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UNITED STATES DEPARTMENT OF THE INTERIOR

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GEOLOGICAL SURVEY

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Professional Paper 186—D

SOME ORGANIC CONSTITUENTS OF A RECENT SEDIMENT
FROM CHINCOTEAGUE BAY, VIRGINIA

BY

ROGER C. WELLS AND E. THEODORE ERICKSON

Shorter contributions to general geology, 1936

(Pages 69-79)



UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON : 1937

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SOME ORGANIC CONSTITUENTS OF A RECENT SEDIMENT FROM CHINCOTEAGUE BAY, VIRGINIA

By ROGER C. WELLS and E. THEODORE ERICKSON

ABSTRACT

This paper is a development of a previous paper by the writers which called attention to the possible existence of calcium and magnesium salts of organic fatty acids as constituents of sediments and as possible source material in the formation of petroleum. Its main purpose, however, is to describe the methods of analysis and nature of the products or groups of products isolated from a recent sediment.

For the present study portions of sediment weighing nearly a kilogram were used for analysis. The material was collected under sea water in Chincoteague Bay, Va. This sediment contained about 0.7 percent of organic matter, from which a number of different substances were isolated by extraction with solvents, fractional precipitations, and other methods of analysis. The three principal reagents used were alcohol, ammonium hydroxide, and sodium hydroxide.

The organic substances or groups of substances found include chlorophyll, cholesterol, sulphur, "algin", wax, humic-acid material soluble in alcohol, humic-acid material insoluble in alcohol, fatty-acid material, pentosans, and acid-soluble organic matter. Different fractions of the waxlike material melted at 89° to 175° C. Different fractions of the free fatty acids eventually obtained ranged in their melting point from below 37° to 71° C. Several facts indicate that at least some of these fatty acids may be present in the sediments as calcium or magnesium salts. Among these facts are the tendency of the inorganic bases to accompany the fatty acids into the final fractions that were separated and the yield of precipitates from somewhat concentrated alcoholic solutions of several of the organic fractions when treated with sea water and with alcoholic calcium acetate solution.

As the organic content of the sample was only about 0.7 percent, the percentages of the different organic fractions obtained were correspondingly small. The main classes of substances found were algin, chlorophyll, sulphur, wax, humic-acid complex, fatty-acid material, and acid-soluble organic matter. These groups were further subdivided by melting-point and other tests. The analytical procedures and results are described in systematic detail.

INTRODUCTION

The work of F. W. Clarke¹ called attention to certain relations among the inorganic constituents contributed to sediments by marine invertebrates, but in that study little or no attention was paid to the organic constituents. In fact, the samples were purposely ignited prior to analysis in order to destroy the organic matter. The organic contributions have long been of interest to economic geologists because of their bearing on the formation of coal and petroleum, and aside from

¹ Clarke, F. W., and Wheeler, W. C., The inorganic constituents of marine invertebrates: U. S. Geol. Survey Prof. Paper 124, 1922.

this feature they are of great interest to the geochemist on account of their variety and the chemical features involved in their progressive changes. Yet the organic constituents have been given relatively little study.

The organic constituents may include plant residues as well as animal remains, debris of microscopic algae, fatty material, the indefinite mixture termed "sapropel"—in short, a great variety of chemical compounds. These substances conceivably differ from one sediment to another, so that many sedimentary deposits should be examined and compared, but on account of the time required to make such studies, progress is necessarily slow, and hence the aim of the present paper is chiefly to describe in some detail what has been found about the nature of the organic matter in a single sediment.

In a recent paper by the writers² it was suggested that fatty acids may find their way into marine sediments in the form of insoluble calcium and magnesium salts. That viewpoint has been kept in mind in the present study. The experimental work here described is almost entirely that of the junior author, but responsibility for certain details of the investigation and for the conclusions is shared by the senior author.

Twenhofel³ has summarized such information as is available on the more general chemical composition of sediments. The content of organic matter in sediments has also been rather fully studied by Trask, Hammar, and Wu,⁴ but in attempting to cover a very large field they were obliged to limit their work for the most part to determinations of carbon and nitrogen, supplemented by the use of the customary solvents for organic matter. A beginning was made in studying the nature of the organic matter in some samples, but much remained to be done, and it seemed well worth while to the writers to make a more extended analysis of the organic matter in a single sediment. They are greatly indebted to Mr. Trask for helpful suggestions in the preparation of this paper.

² Wells, R. C., and Erickson, E. T., The analysis and composition of fatty material produced by the decomposition of herring in sea water: *Am. Chem. Soc. Jour.*, vol. 55, pp. 338-341, 1933.

³ Twenhofel, W. H., *Treatise on sedimentation*, 2d ed., ch. 1-4, Baltimore, Williams & Wilkins Co., 1932.

⁴ Trask, P. D., Hammar, H. E., and Wu, C. C., *Origin and environment of source sediments of petroleum*, Houston, Tex., Gulf Publishing Co., 1932.

David White⁵ gives a concise summary of the present knowledge of the chemical changes in sediments. He points out that the carbonaceous material originally consists principally of the residues of plants or animals, more especially of plants. The early chemical changes are largely covered by the single word "decomposition", including biochemical decomposition, and the later changes are referred for the most part to those observable in the solid residues, the coals.

As a matter of fact, sapropel may also contain a high percentage of coprolitic matter, ejected or non-assimilable by both organisms and bacteria, which tends to accumulate because it is nonassimilable. Just what kinds of compounds may be so classed under marine conditions is not well known. Possibly wax-like substances, carbohydrates, fats, fatty acids, and soaps are the principal types. Anaerobic bacteria tend in the earlier stage of their activity to develop acidity, but where protein material is available the ammonia generated by its decomposition tends to produce an alkaline environment, at least temporarily, and sea water itself has an alkaline reaction. Some bacteria and lower organisms are poisoned by the higher fatty acids and soaps. Other bacteria convert different fatty acids into methane rather than into the expected higher hydrocarbons.⁶ On the other hand, some bacteria and molds produce fats.⁷ Hammar⁸ concludes from an extensive survey of the literature that there is little or no evidence that hydrocarbons higher than methane are formed from other organic substances by bacteria.

These conflicting statements leave some doubt as to the fate of fatty acids from anaerobic bacterial attack, but in any case there will still remain the humus or residue. In this connection Waksman⁹ says: "The marine humus, just as the soil humus, is formed by similar micro-organisms in a very similar manner", but certain differences in chemical nature from land humus are accounted for by the fact that marine residues, from which all or part of the humus is formed, are different in chemical composition from the land residues from which soil humus is formed. Thus, land vascular plants have a notable lignin content, which is lacking in algal material. For the problem here considered the principal differences in the marine environment are the alkaline reaction of the water and the presence of calcium and magnesium salts. The

⁵ White, David, in Twenhofel, W. H., op. cit., p. 415; The origin of petroleum, in Petroleum investigation: 73d Cong. (recess), Hearings on H. Res. 441, pt. 2, pp. 898-906, 1934.

⁶ Thayer, L. H., Bacterial genesis of hydrocarbons from fatty acids: Am. Assoc. Petroleum Geologists Bull., vol. 15, pp. 441-453, 1931.

⁷ Ward, G. E., Lockwood, L. B., and May, O. E., Production of fat from glucose by molds: Ind. and Eng. Chemistry, vol. 27, pp. 318-322, 1935.

⁸ Hammar, H. E., Relation of micro-organisms to generation of petroleum: Problems of petroleum geology (Sidney Powers memorial volume), pp. 35-50, Am. Assoc. Petroleum Geologists, 1934.

⁹ Waksman, S. A., The role of bacteria in the cycle of life in the sea: Sci. Monthly, vol. 38, pp. 35-49, 1934.

present study gives some indication of what kinds of organic matter may survive at an early stage of sedimentation.

SOURCE OF SEDIMENT STUDIED

The sample of sediment used in the present investigation was collected by E. T. Erickson in September 1932, at a locality in Chincoteague Bay, Va., about half a mile northwest of Killick Shoal Lighthouse, at the point marked "X" in plate 15. Here the bottom of the bay was approximately level and was just reached with an ordinary long-handled shovel from a small boat. The nearest land was the eastern member of a group of islands in Chincoteague Channel, about a mile south. Dr. Paul Bartsch, of the United States National Museum, to whom acknowledgment is gratefully made, suggested this locality on account of its marine life and convenient accessibility from Washington. Acknowledgment is also made to Mr. Archie Bradford, of Chincoteague Island, for assistance in collecting the sample and other materials studied. The water is essentially coastal sea water in a marginal lagoon or bay. The land nearby is covered with salt-marsh grass, and the shores are strewn with abundant masses of algae. In the water or on the bottom may be found fishes, sponges, and oysters. But K. E. Lohman, of the Geological Survey, found no Foraminifera or diatoms in the sample collected, which is mainly quartz sand with some mud. Dr. Bartsch suggests that the absence of diatoms may be due to their effective removal from the water by oysters, whose culture has increased in the bay in recent years.

The sample was dark gray when wet, lighter gray when dried. Its inorganic chemical composition is shown in table 1.

TABLE 1.—Analysis of sediment from Chincoteague Bay, Va.

[E. T. Erickson, analyst]			
SiO ₂ -----	76.32	NaCl-----	0.88
Fe ₂ O ₃ -----	1.08	MnO-----	.02
Al ₂ O ₃ -----	10.69	SO ₃ -----	.31
TiO ₂ -----	.46	Loss at 105°	1.55
P ₂ O ₅ -----	.07	Further loss on igni-	
CaO-----	1.46	tion-----	3.34
MgO-----	.77		
Na ₂ O-----	1.65	Total-----	100.50
K ₂ O-----	1.90		

ORGANIC CONTENT OF THE SAMPLE

The organic content of the sample, other than carbonate, was small—approximately 0.7 percent, based on the total products extracted. The material dried on the steam bath gave 0.41 percent of carbon as the average of three determinations (0.38, 0.45, 0.40), and 0.0755 percent of nitrogen as the average of two determinations (0.076 and 0.075). Engler¹⁰ has expressed the view that little nitrogen is to be expected in sedi-

¹⁰ Engler, C., in Potonié, H., Zur Frage nach dem Ur-Materialien der Petrolea: K. preuss. geol. Landesanstalt Jahrb., Band 25, p. 342, 1904.

mentary sapropelic matter, as the nitrogen would be removed by biochemical processes before or during deposition. These are the first changes affecting the chemical composition of the sapropel. Nitrogenous compounds tend to move in a cycle rather than to become fixed in sediment, but according to Trask the nitrogen in most sediments ranges from one-seventh to one-eighteenth of the carbon.¹¹ In living matter the nitrogen-carbon ratio has a wide range, depending on the nature of the material.

The main organic substances or classes of substances separated from the sediment of Chincoteague Bay are as follows:

Percentage of organic substances in sediment of Chincoteague Bay

Algin.....	0.037
Chlorophyll.....	.003
Sulphur.....	.006
Wax.....	.023
Humic-acid complex insoluble in alcohol after isolation.....	.075
Humic-acid complex soluble in alcohol after isolation.....	.116
Fatty-acid material.....	.034
Acid-soluble organic matter.....	.448

It should not be thought that these substances necessarily exist as such in the free state in the sediments. There is clear evidence that some of them do not, although some may. One of the most difficult parts of the problem is to determine in what form they are actually present.

It seems clear that sulphur is present in the free state, though some may also be combined. Chlorophyll is probably present in the free state and in residues of plant tissue. The chemical formula of alpha-chlorophyll is $C_{55}H_{72}O_5N_4Mg$, with a carbon-nitrogen ratio of 11.8. This complex molecule is decomposed by treatment with either strong acids or alkalis and yields methyl alcohol and phytyl alcohol, $C_{20}H_{40}O$, a primary unsaturated, monohydric alcohol, but it apparently dissolved directly without decomposition in the alcohol with which the organic matter was extracted from the sediment. The "algin" appears to be a colloidal complex of a hygroscopic nature. The writers believe that some of the "humic acids" and probably also some of the fatty acids are present as calcium and magnesium salts.

The waxes probably occur in the sediment in the free state; chemically these substances are salts or esters of the higher alcohols and acids. Krämer and Spilker¹² found that "sea-weed wax" obtained from algae appeared to consist of esters of alcohols containing 20 to 22 atoms of carbon united with arachidic, behenic, and lignoceric acids, and that it had a fairly low melting point. The wax from diatoms melted at 50° to 70°

C.¹³ Waxes extracted from peat melt much higher.¹⁴ Wax from the Chincoteague Bay sediment ranged in melting point from 25° to 90°, indicating a great variety of compounds. The survival of waxes in the sediments suggests that they are not assimilated by animals, though no direct evidence on this point is available. They may possibly even be built up by micro-organisms.

Extraction of the sediment with alcohol having removed "algin", chlorophyll, sulphur, wax, and some oily matter, the material was next treated either with ammonia or with dilute caustic soda solution. This procedure raises the question of the nature of the action of these reagents. It is well known that they bring a large proportion of organic matter into solution, but do they act simply as solvents and as hydrolyzing agents or by base exchange, forming salts with weak acids and compounds carrying exchangeable hydrogen? It seems likely that calcium and magnesium salts of organic acids may be present in the sediments rather than the free acids, and therefore the action of the alkalis is probably in part of the nature of a base exchange. If it is desired to minimize decomposition of the higher and complex molecules during the extraction, ammonia is preferable to the caustic alkalis on account of its milder action on the organic compounds.

Schreiner and Shorey¹⁵ emphasize the fact that the organic matter of soils is reactive to the inorganic matter, and the same must be even more true of sediments deposited in sea water, which contain calcium and magnesium ions available to form insoluble compounds with such acids. In other words, the organic "acids" may be present in part as inorganic salts, besides existing as esters and adsorption complexes. Although this is not a new idea, it has received little emphasis heretofore.

At least two lines of evidence offer support for the idea. First, when the organic matter is extracted and concentrated in certain solvents, as described in the following pages, an inorganic part containing SiO_2 , R_2O_3 , MgO , CaO , and a few other oxides tends to accompany the organic matter. Second, when the organic acids are set free with hydrochloric acid, extracted with benzene, then with alcohol after evaporating the benzene, the alcoholic solution gives a precipitate with sea water. A precipitate may also be obtained, if fatty acids are initially present, with an alcoholic solution of calcium acetate—a test proposed by Mr. Erickson. Such a solution is easily made by shaking at intervals during 15 minutes about 1 gram of calcium acetate with 55 milliliters of 95-percent alcohol and filtering, and it appears to be a convenient reagent for some purposes. That the reagent indicates

¹³ Krämer, G., and Spilker, A., *Das Wachs der Bacillariaceen und sein Zusammenhang mit dem Erdöl*: Deutsche chem. Gesell. Ber., Jahrg. 32, Band 3, p. 2940, 1899.

¹⁴ Lewkowitzsch, J., *Chemical technology and analysis of oils, fats, and waxes*, 5th ed., vol. 3, p. 282, 1915.

¹⁵ Schreiner, O., and Shorey, E. C., *Chemical nature of soil organic matter*: U. S. Bur. Soils Bull. 74, 1910.

¹¹ Trask, P. D., Hammar, H. E., and Wu, C. C., *op. cit.*, p. 22.

¹² Krämer, G., and Spilker, A., *Das Algenwachs und sein Zusammenhang mit dem Erdöl*: Deutsche chem. Gesell. Ber., Jahrg. 35, p. 1212, 1902.

the presence of fatty acids only has not been shown. The facts so far established are simply that "organic acids" are present in the sediment in small quantities and may be shown to give calcium precipitates with the reagent, or sea water, and among the acids so far identified are members of the paraffin series generally termed the "fatty acids."

Nitrogen was proved to be present in the humic-acid groups, though partly lost in the extraction. It is probably present largely as protein, but just what kind of protein has not yet been determined.

METHODS OF SEPARATING THE ORGANIC MATTER

The general scheme of analysis and separation of the different groups of constituents is shown in tables 2

and 5. In these tables the following abbreviations are used: R, residue; F, filtrate; P, precipitate; E, extract; S, solution. The figures in the tables refer to weights in grams of the different fractions obtained. The scheme is partly modeled on the work of Odén,¹⁶ partly on that of Schreiner and Shorey¹⁷ and on other work. These methods are the best so far developed for separating the organic matter into classes which can be tested for individual substances. The time has not yet arrived when some specific test can be made for each substance or even for each class of substances. The desired knowledge can be obtained only by successive approximations through a series of steps. The original samples were dried on the steam bath before weighing out portions for study.

TABLE 2.—Outline of method of analysis

Extract with hot alcohol.										
R ₁ . Heat with 600 ml of dilute NH ₄ OH. Filter.					F ₁ . Concentrate and filter off the insoluble inorganic salts.					
R ₉ . Repeat the extraction and finally discard residue.	F ₉ . Evaporate slowly to 150 ml. Filter.				F ₅ . Extract with petroleum ether.				R ₄ . Salts, 3.76.	
	R ₁₀ . "Reversed" precipitate, 0.8641.	F ₁₀ . Add 25 ml of acetic acid and filter.				S ₁ . Residual solution. Extract with benzene.		F ₆ . Petroleum ether extract. Extract with alcohol, 0.333.		
		P ₄ . Treat with hot alcohol.		F ₁₁ . Treat with hot alcohol.		R ₃ . "Algin", 0.300. Extract with absolute alcohol. R ₁₃ , 0.026. F ₁₄ , 0.1509.	E ₂ , 0.055.	R ₆ . See table 3. Wax, 0.190; sulphur, 0.051.	F ₇ . Chlorophyll, etc., 0.024. Oily matter, 0.068.	
		R ₁₁ . Insoluble, 1.462.	F ₁₂ . Soluble, 0.390.	R ₁₂ . Insoluble, 6.671.	F ₁₃ . Soluble, 1.531.					

HOT ALCOHOL EXTRACT

A portion of the sample weighing 811 grams was extracted three times with 95 percent ethyl alcohol on the steam bath in a covered flask, using about 600 milliliters of alcohol each time. The duration of the heating varied from several days for the first extraction to overnight for the last one. While hot, each alcoholic solution was filtered. The first filtrate was noticeably green, the last practically colorless. This procedure left residue R₁, which was reserved for further treatment.

The combined alcoholic extracts (F₁) were evaporated to 50 milliliters and filtered. The insoluble material thus obtained was heated with successive small quantities of alcohol and filtered off until it was practically white and nearly free from organic matter; it then weighed 3.76 grams (R₄).

The combined alcoholic filtrate (F₅), evaporated to about 50 milliliters, was then placed in a separatory funnel and extracted several times with light petroleum ether. The first ether extract had a strong green color; the last was only slightly colored. Some green matter

also remained in the residual alcoholic solution, thus showing at least two kinds of green pigment. Ordinary chlorophyll is soluble in light petroleum ether. The total ether extract weighed 0.333 gram.

PETROLEUM ETHER EXTRACT

The ether extract (F₆), after evaporation of the solvent, was treated with small quantities of alcohol until little coloring matter remained. Waxy matter tended to remain in the insoluble part (R₆). The treatment was continued until from the soluble portion a final green viscous residue was obtained which would all dissolve in about 1 milliliter of alcohol (F₇).

On treating the residue from evaporation of F₇ with still less alcohol the green portion was distinctly more soluble than the remainder. In this solution cholesterol was identified by the digitonin reaction,¹⁸ but the

¹⁶ Odén, Sven, Die Huminsäuren; chemische, physikalische und bodenkundliche Forschungen: Kolloid-chemische Beihefte, Band 11, pp. 76-260, 1919.

¹⁷ Schreiner, O., and Shorey, E. C., op. cit.

¹⁸ Holde, D., The examination of hydrocarbon oils and saponifiable fats and waxes, tr. by E. Mueller, p. 350, New York, John Wiley & Sons, 1922.

quantity was too small for determination or further study. Chlorophyll was definitely identified, aside from its color, by the copper acetate test.¹⁹

The rest of F₇ was then saponified with alcoholic potash solution. A little lead acetate solution was then added, which resulted in precipitation of the lead salts of the organic acids. The lead precipitate was filtered off, washed with hot water, dried, and decomposed with warm 20 percent hydrochloric acid, and the resulting mixture was extracted with benzene. The benzene solution, when separated in a separatory funnel, yielded 0.039 gram of oil-like matter on evaporation. This fraction may represent acids derived from algal oils. The remaining acid solution on evaporation to dryness and extraction with alcohol yielded 0.029 gram of brown amber-colored material that was fairly solid at

room temperature. These acids were not fully studied. The filtrate from the lead salts, which may have contained alcohols, was colorless. It was not studied further.

The residue of wax (R₆) was fractionated with ether to separate sulphur. Repeated slow evaporations of the ether solution yielded small crystals of sulphur, which were picked out with a needle, with the aid of a hand lens, giving in all 0.051 gram of sulphur.

The wax was then subjected to a series of fractionations in different solvents, with tests of each fraction, as shown in table 3. The first fraction of wax represents the portion of R₆ that is relatively insoluble in alcohol but more soluble than in benzene, and similarly for the succeeding fractions.

TABLE 3.—Results of fractionation of the waxlike material (R₆) after separation from sulphur

	Fraction (gram)	Melting point (°C.)	Lieberman's test	Reactivity with alcoholic lead acetate ¹	Properties
Insoluble in benzene but soluble in alcohol.	0.031	-----	-----	-----	Mixture. Some crystalline material.
Soluble in benzene but insoluble in warm ether.	.021	89-----	Slight reddish-brown color.	Small-----	Light-colored and waxlike.
Soluble in warm ether but insoluble in cold ether.	.016	79-81-----	do-----	do-----	Do.
Successively more soluble fractions from cold ether.	.026	70±-----	do-----	do-----	Do.
Do-----	.011	Softened below 100° C. but not clearly melted till 175° C.	do-----	do-----	Strong tendency to threadlike crystals. Practically white.
Do-----	.008	do-----	do-----	do-----	Similar to above.
Do-----	.024	50-60-----	do-----	do-----	Softer, waxlike. A little sulphur present.
Residue-----	.053	Softer or sticky at 25° C.	Fair or somewhat stronger color results. More reddish-greenish than reddish.	do-----	Softer, waxlike, but contains some yellowish-green matter. A little sulphur present.
Total-----	.190				

¹ Test carried out in a similar way with palmitic acid gave a definite precipitate.

The various fractions of wax were tested for cholesterol by the Lieberman test²⁰ and for fatty acids with alcoholic lead acetate, but only small quantities were found. The results of these tests are shown in the fourth and fifth columns of table 3. A wide range of melting point of the waxes is shown. They were not individually identified.

Fractions found in petroleum ether extract

	Gram	Percent of sample
R ₃ . Waxlike material-----	0.190	0.023
R ₆ . Sulphur-----	.051	.006
F ₇ . Oil-like acids soluble in benzene-----	.039	.005
F ₇ . Organic acids insoluble in benzene but soluble in alcohol-----	.029	.004
F ₇ . Chlorophyll fraction (by difference)-----	.024	.003
Total-----	.333	.041

¹⁹ Onslow, M. W., Practical plant biochemistry, p. 32, Cambridge Univ. Press, 1920.

²⁰ Holde, D., op. cit., p. 519.

RESIDUAL ALCOHOLIC SOLUTION

The alcoholic solution (S₁) remaining in the separatory funnel after treatment with light petroleum ether still contained some green material. The mixture was extracted with benzene, yielding two layers; the benzene solution on evaporation left a green residue, E₂, weighing 0.055 gram, which was partly crystalline. On account of its small weight this residue was not studied further.

On evaporating the remaining alcohol to a small volume, a further small quantity of inorganic salt was precipitated, which was added to R₄. The remaining alcoholic solution, which contained considerable organic matter, was evaporated, and yielded approximately 0.30 gram of olive-gray organic matter (R₃) that appeared either dry or moist according to the humidity of the air. This material was thought to be an inorganic salt of an "algin" component. It appeared to be a complex mixture of carbohydrates and other organic matter and some inorganic matter.

When heated directly in a test tube it yielded some volatile tarry or oily matter and left a carbonized residue. Similar material was later found in the alkali extracts. The dried residue was extracted with small volumes of absolute alcohol and yielded 0.0264 gram of insoluble material (R_{13}) and 0.1509 gram of soluble material (F_{14}). These two parts were studied as thoroughly as the rather small quantities would permit.

The insoluble part (R_{13}) was too small to study very thoroughly, but as tests showed that considerable inorganic matter accompanied the organic, a partial analysis was made, with the following results:

Results of partial analysis of inorganic matter in R_{13}

	Percent of fraction	Percent of sediment
SiO ₂ -----	3.7	0.0001
R ₂ O ₃ -----	7.0	.0002
MgO-----	4.7	.0001
CaO-----	4.2	.0001
K ₂ O-----	1.9	.0001
Na ₂ O-----	11.4	.0004
SO ₃ -----	17.9	.0006
Loss on ignition-----	44.0	.0014
Total-----	94.8	.0030

On digesting a portion with hydrochloric acid, evaporating, and extracting with benzene, a soft material resembling a fatty acid was obtained. This material, when dissolved in alcohol, gave a fairly definite precipitate with a solution of calcium acetate in alcohol. This precipitate appears to be one of the fractions in which part of the calcium and magnesium salts of the organic acids became concentrated.

The part soluble in absolute alcohol (F_{14}), which weighed 0.1509 gram, gave the following results:

Results of partial analysis of inorganic matter in F_{14}

	Percent of fraction	Percent of sediment
R ₂ O ₃ -----	5.4	0.0010
MgO-----	1.3	.00024
CaO-----	2.7	.0005
Loss on ignition-----	83.3	.0155
Total-----	92.7	.0194

This fraction was only slightly soluble in benzene, yielding a minute quantity of free sulphur and waxlike material. A small quantity of the dried material, on testing with dilute sodium hydroxide and iodine solution at room temperature, soon gave a persistent odor of iodoform, like that given by levulinic acid. It is thought that this acid or some analogous acid was present in this fraction. Similar material might possibly have been found in portions E_2 , R_6 , and F_7 , if tests had been made.

The same lot of the sediment was heated a fourth time with alcohol, and the alcohol was filtered off hot and evaporated, yielding a further small quantity of inorganic salts and some organic matter. It is unnecessary, however, to give details of these tests, as they yielded results essentially similar to those already described, except that the quantities of material obtained were smaller. Unfortunately lack of time has prevented the determination of the carbon-nitrogen ratios in the different fractions. Such determinations, as Mr. Trask has pointed out in conversation with the writers, would be very desirable for comparison with his results and would help to throw light on the nature of the organic material. Some nitrogen compounds were indeed obtained in the extractions with sodium hydroxide described later, but it was felt that this treatment also caused a partial loss of the nitrogen.

TEST OF THE SALTS FOR ORGANIC ACIDS

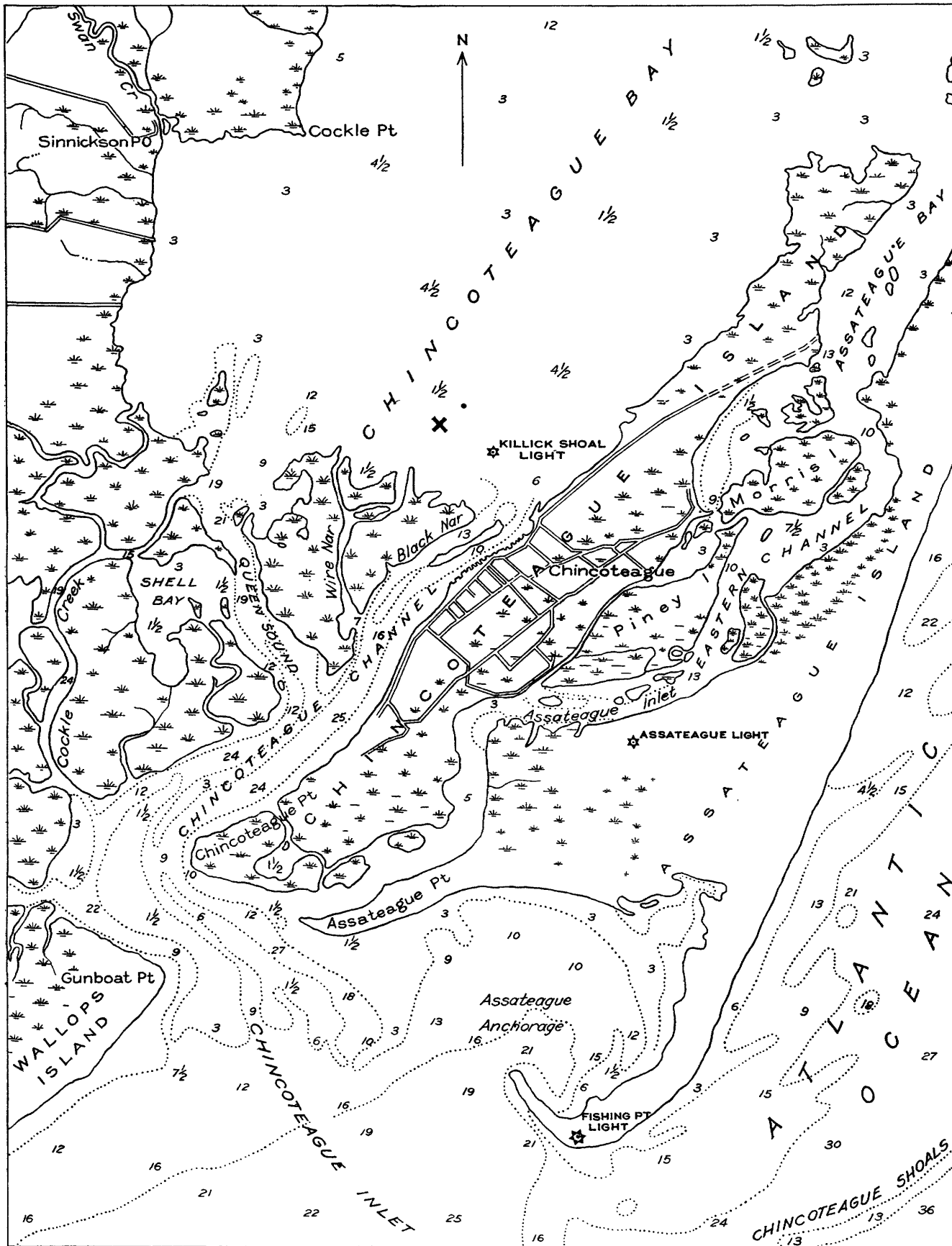
If calcium salts of the fatty acids, for example, were present in the sample, some of them would probably have been dissolved by the large volume of solvent alcohol used, but they would also probably have tended to reprecipitate along with the inorganic salts as the alcohol was evaporated, as the solubility of such salts is generally low. Few exact figures on their solubility are available. Beilstein²¹ gives the solubility of calcium palmitate in alcohol at 20° C. as 0.0103 part per hundred. K. J. Murata, of the Geological Survey laboratory, in studies now in progress, finds 0.0074 part of calcium mucate ($CaC_8H_{15}O_8 \cdot 4H_2O$) per hundred at 25° C.

Two grams of the inorganic salts (R_4) separated by concentration of the original solvent alcohol was heated with 55 milliliters of 10 percent hydrochloric acid and, on cooling, extracted with benzene. Evaporation of the filtered benzene gave a somewhat colored waxlike residue weighing 0.0059 gram. This was treated with 2 milliliters of alcohol, and 0.2 milliliter of the calcium acetate reagent was added to the filtered solution. A precipitate resulted about equal in bulk to that obtained from 1 milligram of palmitic acid. Hence, the quantity of calcium soaps extracted by the alcohol was insignificant compared with the fatty acids found later on treatment with caustic soda (E_1). The writers accordingly conclude that the calcium soaps are held in the sediment in an adsorption complex, from which they are not removed by such a solvent as alcohol but are easily brought into solution by metathesis with the strong alkali.

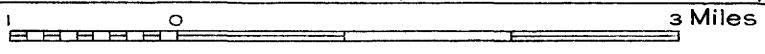
A recent paper by Duncan²² calls attention to the fact that calcium and magnesium salts of certain sulphonated alcohol esters are easily or moderately soluble in water, and hence esters of this type may probably be excluded from consideration as constituents

²¹ Beilstein, F., *Handbuch der organischen Chemie*, 3^e Aufl., Band 1, p. 443, 1893.

²² Duncan, R. A., *The new detergents: Ind. and Eng. Chemistry*, vol. 26, pp. 24-26, 1934.



Base from U.S. Coast and Geodetic Survey charts



MAP OF THE CHINCOTEAGUE BAY REGION, ACCOMAC COUNTY, VA.

of sediments formed in sea water, unless they also should be held in an adsorption complex.

TREATMENT WITH AMMONIA

The residue R_1 , after extraction with alcohol as previously described, was treated five times at about 97° C. with dilute ammonia, in 600-milliliter portions in flasks immersed in a steam bath. Each portion after filtration was slowly evaporated to about 150 milliliters. This treatment resulted in a "reversal" or reprecipitation of dissolved material (R_{10}). Absorption of carbon dioxide from the air may have helped to cause the reprecipitation. The total "reversed" precipitate amounted in all to 0.8641 gram, or 0.31 percent of the sample (811 grams).

The dark-brown ammoniacal filtrate was found to yield a precipitate with sea water. For example, 2 milliliters of the filtrate, on standing 10 days with an excess of sea water, lost its amber-brown color, and a flocculent precipitate appeared in the bottom of the solution.

Table 4 summarizes the results of the extractions with ammonia. The third column gives the weights of the "reversed" precipitate (R_{10}). The next two columns give the material precipitated on neutralization with 25 milliliters of acetic acid, expressed as material insoluble and material soluble in hot alcohol. The last two columns give similar figures for the material remaining in the acetic-acid solution. Extracts 2 and 3 were combined to save time. The terms "1st" and "2d" under "reversed" precipitate refer to successive crops.

TABLE 4.—Results of extraction with ammonia (in grams)

No.	Strength of ammonia (per cent)	"Reversed" precipitate (R_{10})	Precipitated by acetic acid (F_4)		Remainder (F_{11})	
			Insoluble in alcohol (R_{11})	Soluble in alcohol (F_{12})	Insoluble in alcohol (R_{12})	Soluble in alcohol (F_{12})
1	10	{ 0.5264 (1st) .0378 (2d)	0.823	0.269	5.082	1.011
2, 3	10	{ .1583 (1st) .0526 (2d)				
4	15	.0890	.223	.031	.588	.116
5	25					
Total		.8641	1.462	.390	6.671	1.531

¹ The total extract of 0.6414 gram from the fifth treatment was not separated further.

All these fractions contained organic matter, ranging from perhaps 10 to 94 percent, but some also contained considerable inorganic matter. The inorganic matter appeared to be more than would be expected in products of ordinary solubility and must have included some organic compounds, or else the excessive solubility must have been due to the presence of organic matter. Silica was high in some of these extracts, also sulphate.

The two largest portions of the "reversed" precipitate noted in table 4 (0.6847 gram) were partially analyzed, with the following results:

Partial analysis of greater part of "reversed" precipitate

	Percent of fraction	Percent of sediment
SiO ₂	48.9	0.0413
R ₂ O ₃	4.4	.0037
MgO.....	.8	.0007
CaO.....	4.0	.0034
Loss on ignition.....	33.5	.0282
Total.....	91.6	.0773
Soluble in alcohol.....	3.2	.0027
After warming with a little 5 percent HCl, evaporating, and extracting with alcohol.....	24.8	.0209
Ammonia-soluble material in residue.....	8.3	.0070

The material soluble in alcohol after treatment with hydrochloric acid gave a slight precipitate with alcoholic calcium acetate solution. The results were somewhat better if the alcoholic extract was first concentrated to a smaller volume. Blank tests with reagents gave no precipitate.

Similar tests were made of the remainder of the "reversed" precipitate (0.1794 gram) with the following results:

Partial analysis of remainder of "reversed" precipitate

	Percent of fraction	Percent of sediment
SiO ₂	36.4	0.0080
R ₂ O ₃	14.6	.0031
MgO.....	5.4	.0012
CaO.....	3.4	.0008
Loss on ignition.....	39.4	.0087
Total.....	99.2	.0218
Soluble in alcohol.....	1.5	.0003
After warming with a little 5 percent HCl, evaporating, and extracting with alcohol.....	5.9	.0013
Ammonia-soluble material in residue.....	18.6	.0041

The calcium acetate test, when applied to the alcohol extracts, gave results similar to those obtained with the larger fractions. The alcoholic solutions evidently contained other organic matter, in addition to fatty acids. They gave brownish-white precipitates with alcoholic lead acetate. The final ammoniacal extracts noted above were dark, but were not studied further.

The fraction precipitated by acetic acid and insoluble in alcohol (R_{11}) gave the following results:

Partial analysis of fraction R_{11}

	Percent of fraction	Percent of sediment
SiO ₂	1.2	0.0022
R ₂ O ₃	1.2	.0022
MgO.....	Trace	.0000
CaO.....	0.4	.0007
Loss on ignition.....	91.6	.1650
Total.....	94.4	.1701

When examined by the method of Tollens and others,²³ this material appeared to contain pentosans, as it yielded 2.6 percent of methyl pentose and 2.8 percent of pentose. It probably also contained so-called "humic acids."

The fraction precipitated by acetic acid and soluble in alcohol (F_{12}) gave the following results:

Partial analysis of fraction F_{12}

	Percent of fraction	Percent of sediment
R_2O_3	0.9	0.0004
MgO.....	.3	.0001
CaO.....	1.7	.0008
Loss on ignition.....	94.5	.0453
Total.....	97.4	.0466

Fatty acids were definitely obtained from this material by the procedure previously described. The material was low in inorganic matter. It probably also contained pentosans and alcohol-soluble "humic acids."

The remainder of the acetic-acid solution gave 6.671 grams of material insoluble in alcohol (R_{12}). It was analyzed with the following results:

Analysis of fraction R_{12}

	Percent of fraction	Percent of sediment
SiO_2	0.5	0.0042
R_2O_33	.0025
SO_3	22.2	.1826
Cl.....	.5	.0042
MgO.....	.1	.0008
CaO.....	.1	.0008
Na_2O	19.0	.1563
K_2O	3.3	.0271
Loss on ignition.....	51.0	.4195
Total.....	97.0	.7980

Methyl pentose was estimated at 2.1 percent in this fraction and pentose at 4.2 percent. The fraction, though insoluble in alcohol, is soluble in water and is the dominant fraction of organic matter in the sediment. Some sodium sulphate appears to be present. Some marine algae gathered at the time the sediment was collected gave similarly from the dried material 5.9 percent of methyl pentose and 3.7 percent of pentose—a result which strongly suggests that marine algae are a prominent source of organic matter in the sediment. The material in fraction R_{12} forms a precipitate with sea water more slowly than the fraction precipitated by acetic acid (F_{12}). In addition to the pentoses some acids are probably present, though they were not identified. In a similar fraction from

²³ Mayer, Willy, and Tollens, B., Ueber die Fucose und die Bestimmung der methyl Pentosane in Naturprodukten: Jour. Landwirtschaft, Band 55, pp. 261-271, 1907.

soil Shorey²⁴ found saccharic acid $((CHOH)_4(COOH)_2)$, acrylic acid $(CH_2:CHCOOH)$, and some amino acids.

The material of the last fraction (F_{13}) was amber-colored and somewhat sirupy in appearance. That it contained some carbohydrate was shown by the Molisch alpha-naphthol test.

ACTION OF SODIUM HYDROXIDE

FIRST EXTRACTION

As an alternative to the treatment with ammonia use was also made of the more drastic reagent sodium hydroxide. The procedure is outlined in table 5. A separate portion of the original sample weighing 863 grams was extracted with alcohol, as previously described, but beginning with the residue R_1 this material was next heated for a week with about 750 milliliters of 2 percent sodium hydroxide solution in a flask immersed in a steam bath. The neck of the flask protruding from the steam bath was covered with a watch glass to prevent evaporation. The hot solution was filtered off, the residue was washed briefly with water, and the filtrate (F_2), which was dark from organic matter, was made slightly acid with hydrochloric acid. The resulting flocculent precipitate (P_1) was filtered off, washed briefly with water, and transferred while moist to 95 percent alcohol. This mixture was boiled and filtered, thus yielding Odén's "alcohol-insoluble" humic-acid complex (P_2). This material was dried and weighed 0.312 gram. More was obtained from further treatments, as described below, and the weights given in table 5 represent the totals of four extractions (also summarized in table 6).

The alcoholic filtrate of considerable volume from P_2 (F_4), which was also dark from organic matter, was treated with an excess of alcoholic lead acetate, heated on the steam bath, and filtered, yielding the insoluble lead salts (P_3) and a soluble portion (F_7).

The lead salts insoluble in alcohol (P_3) were digested with 20 percent hydrochloric acid, the acid slowly evaporated, and the residue extracted with alcohol. The alcoholic solution was transferred to a porcelain dish and evaporated; the first residue weighed 0.280 gram, and succeeding portions similarly treated gave a total yield of 1.007 grams (R_8). This material, which was not very soluble in benzene, is the analytical equivalent of Odén's "hymetanelansäure", or alcohol-soluble humic acid.

The soluble portion (F_7) was evaporated, digested with 20 percent hydrochloric acid, and extracted with benzene. This procedure left a residue (R_7). The extract (E_1) contained mainly solid fatty-acid material, the first yield weighing 0.0455 gram and the total 0.293 gram. Its treatment is described more fully below.

²⁴ Shorey, E. C., Some organic acid constituents: U. S. Dept. Agr., Bur. Soils, Bull. 88, p. 11, 1913.

TABLE 5.—Scheme of analysis by using NaOH on residue (R_1) extracted with alcohol (table 2)

[Results in grams]

Heat with NaOH 750-ml solution and filter.						
R ₂ . Discard.	F ₂ . Add HCl. Filter.					
	P ₁ . Add to hot alcohol.			F ₃ . Evaporate. Extract with alcohol.		
	P ₂ . Insoluble, 0.648.	F ₄ . Treat with alcoholic Pb(C ₂ H ₃ O ₂) ₂ . ¹		R ₅ . Salts.	E ₂ , 3.87.	
		P ₃ . Decompose with HCl and dissolve in alcohol.	F ₇ . Evaporate alcohol, add dilute HCl, and extract with benzene.			
		R ₈ , 1.007.	R ₇ .			E ₁ . Fatty-acid material, 0.293.

¹ Control experiment: 1 gram of palmitic acid dissolved in 300 milliliters of 95-percent alcohol (heated on the steam bath) gave no indication of precipitation with an excess of alcoholic lead acetate solution. What applies to palmitic acid in this respect very likely applies to other common fatty acids.

The aqueous filtrate (F₃) from the precipitate of humic material, produced on acidifying with hydrochloric acid, was evaporated and the residue extracted with alcohol a few times until the salts were white. The final material soluble in a little alcohol (E₂) weighed 1.80 grams and the total 3.87 grams, as shown in table 6. Practically none of it was soluble in benzene. Its further treatment is also described below with that of the other portions.

ADDITIONAL EXTRACTIONS

The whole extraction with dilute alkali was repeated twice on the same material with 5 percent potassium hydroxide, and a fourth time with 10 percent potassium hydroxide. The yields of the separate fractions are shown in table 6.

TABLE 6.—Yields of fractions extracted from 863 grams of sediment on successive treatments with caustic alkali solutions

Treatment	Humic acid (P ₂)	"Soluble humic acid" (R ₈)	Fatty-acid material (E ₁)	Soluble organic matter (E ₂)
1. 2 percent NaOH	0.312	0.280	0.045	1.80
2. 5 percent KOH	.307	.302	.097	1.58
3. 5 percent KOH	.029	.131	.074	.38
4. 10 percent KOH	Trace	.294	.076	.11
Total	.648	1.007	.293	.387

Probably a stronger alkali would have hastened the extraction somewhat. The final residue after four treatments was light gray when dry and contained very little remaining organic matter, as shown by the fact that on heating some of it in a test tube there was scarcely any detectable odor of organic matter, and no tarry or visible fumes of organic matter appeared.

The same observation was made with the residue remaining from the extractions with ammonia. During the course of the extractions the color of the residues lightened appreciably and the yields of organic matter successively decreased. From these facts it is concluded that practically all the organic matter was removed from the sample.

EXAMINATION OF THE FATTY-ACID MATERIAL (E₁)

Fraction E₁ was examined by the Varrentrap method for the presence of unsaturated liquid and saturated solid fatty acids. The method is directly applicable to mixed free fatty acids.²⁵ The acid material was dissolved in about 25 milliliters of 95 percent alcohol and titrated to neutrality with tenth-normal sodium hydroxide, using phenolphthalein indicator. The alcohol was then largely evaporated, diluted to 50 milliliters with water, and heated, and an aqueous solution of lead acetate was added in excess, which precipitated practically all the fatty-acid material. After filtering, the evaporated filtrate gave a residue consisting of the excess of lead acetate reagent with practically no indication of organic matter. The filtered lead precipitate was carefully dried at low temperature and extracted with ether, which is supposed to dissolve the lead salts of the liquid fatty acids. This separation was apparently not complete, as only 0.0127 gram of liquid acids was obtained out of a total of 0.2925 gram.

Both portions were therefore again combined and then separated into different fractions by fractional precipitation of their magnesium salts with alcoholic magnesium acetate solution, according to the method

²⁵ Fryer, P. J., and Weston, F. E., Technical handbook of oils, fats, and waxes vol. 2, pp. 172-173, Cambridge, 1918.

of Heintz.²⁶ The alcoholic solution was concentrated until an appreciable fraction was precipitated. This was filtered off and the solution concentrated further, giving a second fraction, and so on until seven fractions had been obtained and a final mother liquor remained. Each fraction was then recrystallized from hot alcohol, the filtrate from each fraction being added to the solu-

tion of the next one. A freshly prepared solution of magnesium acetate was used. The several magnesium salts were eventually decomposed with a few milliliters of 20 percent hydrochloric acid, and the free acids were extracted with benzene. The observations made on these acids are given in table 7.

TABLE 7.—Observations on organic acids fractionally precipitated as magnesium salts

Number of fraction	Percent of total fatty-acid material	Character	Further fractionations
1-----	4.7	Solid, light color, melting above 50°	<ol style="list-style-type: none"> 1. The fraction was dissolved in a little alcohol, leaving a small proportion of residue. This was dissolved in hot alcohol, precipitated with $Mg(OAc)_2$, and the precipitate converted into free acid, which melted from 53° to 63°. 2. On adding $Mg(OAc)_2$ to the filtrate an acid was eventually obtained that melted from 68° to 71°. 3. The last filtrate was evaporated to dryness with an excess of $Mg(OAc)_2$. When taken up in a little alcohol the insoluble part yielded an acid that melted from 62° to 64°. The part soluble in alcohol yielded an acid that melted over a wide range below 55°.
2-----	5.6	do-----	<ol style="list-style-type: none"> 1. The fraction was dissolved in a little alcohol, in which a slight insoluble precipitate that was not filtered remained. 2. A partial and direct precipitation with $Mg(OAc)_2$ yielded a light-colored precipitate from which was recovered a little soft free acid material that melted from about 53° to 63°. 3. From a further second precipitation with an excess of $Mg(OAc)_2$ was obtained free light-colored solid acid that melted from 63° to 67°. 4. Filtrate evaporated to dryness with excess of $Mg(OAc)_2$ and taken up with a little alcohol. The insoluble part yielded acid material that melted from 45° to 55°. The acid material from the soluble fraction was somewhat viscous at room temperature.
3-----	7.8	A little soft. Amber color becoming noticeable. Melted from 43° to 48°.	Nos. 3, 4, 5, and 6 dissolved together in a small volume of alcohol.
4-----	15.5	Similar in appearance to no. 3. Melted from 44° to 48°.	<ol style="list-style-type: none"> 1. A little insoluble material was filtered. This material was viscous at 33° and was light straw-colored. On dissolving in a small amount of heated alcohol, a precipitate formed with $Mg(OAc)_2$.
5-----	15.1	Increasing softness and color. Melted below 39°.	<ol style="list-style-type: none"> 2. A first partial precipitation with $Mg(OAc)_2$ yielded light straw-colored acid material that melted from 45° to 48°.
6-----	23.2	Somewhat soft at 33°. Darker amber color than no. 5.	<ol style="list-style-type: none"> 3. A second partial precipitation with $Mg(OAc)_2$ yielded a little darker acid material that melted from 40° to 43°. 4. Further fractionation was obtained from filtrate 3 by evaporation to dryness with an excess of $Mg(OAc)_2$ and taking up with a little alcohol. 4a. The insoluble fraction yielded straw-colored acid material that melted below 37°. 4b. The soluble fraction yielded straw-colored acid material that was soft at ordinary temperature.
7-----	5.8	Somewhat soft at room temperature. Amber-colored.	Dissolved in a small volume of alcohol. Direct precipitate with $Mg(OAc)_2$ slight. After evaporation with an excess of $Mg(OAc)_2$ to dryness, some insoluble material was obtained on taking up in a little alcohol. This yielded viscous acid material that melted below 35°. From the filtrate soft acid material was obtained in which needle markings flowed together.
8-----	22.3	Amber oil-like at 33°. The oil readily flowed together after marking with a needle point.	Dissolved in a small volume of alcohol. Insoluble material slight. A small amount of precipitate formed directly with $Mg(OAc)_2$ which yielded soft viscous acid material. The filtrate yielded liquid oil.

The range in melting point of the fatty acids was thus found to extend from below 37° to 71° and is typical of the fatty acids commonly found as esters in vegetable and mineral oils. Common saturated fatty acids and their melting points are: Stearic, 69.5°; palmitic, 63.5°; myristic, 57.5°; lauric, 47.5°; and

capric, 31.4°. The last two are found in vegetable oils. Although the oils in living matter are generally high in unsaturated constituents, the sediment examined was relatively low in such substances. Thus there seems to be no doubt that fatty acids were identified as such in the final tests and that they were present in the original organic matter in some form insoluble in hot alcohol. With the fatty-acid material there may

²⁶ Holde, D., The examination of hydrocarbon oils and saponifiable fats and waxes, tr. by E. Mueller, p. 378, New York, John Wiley & Sons, 1922.

have been some related acids, as indicated by the low melting points of the first subfractions of fractions 1 and 2 (table 7). Their magnesium salts were less soluble than those of the succeeding fractions of acids having higher melting points. Methods for the identification of such possible compounds, however, will have to await further research.

The portion E₂ was tested for carbohydrates by the Molisch reaction²⁷ with alpha-naphthol, and a distinct positive reaction obtained. The nature of the carbohydrate was revealed further by the aniline acetate test, which showed that some pentose and possibly a little hexose, such as fructose or sorbinose, was present.

A portion of E₂ weighing 0.4168 gram was dissolved in about 25 milliliters of water, 0.25 gram of sodium hydroxide added, and the solution evaporated to

dryness. The material was extracted several times with warm alcohol, which dissolved the remaining sodium hydroxide but very little organic matter. The brown residue, easily powdered, gave 6.3 percent of ash on ignition. Apparently a sodium salt of the material was formed which was insoluble in alcohol, although the material before conversion to the sodium salt was soluble.

Sodium hydroxide has long been used in extracting the organic matter of soils, and a considerable number of compounds have been thus isolated and identified.²⁸ For some constituents, portions weighing 50 pounds were necessary in order to obtain identifiable quantities of the organic compounds. Similar methods might well be applied to sediments.

²⁸ Shorey, E. C., Some organic soil constituents: U. S. Dept. Agr., Bur. Soils, Bull. 88, 1918.

²⁷ Mulliken, S. P., The identification of organic substances, vol. 1, p. 26, New York, John Wiley & Sons, 1904.

