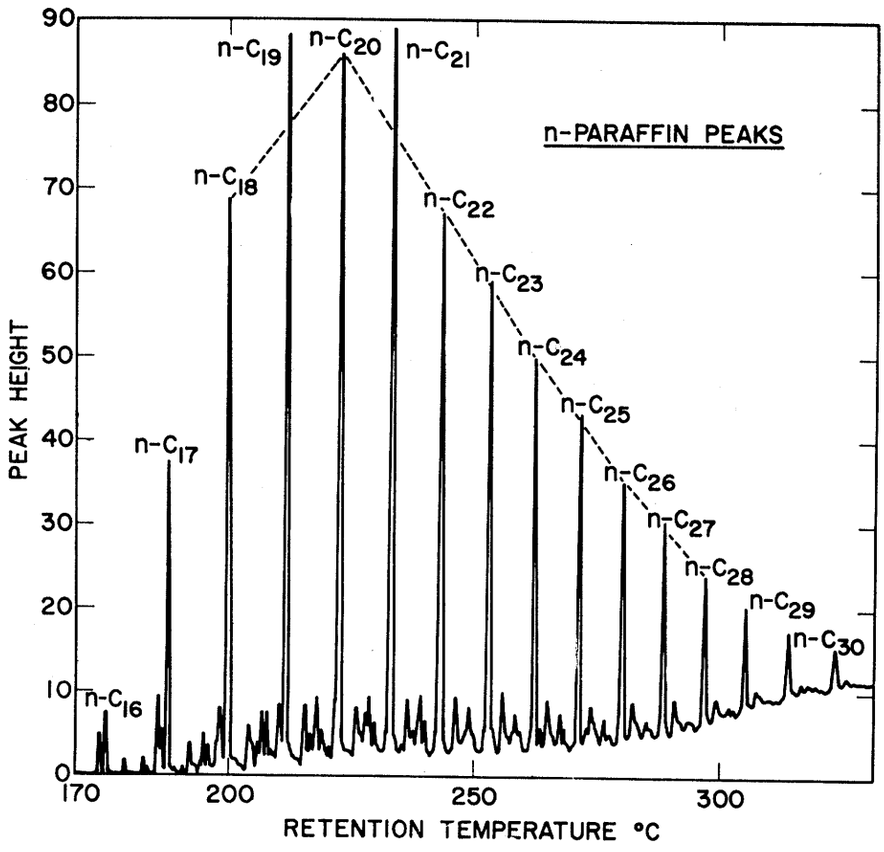

Fig. 5. Gas-liquid chromatographic analysis of alkanes in the *n*-heptane eluate from silica-gel columns of Nonesuch crude oil.

Results and discussion: Hydrocarbons. Crude oils are generally thought 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 (*18*, *24*). Data in Table 2 make it possible to characterize the crude oil and benzene extract from the Nonesuch siltstone. These data, in conjunction with results of other analyses, show that both the Nonesuch samples contain higher concentrations of alkanes and lower concentrations of aromatic hydrocarbons than an average crude oil. Differences in the results reported in

Fig. 4 (left). Physical organization of organic matter in Parting shale unit, Nonesuch shale formation, White Pine, Michigan (all photographs taken in transmitted light). Part 1. Representative of category 2 in text. Composite photograph showing concentric layers of alternating reddish-brown and translucent amber asphaltic-like organic matter interpreted as residual petroleum exhibiting varying degrees of devolatilization. (In the photograph the reddish-brown layers are dark, the translucent layers lighter.) Central area, superimposed from photograph taken at lower focal depth, shows radially oriented condensation cracks. (Thin section; about × 1275.) Part 2. Representative of category 2 in text. Asphaltic-like organic material, mammillary in outline, showing well-defined outer layer of residual petroleum and associated solid spheroidal body (at upper left). (Thin section; about × 1275.) Part 3. Representative of category 3 in text. Anastomosing aggregate of filamentous plant fragments oriented parallel to bedding planes of siltstone; arrow points to cell wall of nonseptate filament. (Thin section; about × 1275.) Part 4. Representative of category 2 in text. Globular structure showing broad, sharply delineated layers of asphaltic-like organic material in varying degrees of devolatilization; inner layer (light in photograph) exhibits poorly defined radial condensation cracks. (Thin section; about × 1275.) Part 5. Representative of category 1 in text. Angular anthraxolitic organic matter occurring as interstitial filling between mineral grains; arrow points to organic material extruded at grain boundaries, suggesting a precompaction origin for this material. (Thin section; about × 1025.) Part 6. Solid organic spheroidal body, probably derived from disaggregation of asphaltic-like organic residues (see parts 1 and 2), about 7 μ in diameter. (Maceration; about × 1325.) Part 7. Hollow, hyaline, spherical organic body, about 5 μ in diameter, associated with poorly defined plant filament. (Maceration; about × 1325.) Part 8. Massive sheet of plant tissue composed of filamentous cellular residues. (Maceration; about × 675.) Part 9. Ovoid solid organic bleb in juxtaposition with branched and unbranched plant filaments; arrow points to filament showing septated cell-like divisions approximately 1 μ wide. (Maceration; about × 1600.) Part 10. Aggregate of filamentous plant fragments in juxtaposition with solid spheroidal organic body; arrow points to branching filament. (Maceration; about × 1325.) Part 11. Sheet of plant tissue composed of occasionally branching, anastomosing filaments. (Maceration; about × 1025.) Part 12. Filamentous plant fragment associated with subangular solid organic particle having a fossil-like appearance. (Maceration; about × 1025.) Part 13. Hollow, hyaline spherical organic body, 42 μ in diameter, containing an internal cluster of hollow spherical nodes ranging in diameter from 1 to 13 μ; origin of this pseudofossil structure apparently involves an emulsification or alveolation process. (Maceration; about × 500.)

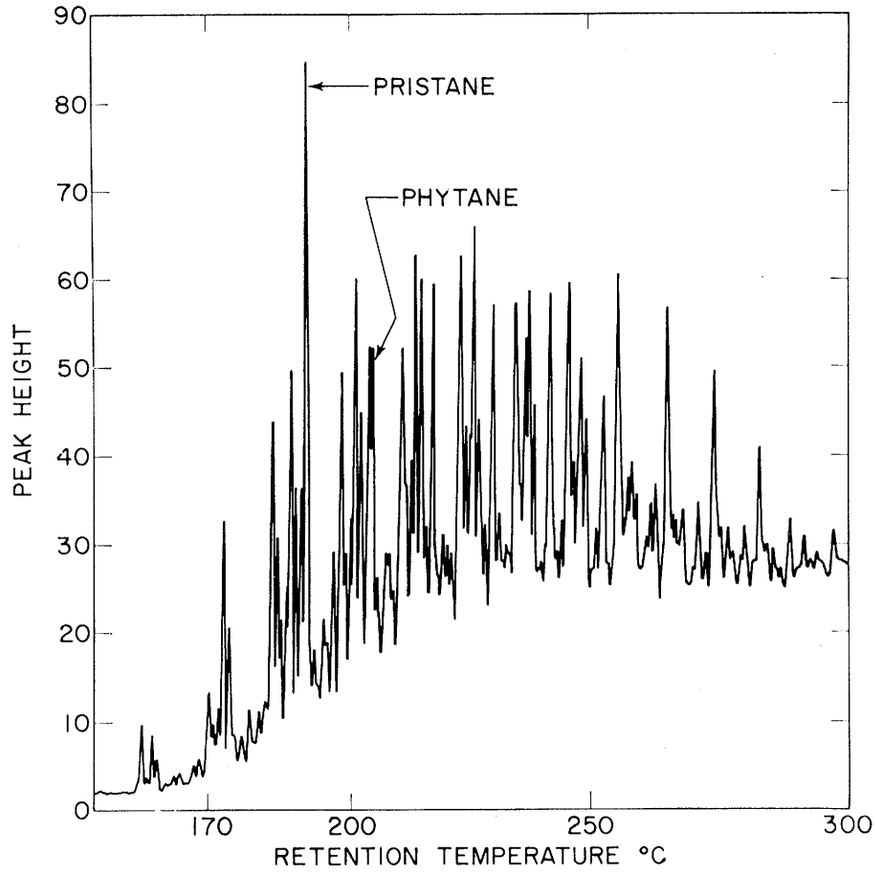

Fig. 6. Gas-liquid chromatographic analysis of the *n*-paraffin-free, first *n*-heptane eluate of Nonesuch alkanes from the alumina column. *n*-Paraffins were removed from this eluate by means of molecular sieves.

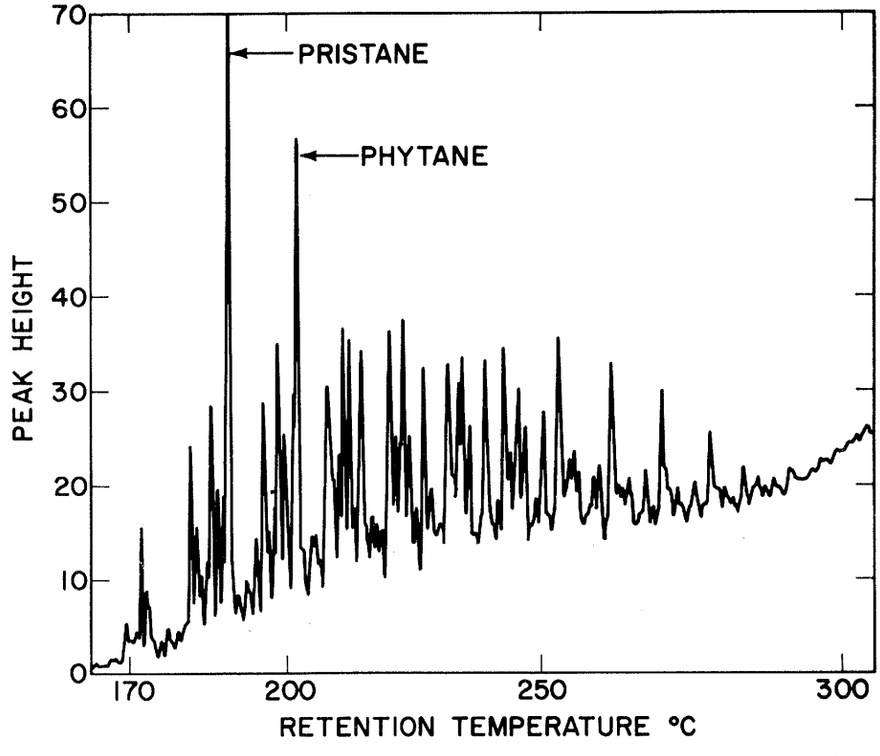

Fig. 7. Gas-liquid chromatographic analysis of the *n*-paraffin-free, first *n*-heptane eluate of Nonesuch alkanes from the alumina column, after the addition of reference pristane and phytane to this eluate.

Table 2 for the Nonesuch oil and extracts 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 (*25*).

Organisms are uniquely capable of synthesizing carbon compounds which possess optical activity (*26*). Alkanes from living organisms (*26*), recent sediments (*24, 27*), and crude oils (*28*) rotate polarized light in a clockwise or positive direction. Values for the optical activities of alkanes from these sources and for the Nonesuch crude oil (*11, 19*) are presented in Table 3. It is apparent from these values that the Nonesuch alkanes have optical rotations comparable in direction and magnitude to those of other biological and sedimental alkanes.

A gas chromatogram of the alkane fraction from the Nonesuch crude oil is presented in Fig. 5. All the large peaks in this chromatogram are produced by *n*-paraffins. In Fig. 5, the tops of successive peaks produced by *n*-paraffins of even carbon number (n-C_{18} to n-C_{28}) have been joined by dotted lines. It is noteworthy that the peaks for *n*-paraffins of odd carbon number 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 (*24, 29*). Although this "odd carbon preference" is not pronounced in the alkanes from some organisms (*30*) and from most ancient sediments, the slightly greater abundances of odd- than of even-carbon-number *n*-paraffins in sedimental alkanes have been reported as evidence that these compounds were made either by formerly living organisms or from biological acids and alcohols (*24, 29*). The "odd carbon preference" of the Nonesuch *n*-paraffins is greater than that of the *n*-paraffins from some organisms (*30*) and from most crude oils, even some oils that are younger than Paleozoic (*29, 31*).

Branched-chain alkanes and n-C_{21} and smaller-molecular *n*-paraffins are concentrated in the first fraction of sedimental alkanes that are eluted by *n*-heptane from alumina columns (*32*), and *n*-paraffins are removed from mixtures of alkanes by molecular sieves

(*33*). A gas chromatogram of this first *n*-heptane eluate of the Nonesuch alkanes, obtained after the removal of *n*-paraffins, is shown in Fig. 6. Peaks labeled "pristane" and "phytane" in this chromatogram are chromatographically equivalent to pristane and phytane as established in Fig. 7. The heights of the pristane and phytane peaks of Fig. 7 had been increased by the addition of reference compounds. The chromatographic equivalence of the reference and Nonesuch pristane and phytane were established on five gas-liquid chromatography 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 1 for these five columns. Mass spectra of the branched-chain concentrate from the Nonesuch alkanes contained large peaks at mass 183. In the mass spectra of pristane and phytane, large peaks at mass 183 are assumed to be caused by 2,6,10-trimethyldecyl ions (*34*).

Polycyclic alkanes and n-C_{25} and larger molecule *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 peaks at mass 372, 218, 217, and 149; such peaks are characteristic of parent sterol hydrocarbons (*16*). Similar polycyclic alkanes are apparently found ubiquitously in biological and sedimental alkanes (*18, 35*).

Geological evidence, the occurrence of plant fragments within the formation, and the distribution of porphyrins discussed above clearly indicate that biological materials in the Nonesuch formation were not derived from younger sediments. The 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 1 billion years ago. Identities or similarities in the structures and optical activities of the Nonesuch alkanes and of modern biological alkanes suggest 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 1 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.

Table 2. Data from silica gel chromatographic analyses of material from the Nonesuch formation.

Material	Percentage eluted by			
	n-Heptane (alkanes)	Carbon tetrachloride (alkanes and aromatics)	Benzene (aromatics)	Methanol (nonhydrocarbons)
Nonesuch samples:				
Crude oil	61.2	22.2	12.7	3.9
Extract	61.5	20.3	15.4	2.8
Average crude oil*	47.2	12.1	32.4	8.3

* Average for 110 crude oils.

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 (*36*), 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 a precursor of the pristane and phytane in sediments (*34, 37*). However, pristane is a constituent of various organisms (*38*), and phytane has been tentatively identified in the bacterium *Vibrio ponticus* (*30*).

Since living organisms synthesize pristane and apparently phytane, and the production of these compounds from phytol has not been accomplished by abiotic reactions that might occur in sediments, pristane and phytane are probably less reliable indicators of the preexistence of photosynthetic organisms than porphyrins are. The metabolic processes of living organisms suggest that complementary information about the biosynthetic pathways of ancient organisms may be obtained through consideration of the porphyrins and alkanes present 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 in lipid biosynthesis (*39*). The biosynthesis of the pyrrole rings in porphyrins has been traced from succinate, a compound of the Krebs cycle (*40*). 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 (*21*).

Because alkanes are minor and chemically inert products of plants and animals, the metabolism of alkanes has received less attention than has that of most other organic compounds. However, alkanes are structurally and isotopically similar to the acids and alcohols in biological fats and waxes (*18, 21*). Furthermore, direct metabolic relationships between *n*-paraffins and fatty acids have been established. *n*-Paraffins are converted by Ω oxidation into fatty acids in the livers of certain verte-

Table 3. Optical activities of alkanes.

Sample	Measured rotation (deg)	α_D^{20}* (deg)	α_{5460}^{35}† (deg)	α_{5780}^{35}‡ (deg)	Reference
Biological					(*26*)
Spirogyra	+1.28				
Hydrodictyon reticulatum	+1.0				
Vibrio ponticus	+0.94				
Recent sediments:					
Gulf of Mexico		+0.514			(*24*)
Atlantic Coast of France		+3.3			(*27*)
Crude oils					(*28*)
Pennsylvania		+0.25			
Rodessa		+0.79			
Mid-Continental No. 1		+0.93			
Mid-Continental No. 2		+1.20			
California		+2.73			
Nonesuch alkanes			+0.541	+1.569	(*11, 19*)

* α_D^{20} Means rotation measured at 20°C in light source of wavelength of the D line of sodium, 5890 Å. † α_{5460}^{35} Means rotation measured at 35°C in light source of wavelength 5460 Å. ‡ α_{5780}^{35} Means rotation measured at 35°C in light source of wavelength 5780 Å.

Table 4. The δC^{13} determinations in Nonesuch shale.

Sample	δC^{13}
$CaCO_3$	− 3.2
$CaCO_3$	− 2.1
"Kerogen"	−28.9
Petroleum	−29.7

brates (*41*). Thus, information about the structural (*24, 39, 40*) and isotopic (*21, 41*) selectivities of biological processes and the limited data on the metabolism of *n*-paraffins provide substantial reasons for presuming that the alkane constituents of plant and animal lipids are biosynthesized either from acetates, as are the acids and alcohols, or from the acids and alcohols (*18*).

Lipid biosyntheses are defined in even greater detail than is the biosynthesis of porphyrins (*39, 42*). 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 (*39*). Equilibria exist between hexoses, pyruvates, acetates, and the tricarboxylates in living cells. Amino acids, building units of proteins, as well as pyrrole rings are made from the tricarboxylic acids participating in the Krebs cycle (*39*).

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 acetates to lipids, and the other, through the Krebs cycle to the syntheses of amino acids and the porphyrins. The presence in the Nonesuch formation of porphyrins and alkanes that resemble porphyrins in geologically young sediments and alkanes in living organisms suggests that life which existed in Precambrian times was metabolically similar to existing life.

Paleontology and Physical Organization of Organic Matter

As previously noted, organic matter is widely disseminated throughout the cupriferous zone of the formation. The organic matter, as seen in thin sections of the rock, exhibits three basic patterns of organization.

1) Irregular, often highly angular discrete or anastomosing opaque bodies occurring as interstitial fillings between mineral grains (Fig. 4, part 5).

2) Globular spheroidal masses, translucent amber to reddish-brown, often mammillary in outline and commonly exhibiting radial cracks or checks which are oriented at right angles to the interface or to the surface (Fig. 4, parts 1, 2, and 4).

3) Threads, or aggregates of threads, of organic matter, often tightly anastomosed and "shreddy" in outline (Fig. 4, part 3).

These filaments vary in color from nearly hyaline to dense brown, and they vary markedly in length, organization, and degree of curvature. Bodies in categories 1 and 2 seem most logically interpretable as structures which have resulted from diffusion of liquid organic material through the porosities of the mineral matrix prior to compaction and from subsequent devolatilization and condensation. Material in category 3 is interpreted as being fragments of plant tissue, ranging from triturated filamentous structures 1 to 15 microns long to aggregates exceeding 100 microns in length.

The putative plant fragments have been rigorously examined with a view to determining whether they are in fact original plant tissue, as opposed to pseudomorphs or secondary aggregates of organic matter unrelated to primary structure. It should be noted that the poor preservation of these filamentous bodies makes their interpretation difficult. However, careful resolution of the structure of these fragments shows the following features characteristic of plant microfossils, which form the bases for our interpretation of their organismal origin: (i) filamentous or laminar form with discrete cell walls and occasional septations; (ii) prevailing orientation of filaments and laminae parallel to the bedding planes; (iii) tendency of the filament aggregates to anastomose; (iv) occasional appearance of true branching; (v) absence of birefringence in polarized light; and (vi) optical differentiation from organic matter of categories 1 and 2 present in the same rock.

Although it seems probable that these threads or filaments constitute remnants of photosynthetic organisms, the great age and poor preservation of these plant fossils makes it impossible to assign a possible phylogenetic position with any degree of certainty. Gross morphology, the depositional environment, and evolutionary considerations would be consistent with an assumed algal or fungal affinity for these forms, but it would probably be unwarranted to base such an assignment on the available evidence.

Preparations of organic residues from the Nonesuch shale were secured by dissolution of the rock in mixtures of hydrochloric and hydrofluoric acids, followed by washing and centrifugation in heavy liquid (ZnBr, specific gravity 2.3). Slurries of the organic fraction recovered in this manner were mounted on clean slides in a medium of purified glycerine jelly. Numerous rounded or subangular solid blebs of organic matter were observed distributed among masses of plant-tissue fragments, often in juxtaposition with the fragments (Fig. 4, parts 6, 9, 10, 12). It is apparent that many of the rounded or subangular solid particles have resulted from disaggregation of the spheroidal or mammillary asphaltic residues observed in thin sections of the shale. Plant fragments, observed in macerations far more clearly than in thin sections, consist of fragments of filaments, aggregates of filaments, and occasional sheets of tissue which appear to show cellular residues (Fig. 4, parts 8 and 11). It is not possible to demonstrate the presence of spores with any assurance. The pseudosporelike bodies seen in Fig. 4, parts 9 and 10, are actually solid blebs of organic material observed in juxtaposition with, or superimposed on, filaments of plant residues. The distinction between what appear to be organized sporelike entities and true plant structures is not easy to make. Our interpretation that the pseudospores are disaggregated particles of condensed spheroidal organic matter and not true microfossils is based on the facts that they are solid, often subangular, and optically readily differentiated from the filamentous or laminar masses, which can be shown to possess lumena in various degrees of occlusion (Fig. 4, part 9). The fact that the solid spheroidal bodies are commonly in physical contact with the filamentous, branched or unbranched plant fragments complicates their paleontological interpretation.

An additional complexity in interpreting the microstructure of the organic residues results from the occasional presence, in macerations, of small organic bodies in the form of perfect and apparently hollow spheres of various sizes. These range in diameter from approximately 1 micron to more than 40 microns. They are gen-

erally hyaline in appearance, but they possess a distinct refractive outer layer, which renders them bubble-like as seen in optical section. In a few cases these spherical bodies possess an internal cluster of smaller bodies of disparate size but perfectly spherical outline (Fig. 4, part 13). These spherical objects are not included in the three major categories of visible organic matter since they are of infrequent occurrence and of highly problematical origin. Their superficial resemblance to spores or related structures of biological origin is notable. However, their variable size, their unusually refractive membranes or walls, and the occasional occurrence of contained alveolar masses renders this interpretation questionable. Although no simple explanation is suggested for the mechanism of formation of these spherical bodies, their appearance, structural features, and variable size suggest phenomena involving emulsification or some mechanism for alveolation of the organic matter either during its emplacement in the shale or possibly during sample preparation by the acid treatment or heavy-liquid separation procedures. Scrupulous care in sample treatment and slide preparation eliminates the possibility that these structures were introduced as laboratory contaminants.

Accessory evidence from isotope studies. The presence of the metal-porphyrin complexes in the Nonesuch shale, probably originating from alteration products of chlorophyll, provides a reasonable basis for postulating a photosynthetic system. One auxiliary line of evidence for this interpretation is of much interest—that is, comparison of the isotopic ratios of the biologically fractionated elements carbon and sulfur.

As shown by the early studies of Nier and Gulbransen (*43*), Wickman (*44*), Rankama (*45*), and Craig (*46*), the ratio of the stable carbon isotopes C^{12} and C^{13} in biogenically synthesized carbon compounds consistently exhibits an enrichment of the lighter isotope C^{12} with respect to the ratio of inorganic terrestrial C^{12} and C^{13} in the atmosphere, in the hydrosphere, and in nonbiogenic sedimentary carbonates. The terrestrial ratio of the two isotopes C^{12} and C^{13} is approximately 90:1 in inorganic environments in which the photosynthetic system does not operate. Reduction of CO_2 during the photosynthetic reaction tends to selectively concentrate the lighter isotope relative to the heavy isotope, with the resulting

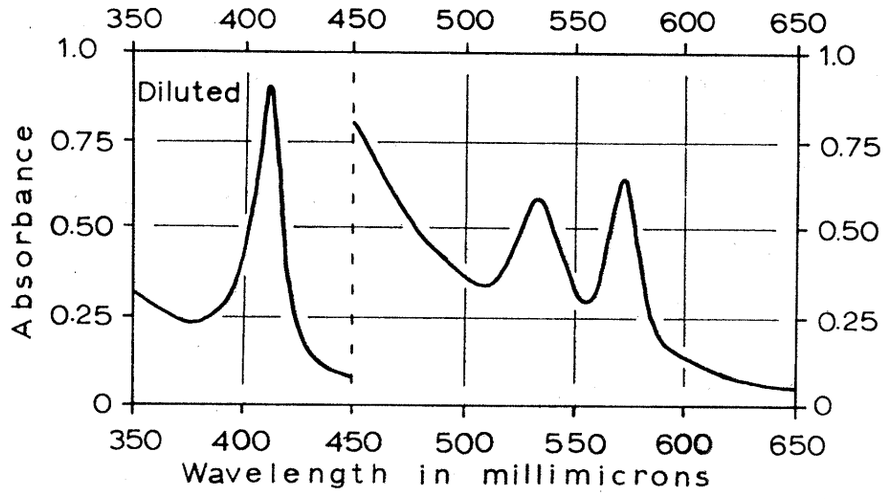

Fig. 8. Visual absorption spectrum of vanadyl porphyrin recovered from benzene-ether eluates of nonasphaltic extractable constituents of Parting shale unit, Nonesuch formation. Approximately 2.5 mg of chloroform per milliliter.

enrichment of C^{12} in biogenic organic matter. As Craig (*46*) expresses it, terrestrial organic matter (that is, organic matter photosynthetically produced and incorporated into the biosphere) and carbonate rocks constitute two well-defined groups in which the carbonates are richer in C^{13}. Organic matter of fossil origin exhibits a preferential enrichment of C^{12}, presumably arising from its initial formation through photosynthesis. However, it has been shown by studies (*47*) subsequent to those of Craig that the fractionation of the two isotopes is biochemically complex (*46*). Although complications exist pertaining to the carbon reservoir of the terrestrial environment and the rate of cycling of carbon, it is evident that ancient organic matter which shows a substantial enrichment of the lighter carbon isotope relative to inorganic carbon of the same deposit is most reasonably interpreted as biogenic in origin. In the case of the Nonesuch shale the stable-carbon-isotope ratios for both the inorganic carbonate carbon and the reduced (organic) carbon have been determined by T. C. Hoering (*48*). These values are expressed in units of δC^{13}, the difference between the C^{13} content of the sample and that of an arbitrary standard, according to the equation

$$\delta C^{13} = \frac{(C^{13}/C^{12})x - (C^{13}/C^{12})s}{(C^{13}/C^{12})} \times 1000$$

in which x is the sample and s indicates the National Bureau of Standards isotope reference sample No. 20 (Solenhofen limestone). The δC^{13} determinations are shown in Table 4.

When the data are considered in conjunction with our evidence of the presence of pristane, phytane, and metal-porphyrin complexes in the organic matter of the Nonesuch formation, it appears reasonable to infer that

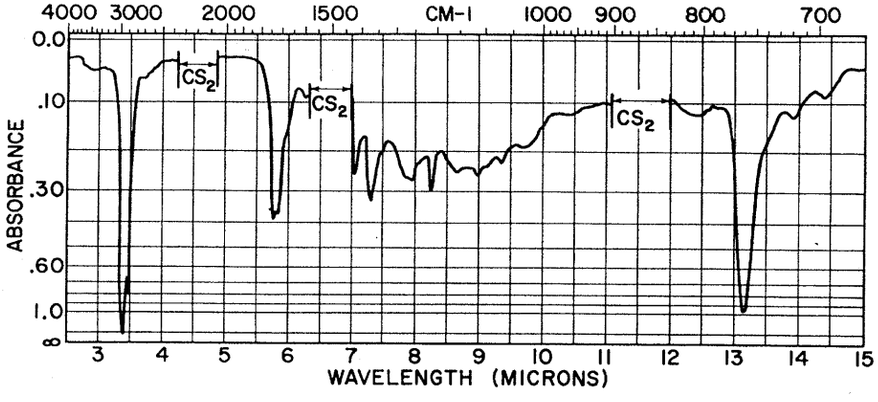

Fig. 9. Infrared absorption spectrum of vanadyl porphyrin aggregate recovered from benzene-ether eluates of nonasphaltic extractable constituents of Parting shale unit, Nonesuch formation. Spectrum solvent is CS_2.

the carbon-isotope fractionation and enrichment of C^{12} constitute corroborative evidence of a photosynthetic system operating in the primary biosynthesis in the Nonesuch basin.

The situation with respect to sulfur isotopes in the Nonesuch shale is comparable to that for the carbon isotopes as indicators of biological processes in Nonesuch time. Of the four stable sulfur isotopes found in nature, only two, S^{32} and S^{34}, are quantitatively important. The light isotope, S^{32}, is preferentially reduced by sulfate-reducing bacteria. Thus, an enrichment of the heavier isotope, S^{34}, relative to a standard of abiotic origin, has been proposed as evidence of the biogenic nature of sedimentary sulfides. In the sulfur isotope investigations of the Nonesuch formation the arbitrary standard used was the Cañon Diablo meteorite ($S^{32}/S^{34} = 22.21$) (*49*). The mean isotopic ratio for oceanic sulfates, relative to the standard, is $S^{32}/S^{34} = 21.76$, whereas the mean value for sulfides of sedimentary origin is 22.49 (*50*). Wiese (*9*) reports that the mean S^{32}/S^{34} value, relative to the Cañon Diablo meteorite, for ten samples of chalcocite from various horizons of the Parting shale unit of the Nonesuch formation is 22.16; the spread of values is from 21.93 to 22.39. Although all ratios from the Nonesuch shale are intermediate between the mean value for oceanic sulfates and the mean value for sedimentary sulfides, the wide range of isotopic values has been interpreted by Jensen, according to Wiese (*9*), as being indicative of biogenically fractionated sulfur.

Summary

Investigations have been made of crude oil, pristane, phytane, steranetype and optically active alkanes, porphyrins, microfossils, and the stable isotopes of carbon and of sulfur found in the Nonesuch shale of Precambrian age from Northern Michigan. These sediments are approximately 1 billion years old. Geologic evidence indicates that they were deposited in a nearshore deltaic environment. Porphyrins are found in the siltstones but not in the crude oils of the Nonesuch formation—evidence that these chemical fossils are adsorbed or absorbed and immobile. This immobility makes it highly unlikely that these porphyrins could have moved from younger formations into the Nonesuch sediments, and the widely disseminated particulate organic matters and fossils in this Precambrian shale are certainly indigenous.

The thermal stability of the Nonesuch porphyrins adds support to the concept of low-temperature emplacement of the copper minerals in the deposit. The concatenation of geologic, geochemical, and micropaleontological evidence strongly indicates that the organic matter, including the crude oil of the Nonesuch deposits, is an indigenous product of primary photosynthetic processes operating in Nonesuch time (*51*).

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