

# PREBIOLOGICAL MEMBRANES: SYNTHESIS AND PROPERTIES

C. E. FOLSOME

*University of Hawaii, Honolulu, U.S.A.*

and

H. J. MOROWITZ

*Yale University, New Haven, Conn., U.S.A.*

**Abstract.** Ultraviolet irradiation of alkanes on aqueous solutions of phosphate and magnesium yields complex structured products which bear many properties similar to simple membranes. The relevance of this product to prebiologic evolution is discussed from the point of view of phase separation.

## 1. Introduction

Abiogenic syntheses under assumed primitive earth conditions of simple organic biological compounds are surprisingly simple and direct. Amino acids, carbohydrates, and related compounds result in high yield from reactions among  $\text{CH}_4$ ,  $\text{NH}_3$ ,  $\text{HOH}$ , and  $\text{H}_2$  subjected to electric discharge (MILLER and UREY, 1959). Nucleic acid constituents and related compounds are obtained from energy driven reactions of  $\text{CN}$  (or  $\text{CH}_4$ ),  $\text{NH}_3$  and  $\text{HOH}$  (ORÓ and KIMBALL, 1961; PONNAMPERUMA *et al.*, 1963; PONNAMPERUMA and KIRK, 1964). Saturated hydrocarbons up to  $\text{C}_{20}$  form from the interaction of electric discharge with  $\text{CH}_4$ ,  $\text{H}_2$ , and  $\text{HOH}$  (WILSON, 1960). These experiments display certain notable common properties. Many of the contemporary ubiquitous biochemical compounds (APPLEWHITE and MOROWITZ, 1966; MOROWITZ, 1968) are formed in at least detectable yields. Also, a requirement for energy exists since the chemical potential of the resultant biochemicals is higher than that of the simpler precursors at equilibrium. The mode by which energy is supplied seems, at this level, not to be highly specific since either electrical discharges, electromagnetic radiation, ionizing radiation, heat, or chemical potential energy can lead to essentially the same results.

The next hierarchical level of prebiologic evolution is less well studied and is concerned with the organization and polymerization of simple biochemical compounds into macro-molecular structures. In experimental practice this level is gradated into the preceding one as ORÓ (1963, 1965) has shown. Polypeptides can be synthesized from dry thermal or phosphate catalyzed mixtures of amino acids (HARADA and FOX, 1960; 1965). Resultant thermally synthesized polypeptides generate spherules when taken into hot aqueous solution, then cooled (FOX *et al.*, 1959; YOUNG, 1965). Again, the *leitmotif* of these experiments is the energy requirement for both polymerization and for more complex structure formation.

To study the stage of the organization of simple biochemicals we require, by analogy with contemporary biology, that a phase separation exist. Such a phase separation immediately generates a multitude of new possibilities. Interior and exterior

reaction rates will differ, products will be localized, shielding of radiation sensitive intermediates or products is likely.

Biological phase separation is accomplished by membranes both within and bounding the cell. We suggest that primitive collections of organic molecules could also obtain boundaries, and thus phase separation, by primitive membranes. The object of this paper is to demonstrate that membrane-like structures can easily be synthesized from alkanes, phosphate, magnesium, and ultraviolet irradiation.

## 2. Methods

*n*-Hexadecane\*, or fuel oil (400–600 °F b.p.) was sterilized by passage through a Millipore GS filter prior to use. 0.01 ml of this alkane was floated onto the surface of 5 ml distilled water contained in an uncovered 60 mm pyrex petri dish.  $\text{PO}_4^-$  as sodium phosphate buffer, pH 7.0, and/or  $\text{Mg}^{++}$  as  $\text{MgCl}_2 \cdot 7 \text{HOH}$  were added to the aqueous phase as required in varying concentrations. Plates were irradiated in a covered box with a General Electric 15T8 15 watt ultraviolet germicidal lamp (predominantly at 2537 Å) 10 mm from the liquid surface for a period of 24 hr. Temperature rise at the liquid surface did not exceed 40 °C.

The product was an oily white scum distributed about the bottom of the petri dish. To harvest the material, excess *n*-hexadecane, or fuel oil, was removed by washing the scum *in situ* with several 5 ml volumes of  $\text{CHCl}_3$ . The residual white fluffy material was then scraped up, suspended in HOH, and washed several times by repeated low speed (2000 g) centrifugation.

Either fuel oil or *n*-hexadecane as the alkane source gave essentially similar products. Results of experiments reported here will be confined to the *n*-hexadecane product.

## 3. Results and Discussion

*Requirement for  $\text{PO}_4^-$ ,  $\text{Mg}^{++}$ , and u.v. irradiation.* A number of dishes each containing 5 ml HOH were prepared. The effect of presence or absence of  $\text{PO}_4^-$ ,  $\text{Mg}^{++}$ , u.v., or *n*-hexadecane was determined. To obtain the fluffy white product all these components were required. Table I summarizes these results.

*Chemical and physical properties of the product.* The density (g/ml) of the HOH washed product was 1.20–1.25 as determined by centrifugation in sucrose solutions of various concentrations.

Elemental analysis of one sample of the washed dried product gave: 16.47% C, 5.33% H, 13.38% P, 14.44% Mg, and 50.38% O. Oxygen was determined by difference.\*\*

A dilute suspension of the product was scanned from 220 mu to 900 mu for major absorbance peaks using a Beckman DK-2A spectrophotometer. No characteristic absorbance peaks were observed.

\* Matheson, Coleman, and Bell, spectroquality.

\*\* Elemental analyses were performed by Truesdail Laboratory Inc., Los Angeles, Calif., U.S.A.

The behavior of the product to various solvents was investigated. The product is insoluble in  $\text{CHCl}_3$ , in 50%–50%  $\text{CHCl}_3$ – $\text{CH}_3\text{OH}$ , 95% ethanol, and acetone. However, the microscopic morphologic properties of the product were destroyed by acetone or alcohol dehydration. In these cases the gross changes involved a change of form from a fluffy white sediment to an amorphous translucent oily mass. Upon resuspension of the mass in water a fluffy white sediment again reformed.

*Morphological properties.* Examination of the washed *n*-hexadecane product under phase contrast, darkfield, and brightfield microscopes showed that the vast majority of the material was composed of refractile spherules arrayed in tiered sheets. The average diameter of a single spherule was 2–4 microns. When acetone or 95% ethanol was added to the product the spherules were noted to coalesce and lose their identity.

TABLE I  
u.v.,  $\text{PO}_4^-$ ,  $\text{Mg}^{++}$ , and alkane requirements

condition	result
all components	white fluffy product
omit $\text{PO}_4^-$	oil droplets, no product
omit $\text{Mg}^{++}$	water soluble ppt.
omit u.v.	oil droplets and slight soluble ppt.
omit alkane	slight soluble ppt.

Reaction mixture: 5 ml d. HOH, 0.04 M phosphate buffer pH 7.0, 0.004 M  $\text{MgCl}_2 \cdot 7\text{HOH}$ , 0.014 M *n*-hexadecane, u.v. – see text, irradiation time – to dryness, about 24 hrs.

Washed spherules were dispersed by brief trituration through a 24 gauge needle and syringe, and then were deposited upon carbon-coated formvar grids and air dried for electron microscopy. Some grids were shadowed at low angle with Pt-Pd, others were negatively stained with 1% phosphotungstic acid, others were untreated. Typical morphologic properties of the product prepared by these modes were shrunken spherules and a wrinkled film which appeared to be of the same composition as the spherules. We stress that the pertinent feature here of interest is the fact that a structured product was observed.

Washed spherules were pelleted, suspended in 3% agar, stained with 0.6% potassium permanganate, dehydrated in ethanol, and embedded in EPON to prepare thin sections for electron microscopic examination. Figure 1 displays the permanganate stained thin sections. Many layers of sheets, or fibrils, are evident. The minimum dimensions of these structures are 35–40 Å, comparable to reconstituted myelin or artificial membranes prepared by other means (KORN, 1966). Not unexpected was the disappearance of the spherules, since it had been found that dehydration converts most of the spherule product into an amorphous mass.

From the elemental composition of the product the mole fraction of each element was directly computed as: 0.126 C, 0.492 H, 0.290 O, 0.039 P, and 0.055 Mg. One interpretation of these data is to assume a C16 skeletal structure and to assign other



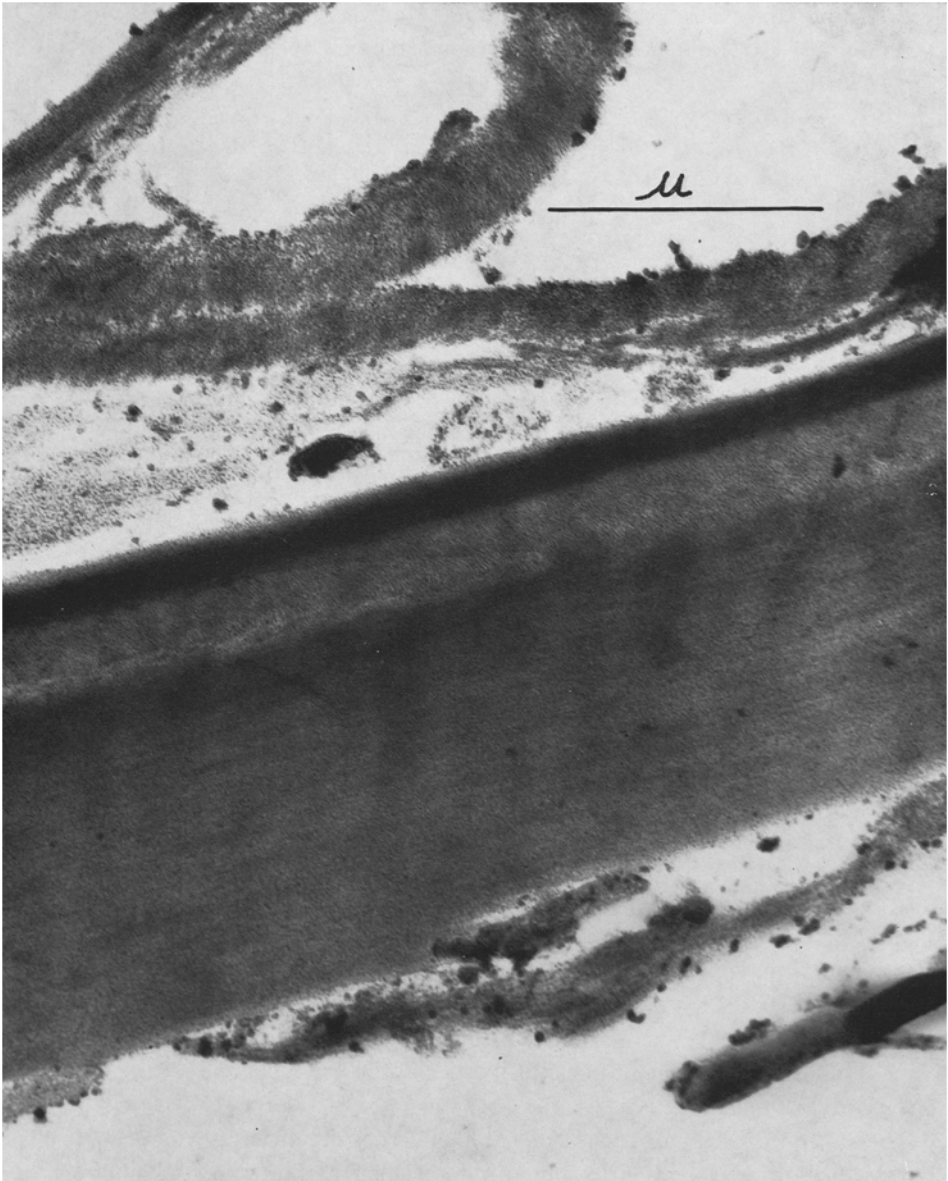


Fig. 1.

The significance of these kinds of structures in the actual prebiological process depends upon the availability of precursors in the primitive environment. The present sea water concentration of magnesium ( $0.04\text{ M}$ ) and of phosphate ( $1.6 \times 10^{-8}\text{ M}$ ) (ALTMAN and DITTMER, 1964) indicates that, given sufficient alkanes, most will be monophosphorylated, and may be complexed by divalent cations. A point in favor

of our suggestions would be to demonstrate the presence of large amounts of alkanes in prebiologic times.

WILSON's experiments (1960) upon electric discharge synthesis of hydrocarbons from reactant mixtures of  $\text{CH}_4$ ,  $\text{HOH}$ , and  $\text{H}_2$  demonstrate that alkanes up to  $\text{C}_{20}$  can be easily formed under assumed prebiologic conditions.

TABLE II  
Effects of lowered  $\text{PO}_4^-$  and  $\text{Mg}^{++}$  concentrations

Experiment	M concentration of:			Product
	<i>n</i> -hexadecane	$\text{PO}_4^-$	$\text{Mg}^{++}$	
I	0.014	0.04	0.004	white powder, spherules, density 1.2-1.25
II	0.014	0.02	0.002	white powder, spherules, density 1.0-1.15
III	0.014	0.01	0.001	white powder, spherules, insufficient material for density determination
IV	0.014	0.005	0.0005	same as III except very low yields

In addition to this mode of hydrocarbon formation, a second, perhaps coincident, source is hydrocarbons derived from carbides and water during the early stages of formation of this planet. ANDERS (1961, 1962) provides some evidence in favor of this suggestion which was originally put forth by BERTHELOT (1886) to account for the genesis of petroleum (MENDELEJEFF, 1877; MOISSAN, 1896).

Mechanisms do exist which appear sufficient to provide hydrocarbons for the formation of prebiologic membranes similar to those investigated here. The existence of some alkanes in the prebiologic environment is a logical presumption.

The significance of the membranous spherules is clear: a phase separation delineating interior from exterior has been created. Phase separation in an Oparin ocean subjected to u.v. irradiation can lead us to the stage where we can profitably investigate events at the next hierarchical stage beyond the synthesis of low molecular weight compounds.

### Acknowledgements

This work was supported by grant GB-4108 from the National Science Foundation, and by the University of Hawaii.

We thank Dr. Hans Hohl and Suzan Hamamoto for their preparation of the electron micrographs.

### References

- ALTMAN, P. L. and DITTMER, D. S. (eds.): 1964, *Biology Data Book*, Fed. Amer. Soc. Exptl. Biol., Wash., p. 539.  
ANDERS, E.: 1961, *Proc. Lunar Planetary Explor. Colloquium*, vol. II (4), 55.

- ANDERS, E.: 1962, *Ann. N.Y. Acad. Sci.* **93**, 649.
- ANDERS, E.: 1963, *Ann. N.Y. Acad. Sci.* **108**, 514.
- APPLEWHITE, P. and MOROWITZ, H. J.: 1966, unpublished studies on ubiquitous biochemical compounds.
- BERTHELOT, M.: 1866, *Compt. Rend.* **62**, 949.
- FOX, S. W., HARADA, K., and KENDRICK, J.: 1959, *Science* **129**, 1221.
- HARADA, K. and FOX, S. W.: 1960, *Arch. Biochem. Biophys.* **86**, 274.
- HARADA, K. and FOX, S. W.: 1965, in *Origins of Prebiological Systems* (ed. by S. W. Fox), Academic Press, N.Y., p. 289.
- KORN, E. D.: 1966, *Sci.* **153**, 1491.
- MENDELEJEFF, D.: 1877, *Ber. Deut. Chem. Gesell.* **10**, 229.
- MILLER, S. L. and UREY, H. C.: 1959, *Science* **130**, 245.
- MOISSAN, H.: 1896, *Compt. rend.* **122**, 1462.
- MOROWITZ, H. J.: 1968, *Energy Flow in Biology*, Academic Press, N.Y.
- ORÓ, J. and KIMBALL, A. P.: 1961, *Arch. Biochem. Biophys.* **94**, 217.
- ORÓ, J.: 1963, *Ann. N.Y. Acad. Sci.* **108**, 464.
- ORÓ, J.: 1965, in *Origins of Prebiological Systems*, (ed. by S. W. Fox), Academic Press, N.Y., p. 137.
- PONNAMPERUMA, C., LEMMON, R. M., MARINER, R., and CALVIN, M.: 1963, *Proc. Nat. Acad. Sci.* **49**, 737.
- PONNAMPERUMA, C. and KIRK, P.: 1964, *Nature* **203**, 400.
- WILSON, A. T.: 1960, *Nature* **188**, 1007.
- YOUNG, R. S.: 1965, in *Origins of Prebiological Systems* (ed. by S. W. Fox), Academic Press, N.Y., p. 347.