Reports

Fossil Membranes and Cell Wall Fragments from a 7000-Year-Old Black Sea Sediment

Abstract. *Lamellar and tubular membranes and organic fragments resembling bacterial cell walls were abundant in Black Sea sediments deposited between 3000 and 7000 years ago. This time period was marked by a gradual transition from a freshwater to a seawater environment. The resulting salinity gradient in the interstitial solutions probably promoted natural chromatography and dissolution, redeposition, and preservation of organic molecules. The preservation of organic structures may have resulted from the lack of dissolved oxygen, high concentrations of metal ions, and structural reorganization during compaction.*

Well-laminated sediment cores collected at a depth of 2000 meters during the cruise of the R.V. *Atlantis II* in the Black Sea in the spring of 1969 contained as much as 50 percent organic matter. The portion of the core that was rich in organic matter extended from 20 to 70 cm below the surface of the sea floor. Although only one core from the eastern basin was studied in detail, similar layers rich in organic material were found in several cores from both the east and west basins and were stratigraphically related to each other. Radiocarbon dating of these layers rich in organic material showed they were deposited 3000 to 7000 years ago. During this period there was a gradual but fluctuating ionic change in the Black Sea from freshwater to seawater (*1*).

One representative core sediment was examined with the electron microscope, and direct studies were made of stained and unstained dispersed sediments from the layers rich in organic material. These sediments were also fixed in 1 percent OsO_4 (by weight), embedded in Epon 812, sectioned with an ultramicrotome (Porter-Blum), and examined with an electron microscope (Philips EM 300). Numerous structures, assumed to be organic because of their staining properties, were clearly visible in these preparations. The most prominent structures were lamellar membranes. These membranes were usually dispersed at random, although occasionally they were aggregated but not enclosed by a limiting membrane. Since the lamellar membranes were not associated with other cell remnants, it is impossible to speculate on their origin.

Two other types of membrane systems were also observed (Fig. 1). One of these, like the lamellar membranes, was not surrounded by a limiting membrane or associated with other cellular components. The membrane of this type was 80 to 90 Å thick and formed a tubular structure approximately 700 to 800 Å in diameter (Fig. 1C). The third type of membrane found also appeared to be of a tubular, branching nature (Fig. 1, A and B), approximately 150 to 200 Å in diameter, but was occasionally aggregated and partially or completely surrounded by a boundary layer.

The origin of these tubular membranes is of particular interest since they are quite rare in contemporary organisms. Tubular membranes have been reported in the cristae of some mitochondria (*2*), in a photosynthetic bacterium (*3*), and in a marine nitrifying bacterium, *Nitrococcus mobilis* (*4*). However, the tubular membranes present in the sediments were far too extensive to have been derived from any of the aforementioned sources. It seems likely that the tubular membranes

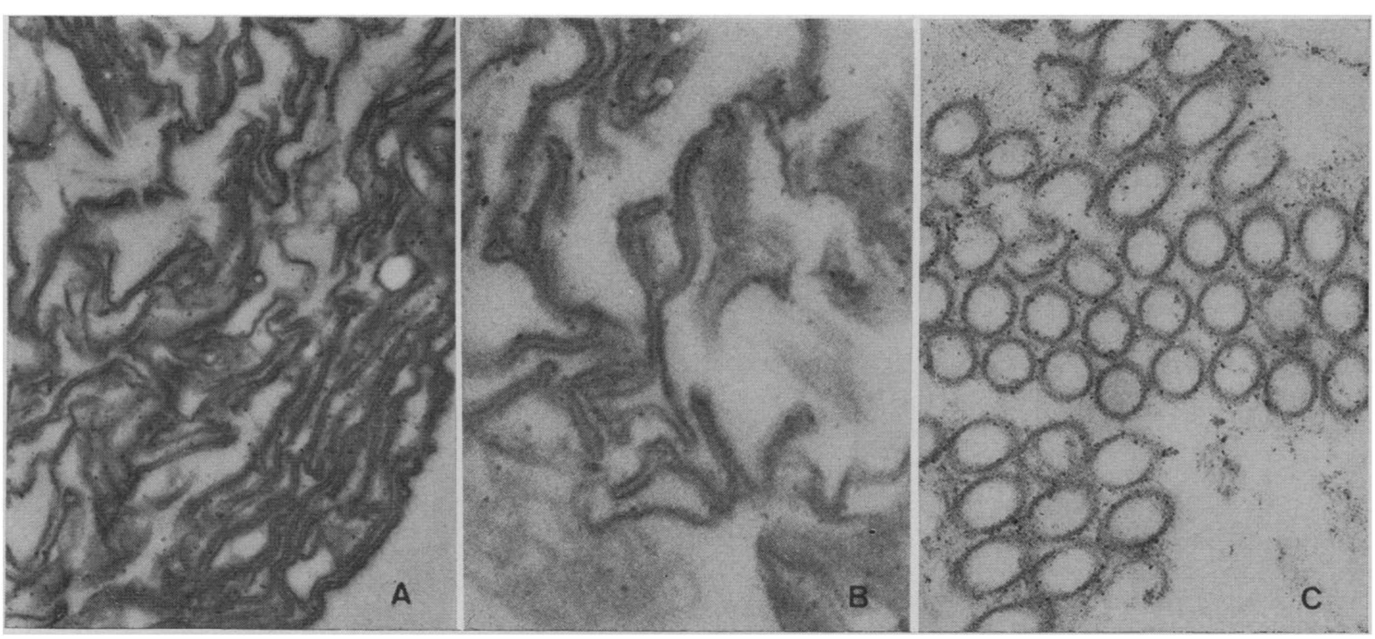

Fig. 1. (A) Branched, tubular membranes aggregated together and bound by a limiting membrane (× 63,800). (B) High magnification of a portion of (A) showing the branched, tubular membranes measuring 150 to 200 Å in diameter (× 159,600). (C) Large tubular membranes having a diameter of 700 to 800 Å and consisting of unit-membranes having a width of 80 Å (× 102,150).

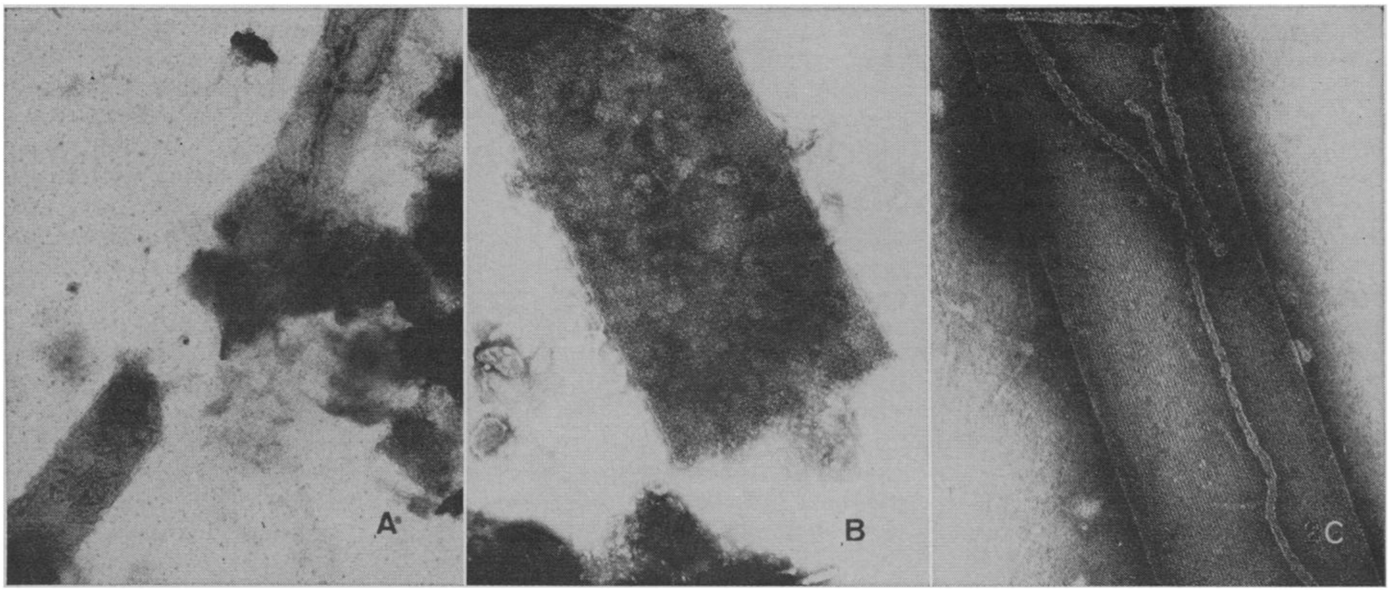

Fig. 2. (A) Organic fragments with angular ends, varying in width from 0.25 to 1.0 μm and in length from 1.0 to 15.0 μm ($\times$ 15,400). (B) Portion of an organic crystal resembling a bacterial cell wall and consisting of a crystalline arrangement of subunits ($\times$ 80,250). (C) Organic crystal resembling a bacterial cell wall and showing a unique pattern of subunits ($\times$ 51,000).

are remnants of some undescribed aerobic, eukaryotic cells which have been preserved in situ for 3000 to 7000 years in the anaerobic sediments of the Black Sea.

The rarity of such extensive tubular membrane systems in present-day organisms has forced us to consider another explanation for their presence in the sediments; namely, that tubular membranes may have been derived from lamellar membranes. Blondin *et al.* have demonstrated that configurational changes in mitochondrial membranes may occur which do not depend on electron transfer or hydrolysis of adenosine 5′-triphosphate (*5*). Rather, these configurational changes are due to fluctuations in the ionic environment and in concentrations of metal ions within the cell. In the sediments during the time period under consideration, fluctuations in the ionic environment and in concentrations of metal ions may have occurred which are similar to those fluctuations which induced configurational changes in the living cell. These environmental changes may have resulted in the transformation of lamellar to tubular membranes. Alternatively, these environmental changes in the sediments may have caused a disaggregation and subsequent reaggregation of membrane subunits. When reaggregation occurred, tubular membranes may have been formed.

Examination by electron microscopy revealed that the membranes and other organic particulate material in these sediments were characterized by a high-contrast image of the sort generally observed only in material stained with a heavy metal. No difference in electron density could be observed between those sectioned membrane materials stained with lead and those that were not stained. We interpret this phenomenon to be a consequence of in situ metal-ion staining by metals dissolved in the interstitial solutions. The abundance of heavy metals in the interstitial solutions may also account in part for the excellent preservation of the membranes for over 3000 years. Metal ions may become coordinated to the oxygen of the organic molecules with the result that polyhedra in which metal ions are coordinated to oxygen atoms will form (*6*).

Fragments composed of repeating subunits arranged in an ordered array and resembling bacterial cell walls (*7*) were present in some sediment layers. Some of these fragments were spherical sac-like structures, but most of them appeared tubular. These tubular structures were 1 to 2 μm wide and 1 to 15 μm long with sharp angular ends giving them a crystalline appearance. The subunits in these structures were organized in at least three distinct arrangements (Fig. 2).

Intact bacteria were rarely, if ever, seen in these preparations; however, some of the fragments observed were partially intact cell walls and reflected the native shapes of the bacteria. The long crystalline-like fragments may be portions of a cell wall from an extremely long bacterium. The possibility must be considered that some of these fractions, although derived from bacteria, may have resulted from the dissolution and reaggregation of subunits in response to changes in the diagenetic environment.

No conclusive statement can be made about the age of these fragments resembling bacterial cell walls. Thus far we have been unable to detect viable cells in these sediments, and it seems likely that some of these bacterial cell walls, like the membranes, have been preserved for periods exceeding 3000 years.

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