ROLE OF AMPHIPHILIC COMPOUNDS IN THE EVOLUTION OF MEMBRANE STRUCTURE ON THE EARLY EARTH

DAVID W. DEAMER
Department of Zoology, University of California, Davis CA 95616, U.S.A.

(Received 26 September, 1985)

Abstract. A variety of amphiphilic compounds have the capacity to self-assemble into membranous structures in the form of bilayers. The earliest cellular organisms must have incorporated such compounds into boundary membranes, and this review discusses amphiphilic components of the prebiotic environment which would be candidates. One possible source is organic material carried to the earth's surface by meteoritic infall. To test this, we have extracted and analysed non-polar substances from the Murchison carbonaceous chondrite, and found that at least some of the components can produce boundary structures which resemble membranes. This observation suggests that membranous boundary structures were present on the early earth, and available to participate in the origin and evolution of the first cellular forms of life.

1. Introduction

As we have gained increasing insight into the biochemical components of contemporary cells, the ability of such molecules to self-assemble into supramolecular systems has emerged as a basic principle underlying cell structure and function. The primary forces driving self-assembly are the hydrogen bonding and ionic interactions which stabilize nucleic acid and protein structure, and the hydrophobic effects which stabilize the association of non-polar molecules such as lipids. The end result of these highly evolved self-assembly processes is the living cell. It follows that our knowledge of the origin of life will ultimately involve an understanding of how organic molecules available on the prebiotic earth could assemble into protocellular structures, most probably through the same physicochemical forces at work in contemporary cells.

One such structure defines all cells. This is the membranous boundary composed of lipid and protein which divides the intracellular substance from the external environment. Within that boundary are contained the systems that characterize the living state: information processing macromolecules which can also replicate their information content, catalytic macromolecules whose synthesis is coded by that information, and regulatory systems which control the rates of these interactive processes. Most of the above systems are also organized in space by membranes, and often require the membrane environment in order to function. Although most research on the origin of life has properly emphasized the replicating/catalytic systems, in the absence of membranous boundary structures, cells could not exist. It is therefore appropriate to investigate the origin and evolution of boundary structures as well.

The objective of this review is to discuss the chemical and physical processes that permitted membranous structures to be produced from the organic components

Origins of Life **17** (1986) 3–25.

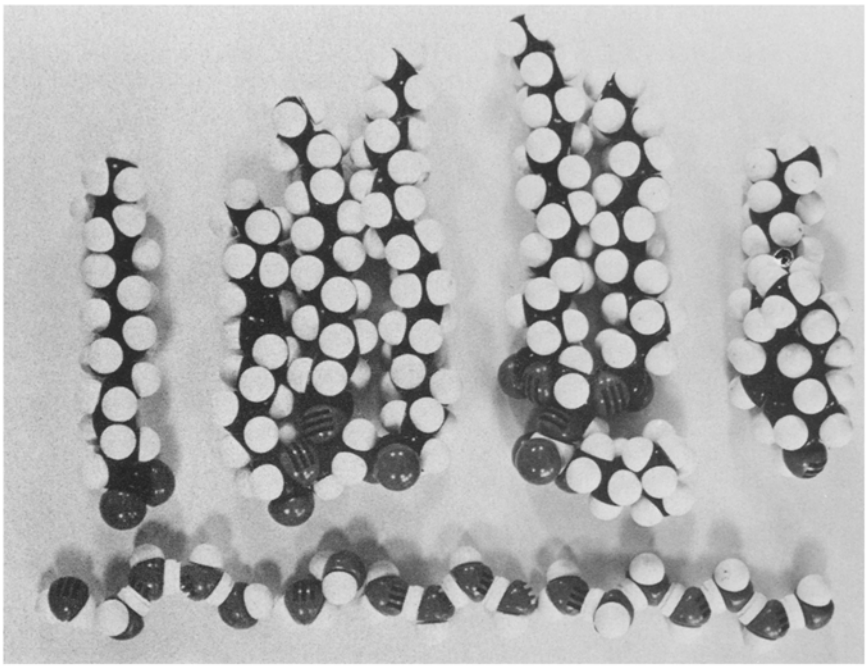

Fig. 1. Several common biological lipids are illustrated, including (from left to right) a fatty acid, a triglyceride, a phospholipid and cholesterol. Most compounds defined as lipids have an extensive hydrophobic hydrocarbon region and a hydrophilic head group, and are therefore called amphiphiles. These properties produce surface activity, and the lipids are shown here as a loose monolayer at the air-water interface. (Normally the polar groups would be dissolved in the aqueous phase, and the hydrocarbon chains would be tightly packed. They are separated here for illustrative purposes.)

available on the early earth. We will also speculate on how such structures could have become organized into protocellular systems. In particular, we will focus on certain lipid-like molecules called amphiphiles that have the ability to self-assemble into boundary structures which form closed microenvironments. The main questions to be addressed are the following:

(1) What physical and chemical properties permit the self-assembly of certain molecules into membranous boundary structures?

(2) What are the most plausible components of the prebiotic environment that might be available for assembly into the earliest membranes?

(3) How could macromolecular systems involved in early life processes become encapsulated in membrane bounded environments?

2. Properties of Amphiphilic Molecules

Amphiphiles are molecules with substantial non-polar portions and one or more polar groups. Lipids are biological amphiphiles, and examples include fatty acids,

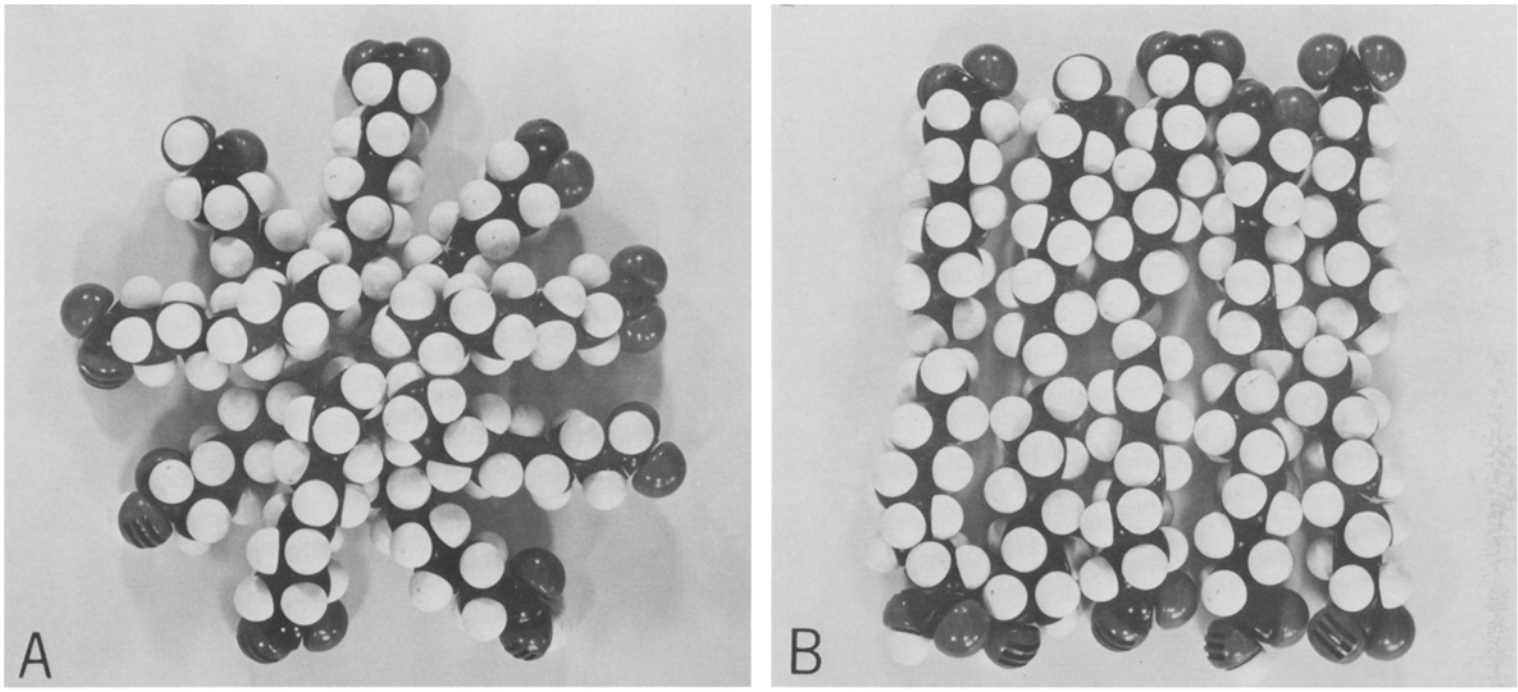

Fig. 2. Above the critical micelle concentration, single chain amphiphiles such as fatty acids typically produce micelles in which hundreds of molecules are associated with their hydrocarbon chains directed inward and the hydrophilic head groups at the aqueous interface. Only a few molecules are shown as a cross section through a small micelle (A). At sufficiently high concentrations and appropriate conditions of pH and temperature, many fatty acids and other single chain amphiphiles produce bilayer structures (B). Double chain amphiphiles like phospholipid typically form bilayers, rather than micelles.

glycerol esters of fatty acids, sterols and phospholipids (Figure 1). Because of the presence of polar and non-polar components on the same molecule, amphiphiles have remarkable properties of self-assembly. One such property common to all amphiphiles is surface activity. That is, amphiphiles tend to accumulate at air-water interfaces with the non-polar groups extending into the air and the polar (hydrophilic groups) interacting with the water. This self-assembly process results in monomolecular structures referred to as monolayers. Surface activity is relevant to our later consideration of the origin of boundary structures, since it provides a concentrating mechanism largely unavailable to other kinds of biomolecules. This was recognized in early papers by Goldacre [1] and Shah [2] who proposed mechanisms by which prebiotic membranes could be produced from monolayers of amphiphilic substances.

Micelles represent a second self-assembly structure of amphiphiles (Figure 2). Above a certain concentration (the critical micelle concentration) some amphiphiles form aggregates of defined size which are in equilibrium with single molecules in solution. The third self-assembly structure of amphiphiles can be thought of as a micelle extended in two dimensions, and appears when the concentration of an amphiphile in solution is increased to the point where micelles can no longer remain stable, and begin to fuse. The result is a multilamellar structure composed of bilayers (Figure 2), or, more rarely, a hexagonal array of amphiphile cylinders. Although single chain amphiphiles like fatty acids can form stable bilayers only at relatively high concentrations, double-chain amphiphiles such as phospholipids have extremely low critical micelle concentrations and form bilayers at all practical concentrations. This property is essential to formation of stable membranes, and will be discussed in detail later.

The physical properties of amphiphilic self-assembly structures are modulated by a number of factors, including temperature, concentration of the amphiphile, chemical composition of the non-polar moiety, and the polar groups and their interaction with the ionic components of the medium. An important consideration here is the stability of the bilayer itself, which in turn is related to the relative solubility of its components. Typically, the hydrocarbon chains of membrane lipids must be 12 carbons or longer in order to produce stable bilayer structures. Chain lengths of 10 carbons or less tend to be present as micelles, rather than bilayers. It follows that if early membranes assembled from typical amphiphiles derived from hydrocarbon chains, a source of relatively long chains must have been available.

A second basic property cell membranes is the relative fluidity of the bilayer structure, and this is also affected by chain length and conformation. For instance, in the biological temperature range, lipids containing normal saturated hydrocarbon chains of 16 carbons or more form relatively solid structures, while chains of 14 carbons or less are 'melted' or fluid. Essentially all cell membranes function only in the fluid state, and therefore some mechanism must exist to introduce fluidity. This is most commonly provided by chain conformation. For instance, when phosphatidylcholine contains saturated C18 chains, its phase transition occurs at

60 °C, but a single cis double bond in each chain lowers the phase transition temperature to −22 °C. Chain branching has much the same effect.

These two properties – chain length and chain conformation – are central to our consideration of the origin of membrane structure. Stable and fluid bilayer structures are essential to contemporary cell function, and there is no reason to expect that similar constraints would not also operate in the earliest cells. It follows that we must search for plausible mechanisms by which amphiphilic molecules with hydrocarbon chains at least 14 carbons long could be synthesized. Furthermore, the resulting membrane must be fluid. If the ambient temperature were sufficiently high – say, 50–60 °C – straight chains up to 18 carbons long would be fluid, while at 40° 16 carbon chains would be fluid. At lower temperatures, fluidity could be produced in several ways. Chain branching is the simplest, and in fact is used by primitive microorganisms today such as the archaebacteria. Alternatively, admixtures of other amphiphiles also produce increased fluidity. For instance, cholesterol addition to phospholipid bilayer membranes markedly lowers the phase transition temperature [3]. Early membranes were probably not pure lipid systems, and if the ambient temperature was relatively high the first membrane boundary structures would probably be sufficiently fluid. A more serious problem is to establish mechanisms that could produce hydrocarbon chains long enough to form stable membranes.

3. Structure and Function in Contemporary Membranes

In recent years there has been a remarkable expansion of our knowledge of membranes as basic structural units in cells. It has always been clear that the outer or plasma membrane of cells differentiates the external from the internal environment and provides a selective diffusion barrier which actively or passively permits useful solutes to pass, but essentially excludes all others. It is now understood that the primary barrier property arises from the lipid bilayer moiety of membranes. The presence of a barrier permits gradients of various solutes to be maintained by enzymatic transport systems. Such gradients are essential for cell function, an obvious example being the proton gradient which mediates chemiosmotic energy transduction and synthesis of ATP [4]. A second example is the sodium-potassium gradient necessary for electrical activity in nerve cell membranes. These gradients are transformed into cell function through specialized proteins embedded in the fluid lipid bilayer, and the term 'fluid mosaic' has been accepted as a useful general term describing biological membrane structure [5]. Some of the proteins act as channels for the passive or facilitated diffusion of solutes, example being the potassium channel of the axon, and the glucose carrier protein of most membranes. Other membrane proteins are transport enzymes, specifically using the energy of ATP hydrolysis to drive active ion translocation. Examples include the sodium-potassium pump of most membranes, the calcium pump of sarcoplasmic reticulum, and the proton pump of lysosomes, secretory vesicles and gastric mucosa.

Lipid bilayers act as an organizing matrix for certain enzymes that require a non-polar environment, for instance, the cytochrome system mediating electron transport in mitochondrial membranes. They also provide a site for the energy transducing pigment systems of photosynthetic membranes which bring chemical energy into the biosphere. Finally, they have the general function of organizing many cellular enzymes into a two-dimensional space, thereby enhancing the efficiency of linked biochemical reactions.

To summarize, the lipid bilayer is an essential component of all contemporary cells. It is therefore appropriate to ask how bilayer membranes could have been produced on the prebiotic earth, and to consider how such structures might have contributed to the organization and function of early cellular life. Over the past decade, numerous laboratories have focused on the properties of lipid bilayers prepared as liposomes. During the course of this work, it became apparent that liposomes could also provide a useful system for investigating membranous microenvironments relevant to prebiotic evolutionary processes.

4. Formation and Properties of Liposomes

Bangham and co-workers reported in 1965 that phospholipids were able to assemble into vesicular structures which encapsulated an aqueous phase [6]. The term liposome was first used in 1967 to describe any vesicle produced from lipid [7] and the remarkable properties of liposomes have since led to their wide application as model membrane systems. The first liposomes were produced by hand shaking a dried lipid film with an aqueous phase. As the water penetrated the dried film and hydrated polar head groups in the layers of lipid, the lipid first swelled, then broke up to form cell-sized multilamellar vesicles (Figure 3). Later research established that more energetic dispersal of the hydrated lipid by sonication produced smaller vesicles 30–50 nm diameter formed from a single bilayer of lipid. Larger unilamellar vesicles could be produced as well by a variety of methods such as detergent dialysis or solvent evaporation. (For reviews see [8, 9]). One of the most important observations arising from liposomes and other lipid bilayer model membrane systems is that in comparison with biological membranes, lipid bilayers are orders of magnitude less permeable to ions such as sodium or potassium. This relative impermeability arises from the energy barrier that must be overcome when a hydrated ion migrates from an aqueous phase into a non-polar phase as it crosses the bilayer. If bilayers represent the primary barriers to free diffusion of ions in biological membranes, ion specific permeability must arise from specialized defects in the bilayer. As noted earlier, it is now understood that both active and passive ion transport across membranes are mediated by proteinaceous transmembrane channels and transport enzymes.

In summary, the properties of liposomes are clearly relevant to the origin of membrane structure. First, liposomes are readily produced by conditions which would have been common on the early earth. If amphiphilic molecules could be produced through chemical evolution, and were then exposed to aqueous phases,

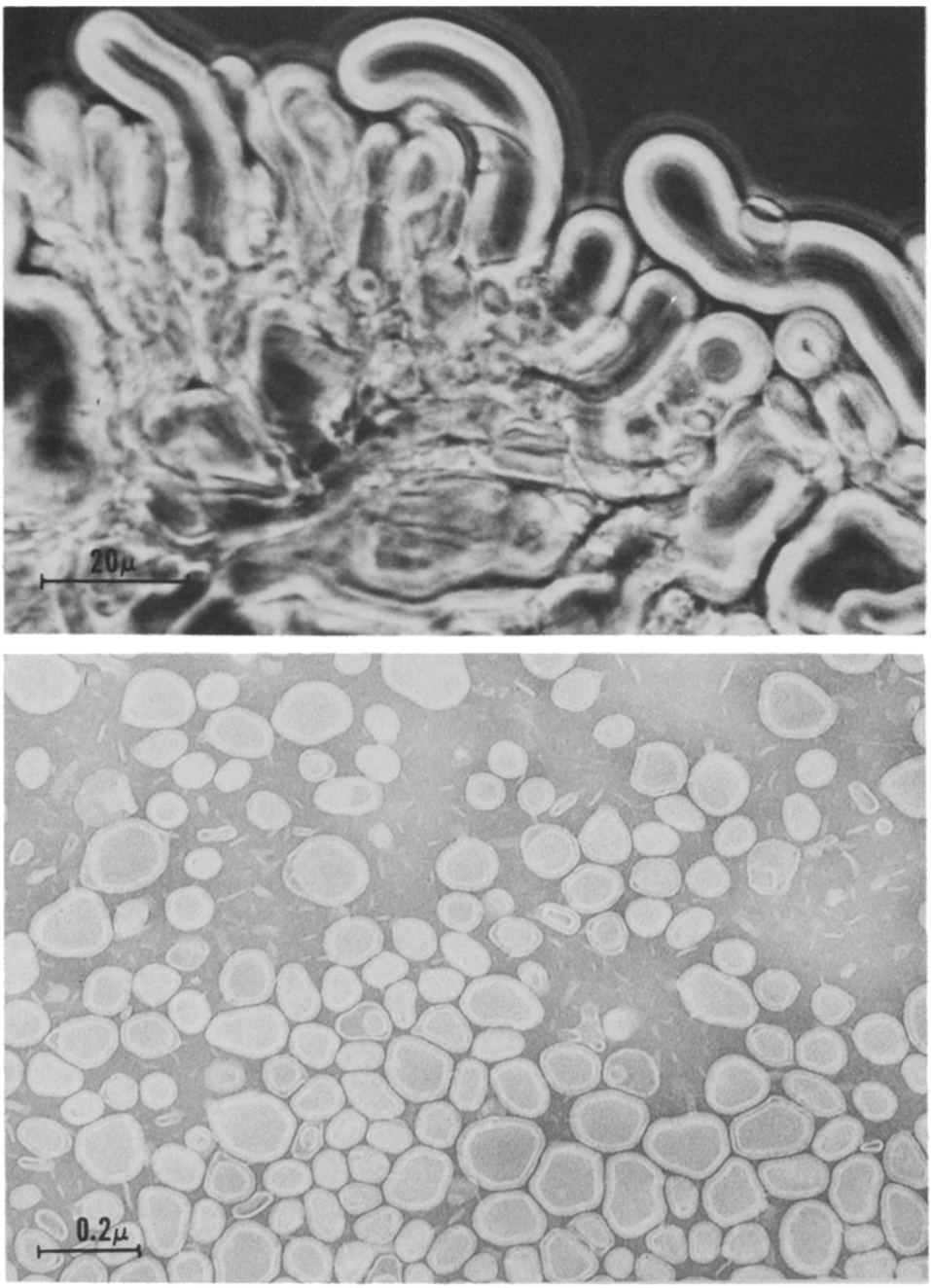

Fig. 3. When phospholipids interact with aqueous phases, their amphiphilic properties cause them to self-assemble into bilayer structures. At the light microscopic level, the bilayers are seen as multilamellar structures such as the myelin figures shown here. Each cylinder is composed of thousands of concentric bilayers. If such structures are dispersed by agitation, for instance, by sonication smaller unilamellar vesicles are produced which can be visualized by electron microscopic methods. A preparation of such liposomes is shown as a negative stain in the bottom micrograph.

they would be present as membranous vesicles. Furthermore, if such membranes were dried in the presence of a solute, the solute would become encapsulated in the resulting vesicles upon rehydration. If the solute happened to be a crude system of replicating molecules associated with catalytic molecules, protocellular structures would result. Finally, if non-polar pigment molecules had evolved as well, they would partition into the hydrocarbon phase of the membranes and could potentially function to trap light energy.

The properties of liposomes also point to a number of significant difficulties in understanding the origin of membranes. First, it is not obvious that sufficient amounts of amphiphilic compounds would have been available through known abiotic synthetic processes, or that their non-polar moiety would be large enough to permit membrane formation, rather than micelles. Second, although simple lipid-like molecules such as single-chain hydrocarbon derivatives are fairly stable, more complex lipids formed through ester linkage formation break down with half times measured in days to weeks. The prebiotic environment would be chemically energetic, and it follows that membranes were either produced from relatively simple amphiphiles, or that some mechanism was available that continuously synthesized complex lipid-like amphiphiles. Finally, the fact that bilayers are relatively impermeable also tends to limit their usefulness: how might the membranes permit replicating molecules to have access to monomers necessary for growth of the system? Or assuming that some transport mechanism evolved, how would a membrane-enclosed replicating system be able to divide when the growth process reached the limits of the enclosed volume? Each of these questions deserves careful attention in any model of protocellular evolution.

5. Simulations of Early Membrane Formation

The first question we will address concerns the minimal conditions required for boundary membranes to be produced. In early studies, Gebicki and Hicks [10] recognized the significance of this question, and reported that membranous vesicles could be produced under certain conditions by oleic acid, a single chain 18 carbon amphiphile with one cis double bond at the 9–10 position. The primary condition for stability was that the pH of the solution needed to be adjusted to near 8.5, where the oleic acid exists as its 'acid soap' in which half the molecules are present as the acid, and half as carboxylate anion. In later work [11] we extended this basic observation to other fatty acids and to alkyl sulfates and alkyl phosphates with admixtures of fatty alcohols. It became apparent that bilayers of these compounds self-assemble when the following conditions are met:

(1) The minimal carbon chain length for bilayer formation is 10–12 carbons

(2) The amphiphile must be above its phase transition temperature

(3) The conditions must permit formation of hydrogen bonded complexes which resemble double-chained amphiphiles containing charged head-groups.

Oleic acid is representative of these conditions. It is an 18 carbon chain with a carboxyl group at one end, and a cis double bond at the 9–10 position. This 'kink' in the chain prevents close packing of the hydrocarbon chains, and the oleic acid is therefore fluid throughout the biological temperature range. Oleic acid also illustrates the formation of hydrogen bonded complexes. For instance, if oleic acid is entirely titrated to the charged anion in an aqueous dispersion (pH > 9) only micelles form, and if it is entirely in the form of the neutral acid (pH < 7) droplets are produced. The acid soap which forms at pH 8.5 provides intermolecular hydrogen bonding opportunities between the neutral molecule and the carboxylate anion, and the resulting complex carries a negative charge. Another example is sodium dodecyl sulfate, an anionic detergent which by itself forms micellar solutions, but readily produces membranes when admixtures of dodecyl alcohol are introduced. The dodecyl alcohol apparently can form hydrogen bonded complexes with the sulfate oxygens of the detergent, and the resulting vesicles trap dyes like carboxyfluorescein for periods up to several weeks.

The fact that membranes produced by single chain amphiphiles are stable only under a narrow range of conditions suggested that more complex chemical structures might have evolved to provide stability for early membrane structures. This led to our second question, which concerns the possible synthesis of more complex lipids under simulated prebiotic conditions. To this end, Hargreaves *et al.* [12] demonstrated that fatty acids and glycerol readily form ester bonds under mild conditions of dehydration (drying under nitrogen at 60 °C for one week) to produce mono-, di-, and tri-glycerides. Furthermore, in the presence of phosphate, fatty acid and cetyl alcohol, phospholipids were produced which could form stable lipid bilayer membranes. Oro and co-workers [13, 14] independently obtained similar results and furthermore demonstrated that in the presence of a condensing agent such as cyanamid or imadazole, phosphatidic acids could be produced in yields approaching 50%. (See [15] for review.)

These observations suggested that membranous structures were probably available on the primitive earth in the form of lipid bilayers assembled from fatty acids and their reaction products. The next question concerned how such structures might interact to form microenvironments that would be able to potentiate the ability of replicating systems to become organized into the first microorganisms. We addressed that question by investigating possible mechanisms by which macromolecules such as nucleic acids could become encapsulated in membrane bounded environments under simulated prebiotic conditions [16]. In this work, dispersions of lipid were mixed with a solute such as DNA and then dried, the analogy being a tide pool going through cycles of drying and rehydration. We found that under these conditions the lipid membranes fused to 'sandwich' the solute, and upon rehydration the membranes were dispersed again in the form of vesicles, but now with the solute encapsulated (Figure 4).

In summary, it is clear from laboratory simulations that a variety of amphiphilic molecules are able to form membrane structures which in turn can encapsulate

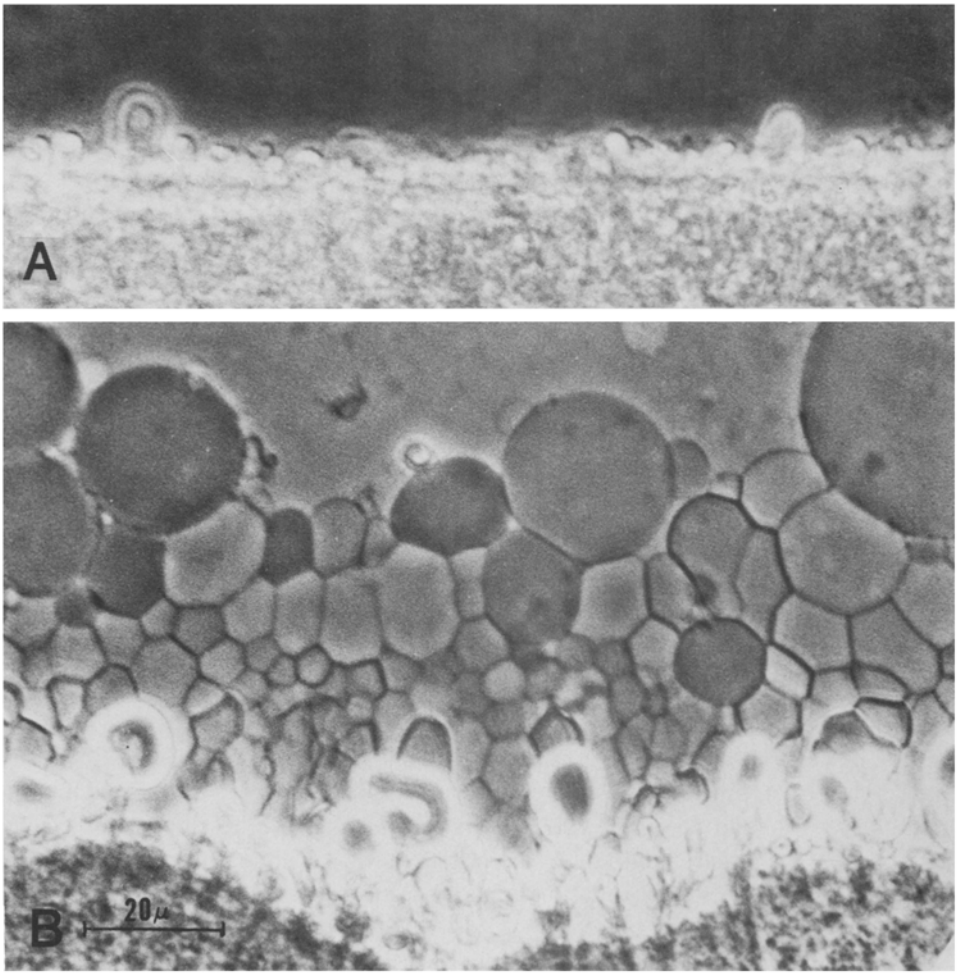

Fig. 4. If lipid vesicles are dried in the presence of solute, as might occur in a cycling tide pool environment, the vesicles fuse to form multilamellar structures which 'sandwich' the solute. Upon rehydration, new vesicles form which encapsulate the solute. The micrographs show such a system with hemoglobin as a solute just after water was added (A) and a few minutes later (B).

macromolecules. We can now go on to investigate possible lines of chemical evolution that would link the primitive mixtures of organic components to the emergence of useful membrane structures available for early life forms.

6. Lipid-Like Molecules on the Prebiotic Earth

Perhaps the most convincing evidence supporting the presence of amphiphilic compounds in the prebiotic environment came from the discovery that carbonaceous

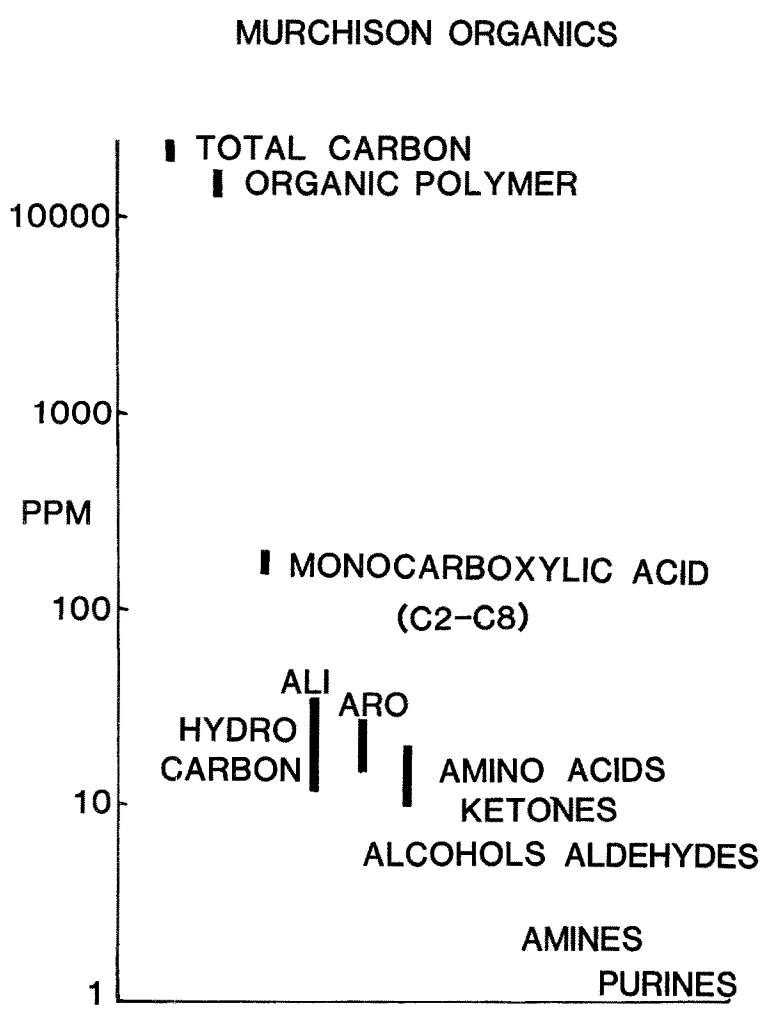

Fig. 5. The organic content of the Murchison carbonaceous chondrite is represented here. Bars represent ranges of data where available. Drawn from Table 7 in [21].

chondrites contain a rich array of organic molecules (Figure 5). Carbonaceous chondrites apparently represent preserved samples of compounds synthesized by abiotic processes in the early solar system [17, 18, 19, 20, 21]. The detailed composition of this complex mixture is still under investigation, and varies considerably among classes of chondrites [22, 23]. The main organic component is a relatively insoluble substance generally referred to as organic polymer. It composes up to 70% of the total carbon in some chondrites, and resembles kerogen in its chemical composition. Organic polymer can be dissolved by rigorous chemical and physical treatment, and when analysed by acid hydrolysis and chromatographic methods is found to consist primarily of polycyclic aromatic sub-units [24].

The minor components of the chondrites are also of considerable interest. These include a series of alkanes and hydrocarbon derivatives such as fatty acids. Long chain hydrocarbon derivatives are a basic component of lipid molecules in contemporary organisms, and were presumably involved in the earliest membrane structures. Furthermore, because of their surface activity, fatty acids and other long chain amphiphiles have an important and ubiquitous concentrating mechanism which would permit them to associate as surface films.

Early reports of long chain fatty acids in chondrites were shown most likely to be due to terrestrial contamination [25]. However, in a recent study, Lawless *et al.* [26] found that fatty acids up to 8 carbons long could be demonstrated in hydrolysed aqueous extracts of interior samples of the Murchison meteorite. The total fatty acid content was in the range of 100–200 ppm.

Is there any meaningful relationship between the organic content of carbonaceous chondrites and the possible components available on the early earth? Clearly we must be cautious. The best evidence suggests that the organic compounds in chondrites were synthesized by at least three separate processes, even including interstellar origin [27]. Furthermore, the chemical mechanisms capable of producing long chain hydrocarbons are still not clear, although several investigators have proposed that Fischer–Tropsch reactions could account for at least some of the synthesis [28, 29, 30]. For instance, Nooner et al. [30, 31] showed that a Fischer–Tropsch synthesis catalysed by meteoritic nickel-iron was able to produce a series of fatty acids up to 21 carbons long, the relative abundance of which resembled that of the Murchison meteorite.

On the other hand, the organic constituents of the carbonaceous chondrites represent the only valid sample we have of the kinds of compounds which were synthesized in the early solar system. Since there is no particular reason to exclude the components as possible constituents of the early earth's environment, it seems reasonable to assume that some mechanism was available which produced significant amounts of hydrocarbons and their derivatives, and that these were in continuous interaction with early lakes and oceans, particularly in intertidal zones. We will further assume that the hydrocarbon content of carbonaceous chondrites represents a useful model of the kinds of non-polar compounds available for chemical evolution on the prebiotic earth.

7. Amphiphilic Compounds in the Murchison Carbonaceous Chondrite

The results to be discussed below were described in an earlier paper [32] which investigated amphiphilic compounds present in carbonaceous meteorites, and asked whether such components are capable of self-assembly into boundary structures. Two carbonaceous chondrites, the Murchison and the Allende, were extracted in solvent systems which would be expected to solubilize amphiphiles. The Murchison chondrite is relatively rich in organics, while the Allende chondrite has little organic content and therefore served as a control for possible contamination introduced by

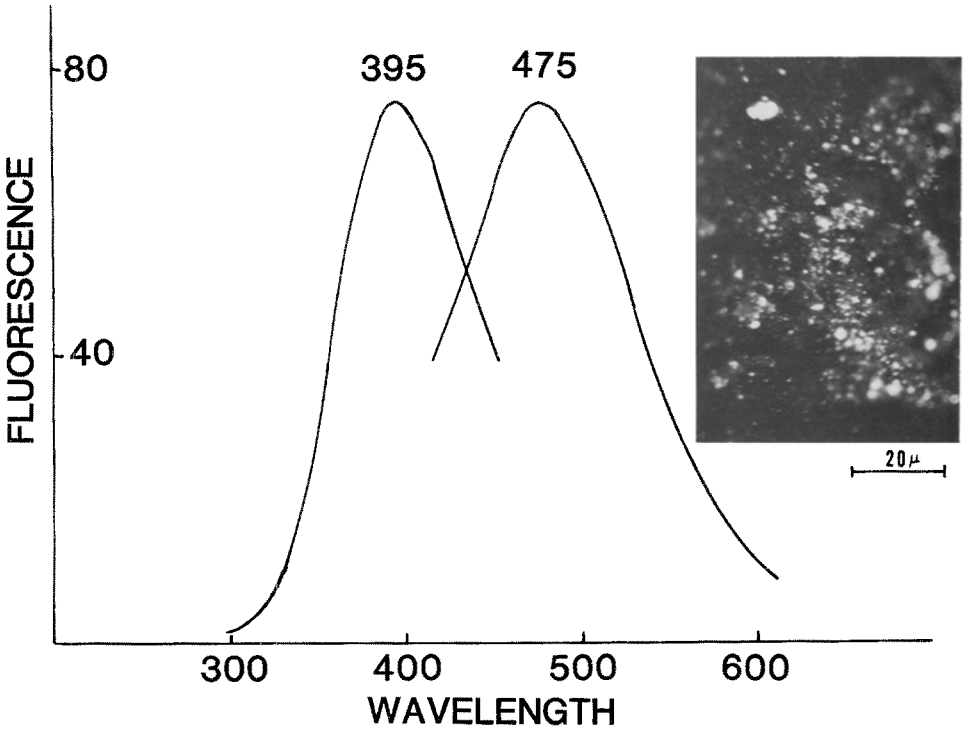

Fig. 6. Fluorescence excitation-emission spectrum of non-polar components extracted from the Murchison meteorite. The insert shows the distribution of fluorescent material in a fracture surface of the meteorite itself, as visualized by epifluorescence microscopy.

the extraction procedure. The solvent systems contained both polar and non-polar phases in order to permit partitioning of organic components between the two phases. The material soluble in the non-polar phase (chloroform) was studied with infrared and fluorescence spectrophotometry, acid-base titration, and light and electron microscopy. Surface pressure isotherms were obtained as well.

The non-polar extracts had a distinct yellow color when dissolved in chloroform, and were fluorescent, with excitation-emission peaks at 395 and 475 nm, respectively (Figure 6). It was important to show that this material was indigenous to the meteorite, and not introduced by the extraction process. Therefore fracture faces of the Murchison meteorite were examined by epifluorescence microscopy immediately after fracture, and following gentle smoothing by a diamond polishing disk. Both fractured and smoothed surfaces showed numerous yellow fluorescent particles in the micron size range (Figure 6, inset) as well as larger structures with deep red fluorescence, presumably calcite. Only the yellow fluorescent particles were substantially reduced in number following a brief chloroform rinse, and presumably represent the *in situ* appearance of the organic components of the Murchison chondrite.

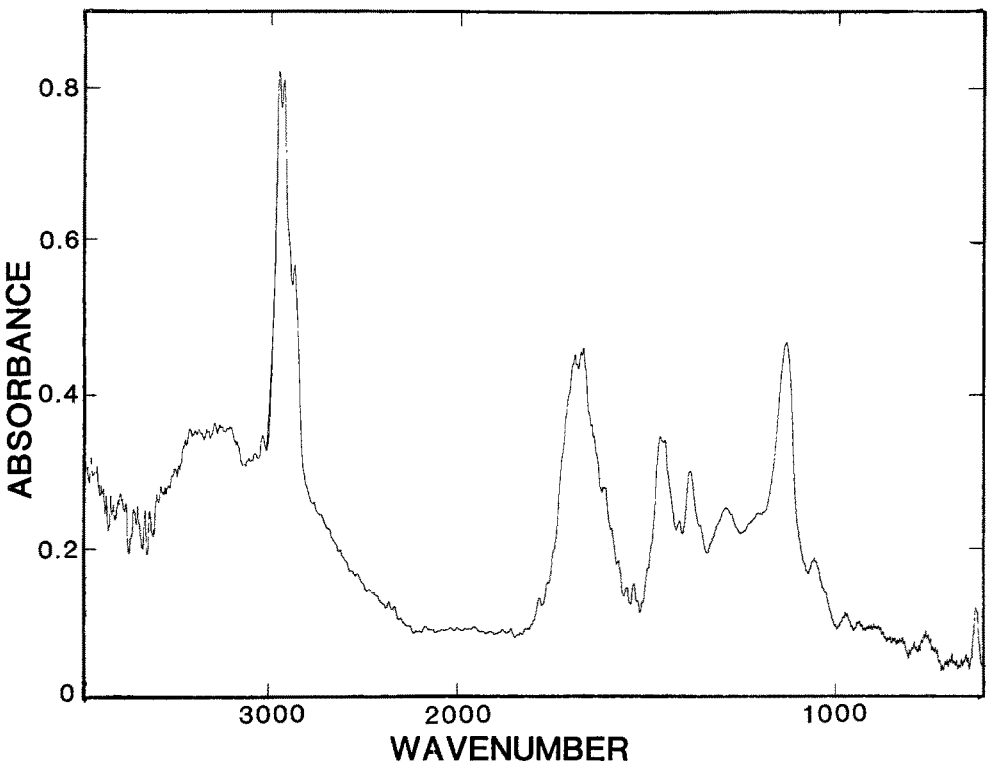

Fig. 7. Infrared spectrum of the non-polar organic components extracted from the Murchison meteorite. (See text for details.)

Infrared spectra showed absorption peaks at wavelengths characteristic of C–H stretching, C–O and carbonyl stretching, and O–H stretching and deformation. A broad absorption between 2000 and 4000 wavenumbers was also present (Figure 7). Titration of the non-polar components dispersed by sonication in water showed that substances with pK values in the range of 6.5 and 9.3 were present. These results, taken together with the infrared spectra, suggest the presence of carboxylate and phenolic groups. The C–H stretching peaks also indicate the presence of hydrocarbons, and the broad absorption is characteristic of polycyclic aromatic hydrocarbon derivatives. These results are consistent with previously published organic analyses of the Murchison chondrite [118, 19] which reported the presence of aliphatic and aromatic hydrocarbons, monocarboxylic acids, and phenolic compounds.

Aliquots of the chloroform extract were spread at air-water interfaces and surface pressure isotherms were obtained. The non-polar components displayed surface activity, and readily spread to form a film which could be compressed to surface pressures as high as 28 dynes cm^{-1} before collapsing (Figure 8). The area of the film ranged from 500 cm^2 per mg at high surface pressures to 4500 cm^2 per mg at low

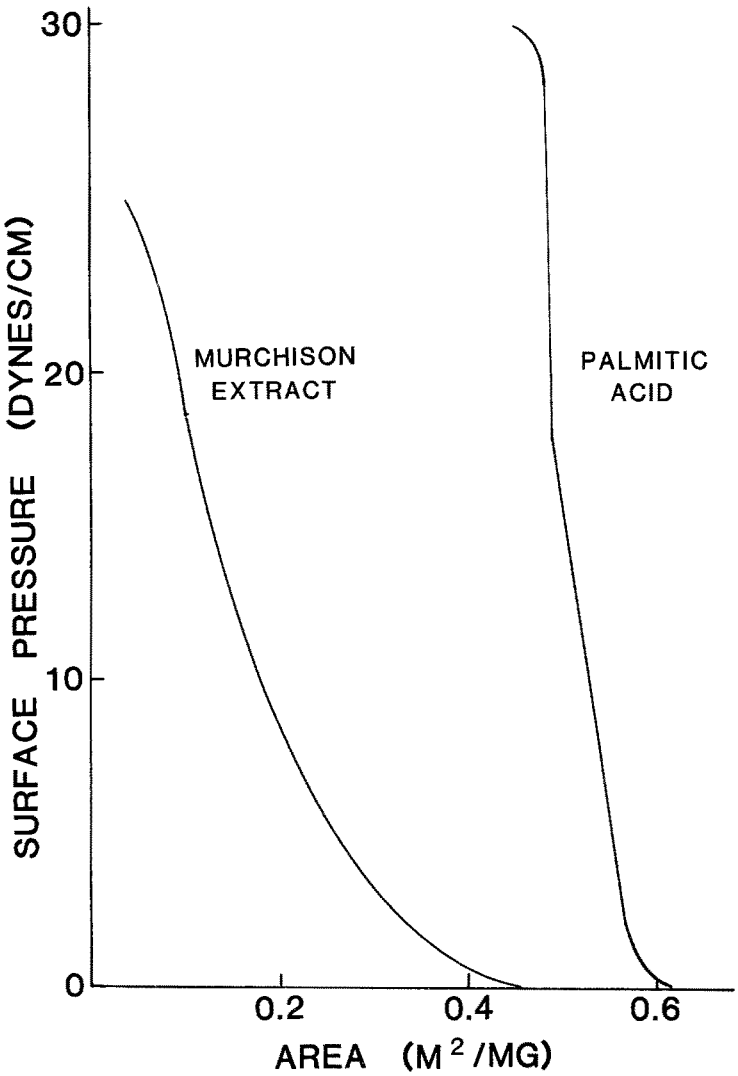

Fig. 8. Surface pressure isotherm of the non-polar components extracted from the Murchison meteorite. For comparison, an isotherm of palmitic acid is shown on the same scale.

surface pressures. For comparison, a monolayer of a pure fatty acid such as palmitic acid ranges from 4800 to 6000 cm^2 per mg over the same surface pressures [33]. A likely explanation for the much larger range of the Murchison material is that the surface active components spread from a second phase that is not surface active and exists as a bulk phase at the interface.

Many surface active substances are also able to assemble into lamellar structures when they interact with aqueous phases, and often the lamellae produce stable membranes. Therefore conditions were established under which phase boundaries

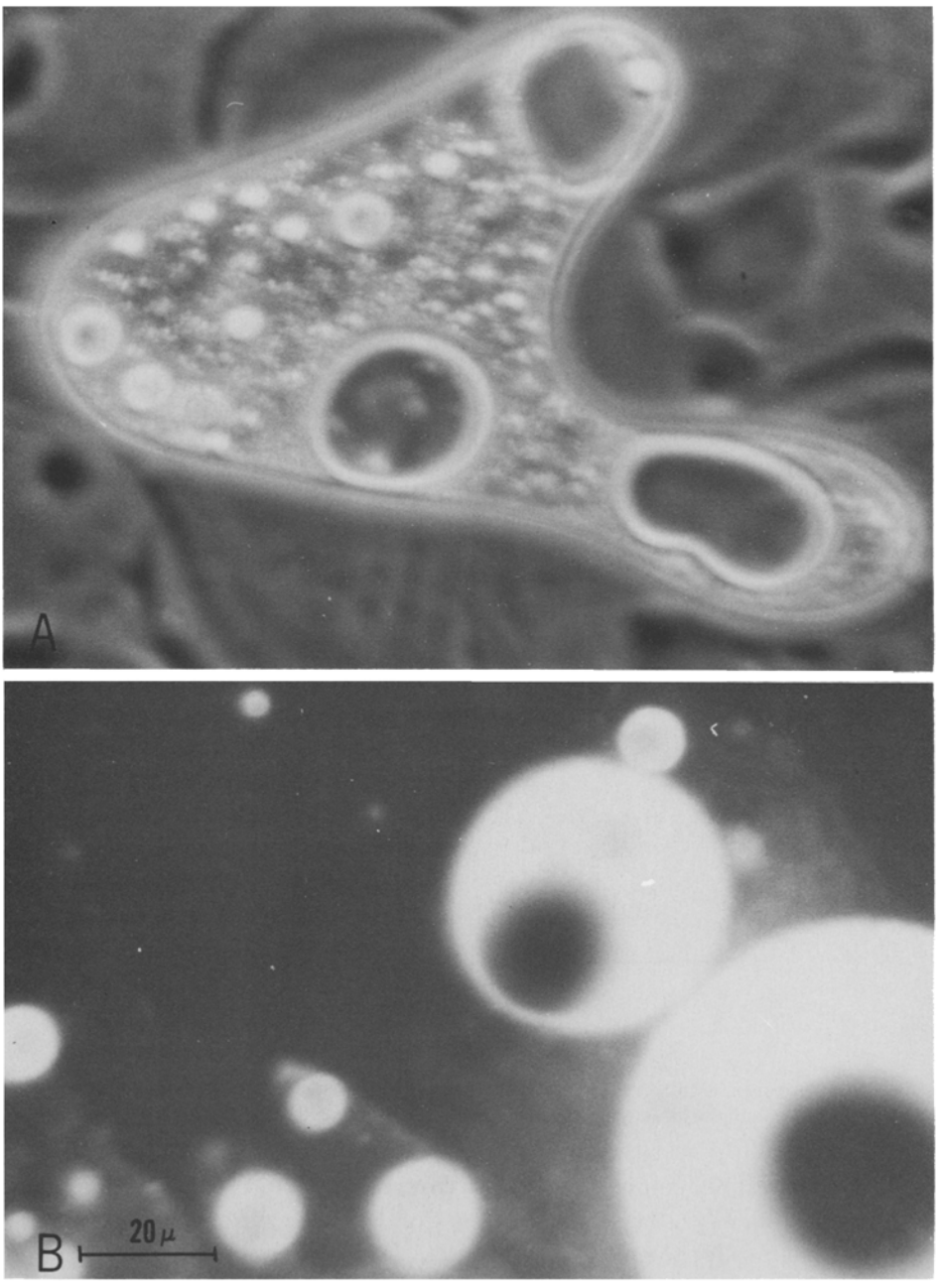

Fig. 9. Phase and fluorescence micrographs of non-polar components extracted from the Murchison meteorite. Aliquots of the extract were dried on a glass slide and alkaline buffer was added, followed by a cover slip. Under these conditions, fluid droplets are produced (A). These have a distinct yellow color, and are highly fluorescent (B).

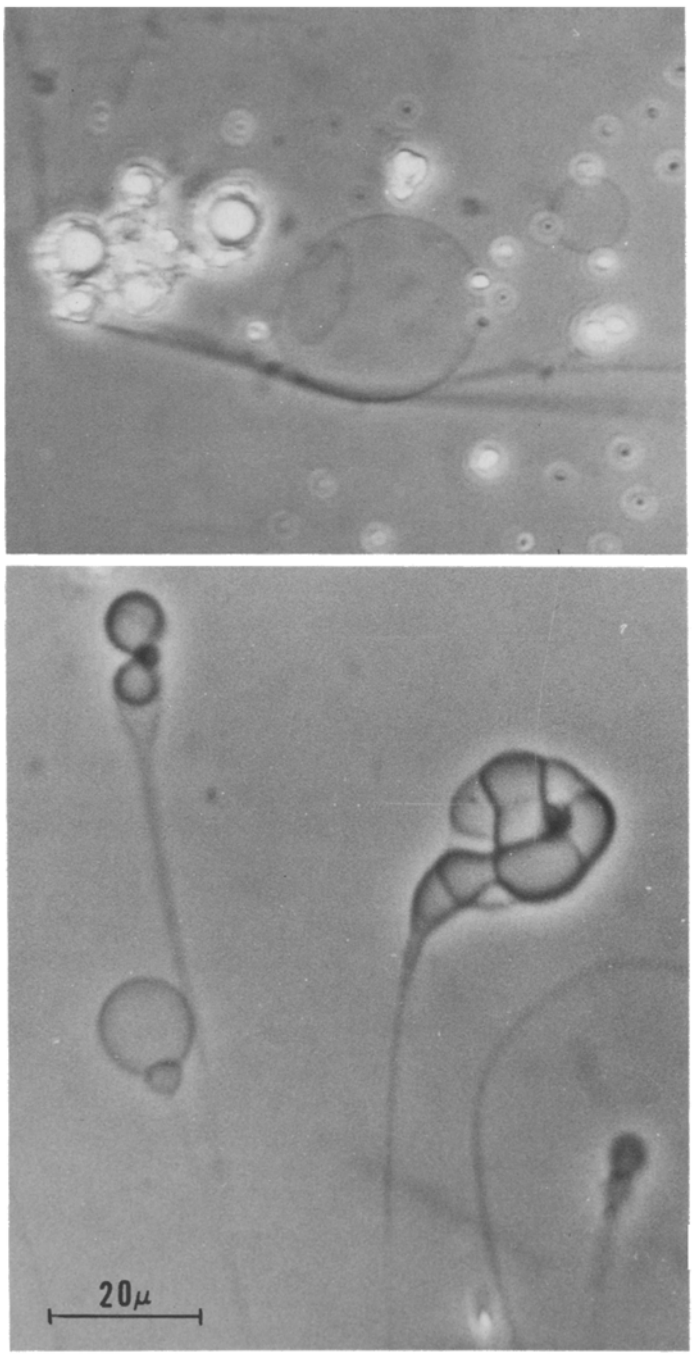

Fig. 10. In some non-polar extracts of the Murchison chondrite, vesicular structures appeared near the surfaces of fluid droplets such as those shown in Figure 9.

might be produced, and light microscopy was used to monitor the non-polar components as they interacted with various aqueous phases. In these experiments, 20 μl aliquots of the chloroform extract were dried on microscope slides, followed by addition of an equal volume of aqueous buffer and a cover slip. Under these conditions, the non-polar components formed yellow microstructures which adhered firmly to the glass. These microstructures were again highly fluorescent when viewed by fluorescence microscopy, and typically contained numerous inclusions which appeared to be separated from one another by relatively stable boundary layers that prevented fusion (Figure 9). Because earlier experiments had shown that there were titratable groups in the non-polar Murchison components, the properties of the microstructures were examined over a range of pH values. At alkaline pH ranges (9 and above) the microstructures were viscous fluid droplets. If an acidic buffer was used, or if the pH was lowered to 8 or below by addition of buffer at the edge of the coverslip, the droplets underwent a marked transition to solid phase.

At high pH ranges ([10] and above) the droplets became increasingly fluid. In 10 mM sodium hydroxide, extracts from three of eleven samples formed thin walled structures at the interface. Over a period of several minutes, these structures produced large numbers of membranous vesicles and long strands which drifted away from the droplet with the flow of aqueous phase (Figure 10). The vesicles were clearly membranous, and had open interior volumes in which Brownian motion of smaller particles could be observed. The largest vesicles were 50 μm in diameter. It is significant that if the extracts dried on slides were placed under vacuum for an hour before being rehydrated, the droplets were considerably more viscous and in three experiments thin walled vesicles were not observed. This suggests that the presence of volatiles aids formation of the vesicles, either fluid hydrocarbons extracted from the meteorite or traces of solvent (chloroform) which remain after air drying.

Thin sectioning and freeze-fracture electron microscopy were also carried out in order to confirm the presence or absence of boundary structures, and Figure 11 shows some typical observations. At least three different fracture patterns were identified. The first was a pattern which would be expected from the fracture of membranous vesicles along the non-polar plane of bilayers (Figure 11A). In a second pattern, the vesicles appeared to be filled with numerous smaller vesicles (Figure 11B). Finally, numerous examples of multilayered structures could be found in the neighborhood of vesicles (Figure 11C). Although it is not clear how these patterns arise, the results are generally consistent with the presence of bilayer boundary structures which guide the fracture planes.

In a second series of experiments, aliquots of meteoritic extract were again dried from chloroform and rehydrated with alkaline buffer, but with gelatin or agar added to act as support media. The specimens were then fixed with osmium tetroxide, embedded and sectioned by standard methods. Figure 12 shows examples of typical images. The sectioned material contained numerous empty vesicles which appeared to be surrounded by membranes. Under high magnification, the material fixed in gelatin had obvious areas of trilaminar structure typical of what has been

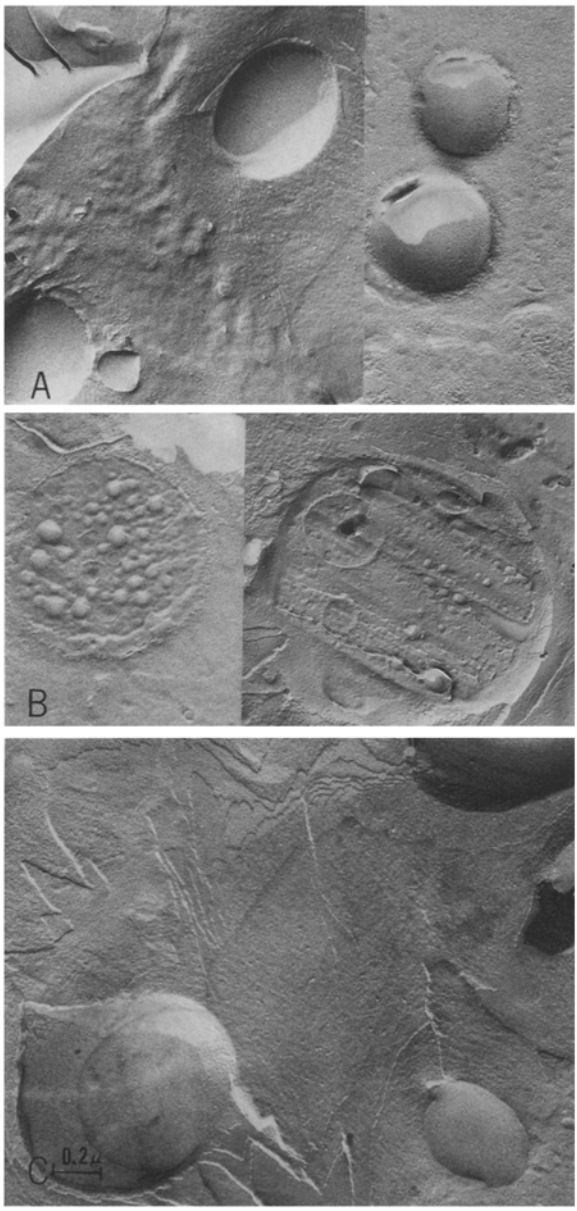

Fig. 11. Freeze-fracture appearance of Murchison non-polar components. Fracture faces usually appeared to represent the surfaces of small droplets (A) but sometimes passed through the droplet, revealing internal structures which are consistent with the structure of larger droplets shown in Figure 9A. In 11C multilamellar fracture planes dominate the region near droplets.

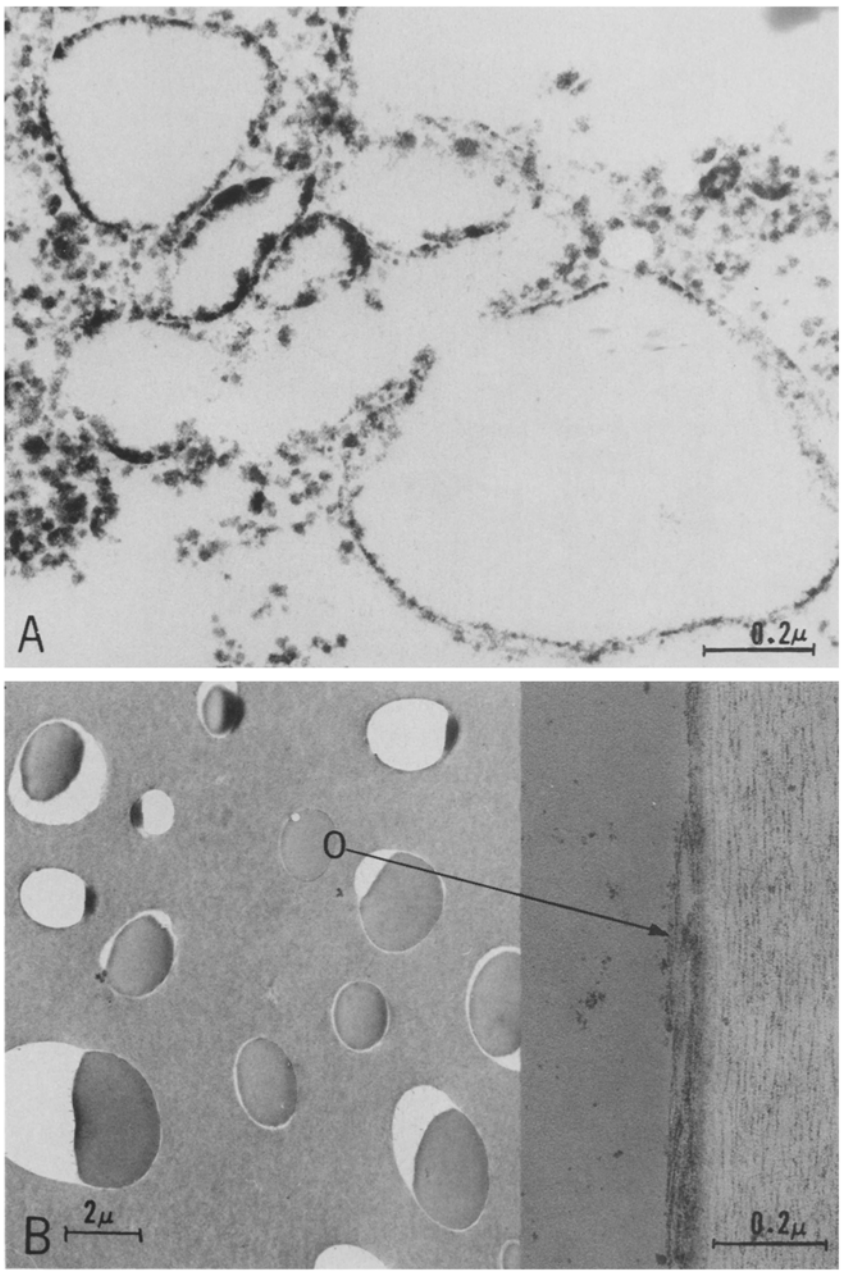

Fig. 12. Membranous structures were commonly observed in non-polar components prepared for electron microscopy by first supporting the fluid droplets in agar (A) or gelatin (B) followed by osmium fixation-staining, embedding and sectioning. In the agar supported specimens, some of the non-polar components have assembled into boundary structures near the surfaces of the droplets. In the gelatin support, numerous droplets could be seen. If the surface of the droplet was examined at higher magnification (circled area) boundary structures were observed which had the trilaminar appearance typical of lipid bilayers prepared under the same conditions.

observed in similarly fixed lipid bilayers. These electron microscopic results generally confirm that a small fraction of non-polar organic components in the extract are able to form boundary structures resembling membranes.

8. Potential Contaminants

When powdered samples of the Allende chondrite were extracted in control experiments, only a thin film was produced on the microscope slide. The film was not affected by alkaline solutions, and did not produce membranous material. It follows that the organic material extracted from the Murchison meteorite is intrinsic, and was not introduced during extraction procedures. However, there remains the question of contamination of the meteorite itself. Such contamination has clearly been demonstrated in the Orgueil chondrite which fell in the last century, and it is possible that the Murchison may be contaminated as well. There are two kinds of contamination that must be ruled out. The first is that microorganisms may have grown in the relatively loose interstices of the meteoritic grains, using the carbon compounds as a source of nutrient. This is unlikely on two counts. First, before beginning studies with non-polar components extracted into chloroform-methanol, numerous attempts were made to find organized structures in various aqueous extracts. This included extensive searches at low and high magnifications, using phase and fluorescence microscopy. Although a variety of mineral microcrystals and amorphous fluorescent particles of various shapes and sizes were observed, no formed elements resembling bacteria or yeasts were seen.

The second line of evidence tends to rule out other contaminants absorbed to the meteoritic samples. The specimens used in this study were interior material produced by breaking larger fragments. If contamination of the fragments had occurred, one would expect a gradient of contaminant from the surface of a fragment toward the interior. The fluorescent particles can be used as a marker for such gradients, since they presumably represent the organic compounds that dissolve in a chloroform-methanol wash. The fluorescent particles were randomly distributed throughout all fracture faces examined, with no tendancy to be concentrated near surfaces.

As noted earlier, probably the most significant potential source of contamination is traces of solvent (chloroform) remaining with the droplets after air drying. Indeed, the droplets become considerably more viscous if the slide is placed under vacuum for an hour before being exposed to aqueous media. However, there is also the possibility that meteoritic volatiles are removed by the vacuum, so this point is not yet decided. The most convincing evidence in favor of amphiphilic substances in chondrite is the fact that the extracted material is surface active and readily forms films at air-water interfaces. This property could not arise from solvent traces.

Despite evidence weighing against significant contamination, a final determination must await detailed analyses of the components of the non-polar extract. Such analyses are underway.

9. Summary

In conclusion, non-polar substances extracted from the Murchison carbonaceous chondrite form two kinds of microstructures when exposed the aqueous media. Most common are viscous fluid droplets at alkaline pH ranges, or solid structures at neutral and acid pH ranges. When the fluid droplets are examined by freeze-fracture electron microscopy, or as fixed and sectioned specimens, there is suggestive evidence that certain components of the extract assemble into structures which resemble lipid bilayers in their properties. That is, fracture planes are directed along the surface of the droplets, which would be expected if a bilayer structure was present which fractures along the plane of its hydrocarbon tails. Furthermore, in fixed specimens a trilaminar structure is observed which is also commonly produced by the lipid bilayer structure in model and biological membranes.

The second structure takes the form of actual membranes. These arise from the surfaces of the droplets described above, and again require alkaline pH ranges for stability. Not all samples produced membranes under the conditions described here, perhaps because the membrane forming compounds are not uniformly distributed throughout the Murchison material. Because of the small quantities available, and the complexity of the mixture, the composition of the membrane forming components has not yet been determined. However, the fact that the membranes only form and remain stable at alkaline pH ranges suggests that they contain a titratable group, perhaps phenolic in character, which must become charged before it is able to assemble into membrane structures.

Although the results described above represent the first report of boundary structures forming in aqueous phases from non-polar organic components of carbonaceous chondrites, Pflug [34] has observed microstructures in the 0.1 micron range in Murchison samples prepared for scanning electron microscopy. Alpern and Benkheiri [35] earlier demonstrated similar structures in the Orguiel meteorite which they related to its organic content. Presumably the non-polar material extracted here is derived from the structures described by these investigators. Its relevance to the origin of membrane structure is not yet clear, however. There is no reason to expect that early membranes would have required alkaline pH ranges for stability. Furthermore, some of the properties described here may result from solvent traces, or from hydrolytic or oxidized derivatives of the original organic components, which presumably formed in anhydrous, and anoxic conditions. When such components are exposed to an oxidizing atmosphere, or to an aqueous environment during extraction, it is possible that products are produced which were not originally present in the meteoritic material. Even with these reservations, the formation of boundary structures from non-polar components of carbonaceous meteorites is intriguing. It is likely that further analysis of their chemical and physical properties will provide useful insights about the evolution of membrane structure on the early earth.

References

[1] Goldacre, R. J.: 1958, in J. F. Danielli (ed.), *Surface Phenomena in Chemistry and Biology*, Pergamon Press, New York, p. 278.
[2] Shah, D. O.: 1972, in C. Ponnamperuma (ed.), *Frontiers of Biology*, Vol. 23, North-Holland Publishing Co., London, pp. 235–265.
[3] Lanbroke, B. D., Williams, R. M., and Chapman, D.: 1968, *Biochim. Biophys. Acta* **150**, 333–340.
[4] Mitchell, P.: 1961, *Nature* **191**, 144–149.
[5] Singer, S. J. and Nicholson, G. L.: 1972, *Science* **175**, 720–731.
[6] Bangham, A. D., Standish, M. M., and Watkins, J. C.: 1965, *J. Mol. Biol.* **13**, 238.
[7] Sessa, G. and Weissman, G.: 1968, *J. Lipid Res.* **9**, 310–318.
[8] Szoka, F. and Papahadjopoulos, D.: 1980, *Ann. Rev. Biophys. Bioeng.* **9**, 467.
[9] Deamer, D. W. and Uster, P.: 1983, in M. Ostro (ed.), *Liposomes*, Marcel Dekker, New York, pp. 27–51.
[10] Gebicki, J. M. and Hicks, M.: 1973, *Nature* **243**, 232–234.
[11] Hargreaves, W. R. and Deamer, D. W.: 1978, *Biochemistry* **17**, 3759–3768.
[12] Hargreaves, W. R., Mulvihill, S. J., and Deamer, D. W.: 1977, *Nature* **266**, 78–80.
[13] Epps, D. E., Sherwood, E., Eichberg, J., and Oro, J.: 1978, *J. Mol. Evol.* **11**, 279–292.
[14] Rao, M., Eichberg, J., and Oro, J.: 1982, *J. Mol. Evol.* **18**, 196–202.
[15] Deamer, D. and Oro, J.: 1980, *BioSystems* **12**, 167–175.
[16] Deamer, D. W. and Barchfeld, G. L.: 1982, *J. Mol. Evol.* **18**, 203–206.
[17] Bunch, T. E. and Chang, S.: 1980, *Geochim. Cosmochim. Acta* **44**, 1543–1577.
[18] Hayatsu, R. and Anders, E.: 1981, *Top. Curr. Chem.* **99**, 1–37.
[19] Becker, R. H. and Epstein, S.: 1982, *Geochim. Geophys. Acta* **46**, 97–103.
[20] McSween, H. Y.: 1979, *Rev. Geophys. Space Phys.* **17**, 1057–1078.
[21] Wood, J. A. and Chang, S. (eds.): 1984, *The Cosmic History of the Biogenic Elements and Compounds*, NASA Publ. 476.
[22] Kvenvolden, K. A.: 1970, *Nature* **228**, 923.
[23] Holzer, G. and Oro, J.: 1979, *J. Mol. Evol.* **13**, 265–270.
[24] Hayatsu, R., Randall–Winans, E., Scott, R. G., McBeth, R. L., Moore, L. P., and Studier, M. H.: 1980, *Science* **207**, 1202–1204.
[25] Smith, J. W. and Kaplan, I. R.: 1970, *Science* **167**, 1367–1370.
[26] Lawless, J. G. and Yuen, G. U.: 1979, *Nature* **282**, 396–398.
[27] Yang, J. G. and Epstein, S.: 1984, *Nature* **311**, 544–547.
[28] Anders, E., Hayatsu, R., and Studier, M. H.: 1973, *Science* **182**, 781.
[29] Leach, W. W., Nooner, D. R., and Oro, J.: 1978, in H. Noda (ed.), *Origins of Life*, Academic Press, Tokyo.
[30] Nooner, D. R., Gilbert, J. M., Gelpi, E., and Oro, J.: 1976, *Geochim. Cosmochim. Acta* **40**, 915–924.
[31] Nooner, D. R. and Oro, J.: 1979, in E. L. Kugler and Steffgen, F. W. (eds.), *Hydrocarbon Synthesis from Carbon Monoxide and Hydrogen*, Adv. Chem. Series, Vol. 178, American Chemical Society, Washington, D.C.
[32] Deamer, D. W.: 1985, *Nature* **317**, 792–794.
[33] Adam, N. K.: 1968, *The Physics and Chemistry of Surfaces*, Dover Press, New York, p. 47.
[34] Pflug, H. D.: 1984, *Naturwiss.* **71**, 531–533.
[35] Alpern, B. and Benkheiri, Y.: 1973, *Earth Planet. Sci. Lett.* **19**, 422–428.