bodies of water. Some of those implications are discussed below.

An immediate outcome of algal-clay flocculation is the formation of larger particles having a higher sedimentation velocity. Thus, flocculation leads to the removal of suspended inorganic particles and algae from the water and to their accumulation at the bottom of water bodies. Sedimentation of algae leads to a downward displacement of nutrients they had taken up from the euphotic zone of the water column.

The sedimentation of algae with clay may be a selective mechanism leading to the preferential accumulation of flocculating algae in the sediment and the preferential survival of nonflocculating algae in the water. This selectivity may be due to taxonomic or physiological differences among the algae. As for bacteria, the flocculation tendency is higher during the declining growth and death phases or when the culture is under stress (*1*). Thus it is possible that the algal-clay flocculation is a population control mechanism leading to selective flocculation and sedimentation of the older and less active algal cells.

Another interesting implication relates to the harvesting of algae by filter feeders. These organisms can consume only particles larger than the openings in their filters. Thus, for example, the silver carp, which can utilize only particles larger than 20 to 50 μm (*13*), cannot feed on the cells of the algae that we used in this work but might well be able to harvest the clusters they form with clay.

Most researchers believe that algal growth in turbid water is limited because of the absorption and scattering of light by the clay particles. Possibly, flocculation and sedimentation of algae are other processes that limit algal growth.

In addition to the interesting ecological implications, the mechanism studied here may provide the means for managing lakes and other bodies of water. It is possible that turbid water could be clarified through the enhancement of algal growth or that algal-rich water could be clarified through the addition of clay to the water.

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Influence of Cholesterol on Water Penetration into Bilayers

Abstract. *X-ray diffraction and capacitance measurements have been used to calculate the depth to which water penetrates in fully hydrated bacterial phosphatidylethanolamine bilayers in the presence and absence of cholesterol. The data indicate that cholesterol decreases the depth of water penetration by about 2.5 angstroms.*

The depth to which water penetrates bilayer membranes and the role cholesterol plays in modulating this penetration are of interest for several reasons. (i) This information is vital for calculations of the total hydrophobic contribution to the free energy of bilayer formation (*1*). (ii) A better interpretation of high- and low-frequency membrane capacitance as well as reflectance measurements could be made if interfacial boundaries were more precisely defined (*2, 3*). (iii) The energetics of molecular adsorption to and transport through bilayers involves the displacement of interfacial water (*4*). (iv) The depth to which water penetrates micelles is a controversial issue (*5, 6*), to which the data obtained from these measurements on bilayers are relevant. (v) This information may be correlated with the potential energy profile across bilayer membranes (*7*). (vi) Some of the biochemical and physiological effects that occur when cholesterol is added to or removed from membranes (*8–10*) may be related to the influence of cholesterol on water penetration.

Data that can be related to the penetration of water into bilayers have been obtained by several techniques, including electron spin resonance, capacitance, and neutron diffraction measurements. Griffith *et al.* (*11*) used electron spin resonance to determine the polarity profile across liver microsomal and myelin membranes. From this profile, it was inferred that water penetrates roughly one-third of the distance from each surface into the bilayer. However, the influence of the bulky nitroxide spin label on this measurement is not known (*12*). The dielectric thickness for a variety of planar bilayers has been obtained by capacitance measurements (*13, 14*). In these capacitance experiments, care must be taken to use "solventless" planar bilayers to make structural comparisons with solventless multilamellar vesicles (*15*). Neutron diffraction experiments provide direct information on the depth of water penetration in phospholipid bilayers. By this technique, water penetration has been measured for partially hydrated samples of gel-state dipalmitoyl phosphatidylcholine (*16*) and liquid crystalline dimyristoyl phosphatidylcholine : cholesterol (2:1) and egg lecithin : cholesterol (2:1) multilayers (*17*). It was shown that water penetrates to the lecithin carbonyl group in dimyristoyl phosphatidylcholine : cholesterol (2:1) bilayers (*17*). The effect of cholesterol on the depth of water penetration into fully hydrated liquid crystalline bilayers has not previously been measured.

We combined x-ray diffraction data from fully hydrated bacterial phosphatidylethanolamine (BPE) bilayers, in the presence and absence of cholesterol, with capacitance data from solventless planar bilayers made from the same lipids under identical ionic conditions. The results show that cholesterol may decrease the penetration of water into the bilayer by about 2.5 Å, displacing water from the deeper carbonyl group in BPE.

For x-ray diffraction experiments, hydrated lipid samples were made as follows. A chloroform solution of the appropriate lipid was evaporated to dryness and then excess (≥ 70 percent by weight) 0.1*M* NaCl was added to the

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lipid. Extensive vortexing was followed by several hours of incubation at 20° ± 2°C. The specimen was sealed in an x-ray capillary tube and the diffraction patterns were recorded and analyzed (*18*). Specific capacitances at 30, 100, and 5000 Hz were obtained from planar bilayers (*19*).

X-ray diffraction patterns from both hydrated BPE and hydrated (BPE : cholesterol) contain wide-angle bands centered at 4.6 Å and four sharp low-angle lamellar reflections. The repeat periods are 53.3 ± 0.4 Å (mean ± standard deviation) for BPE, 54.3 ± 0.5 Å for BPE : cholesterol (mole ratio 2:1), and 54.1 ± 0.5 Å for BPE : cholesterol (1:1) bilayers. The greatest difference among the three patterns is in the intensity distribution for the low-angle reflections, which produces substantial changes in the electron density profiles (Fig. 1). In the profiles, the highest density peaks correspond to the lipid head groups. Adjacent to these peaks are relatively broad, medium density regions, which correspond to the methylene groups of the lipid hydrocarbon chains. The lowest density trough, in the geometric center of the bilayer, is due to the lipid methyl groups. Cholesterol induces changes in the electron density profiles. In both 2:1 and 1:1 bilayers the overall thickness (measured by head group peak separation) and the repeat period are only slightly increased. Cholesterol slightly increases the density of the methylene group region of the profile while increasing the depth of the terminal methyl trough. These changes are consistent with the idea that cholesterol resides between adjacent lipid hydrocarbon chains and tends to order them (*20*).

The specific capacitances for BPE, BPE : cholesterol (2:1), and BPE : cholesterol (1:1) bilayers in 0.1*M* NaCl are 0.69 ± 0.01, 0.63 ± 0.01, and 0.57 ± 0.03 μF/cm^2, respectively. The same results are obtained at 30, 100, and 5000 Hz. Following Requena and Haydon (*14*), we assign a value of 2.2 for the dielectric constant of the hydrocarbon moiety of BPE bilayers. This should not be changed by the addition of cholesterol, which also has a dielectric constant of 2.2 (*13*). Therefore the differences in capacitance can be attributed entirely to an increase in dielectric thickness. The dielectric thickness, d_e, is defined as the thickness of a parallel plate capacitor whose dielectric constant is e. The values of d_e calculated from the capacitance data for BPE, BPE : cholesterol (2:1), and BPE : cholesterol (1:1) are 28.2 ± 0.4, 30.9 ± 0.5, and 34.2 ± 1.8 Å, respectively. These values of d_e are shown superimposed on the electron density profiles in Fig. 1. Note that for pure BPE planar bilayers d_e corresponds closely to the hydrocarbon region of the electron density profile from multilayers. However, with cholesterol in the bilayer, d_e includes appreciable portions of the head group peaks in the electron density profiles. The x-ray diffraction data show that cholesterol only slightly increases total BPE bilayer thickness, whereas the capacitance measurements show that it significantly increases the dielectric thickness of planar BPE membranes. The increases are 2.7 and 5.8 Å for cholesterol mole fractions 0.33 and 0.50, respectively. The simplest explanation of these data is that the addition of cholesterol displaces some of the interstitial water and replaces it with nonpolar material, thereby increasing the dielectric thickness.

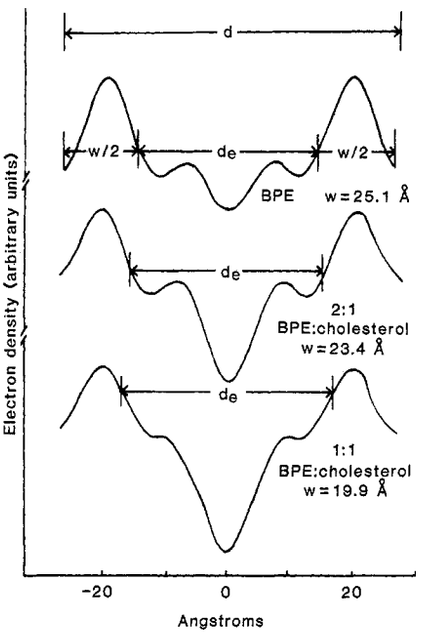

Fig. 1. Dielectric thickness, d_e, obtained from capacitance measurements, superimposed on electron density profiles calculated from x-ray diffraction experiments. The capacitance and x-ray measurements were made with the same lipids in 0.1*M* NaCl solutions. The width of the water layer, w, decreases when cholesterol is added to the bilayer.

Griffith *et al.* (*11*) found that the depth of water penetration is less for myelin membranes than for microsomes. They attributed this to differences in membrane composition. Our data suggest that cholesterol is the key factor in modulating the depth of water penetration, since myelin has a higher cholesterol content than liver microsomal membranes (*21*).

We can now use the x-ray diffraction and capacitance data to determine the depth to which water penetrates BPE bilayers with and without cholesterol (*22*). Referring to Fig. 1, the width of the water layer, w (*23*), can be calculated from $w = (d - d_e)$, where d is the x-ray repeat period. The values of w are 25.1, 23.4, and 19.9 Å for BPE and BPE : cholesterol bilayers (2:1 and 1:1), respectively. From the electron density profiles, the depth to which water penetrates relative to the center of the head group peak is 5.5 Å in BPE and 4.7 and 3.1 Å in BPE : cholesterol (2:1 and 1:1, respectively). We assume that the high-density phosphate group is located near the center of each of these head group peaks. Previous studies have shown that the distance between the phosphate and deeper carbonyl groups is between 4.5 (*17*) and 6.1 Å (*24*). Therefore, water penetrates approximately to the deeper carbonyl group in BPE membranes and cholesterol may reduce this penetration, displacing water to about the glycerol backbone.

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Cultivation of Bacteria-Free *Hydra viridis*: Missing Budding Factor in Nonsymbiotic Hydra

Abstract. *Bacteria-free hydra, cultured in sterile media, were fed bacteria-free larvae of* Artemia salina. *Normal growth and budding were obtained in symbiotic and aposymbiotic* Hydra viridis. *Two nonsymbiotic hydra species did not form buds under bacteria-free conditions. When these hydra were fed nonsterile* Artemia, *or if the medium was reinoculated with bacteria isolated from budding stock cultures, normal budding was resumed. An exogenous budding factor, which can be provided by nonsterile* Artemia *larvae, or even by some bacteria, appears to be required by these nonsymbiotic hydra. This factor is endogenous in* Hydra viridis.

The simple freshwater hydra is a valuable animal for research in cell and developmental biology (*1*). The green symbiotic *Hydra viridis* has been used for research of nutritional aspects of the algae-hydra symbiosis (*2*). For many investigations the culture of hydra in the presence of bacteria does not cause problems. However, for critical nutritional and biochemical studies of hydra, the organisms grown in bacteria-free media are necessary to ensure that the measured parameters are those of the hydra and not of contaminants. We now report the successful maintenance and growth of bacteria-free *Hydra viridis* fed bacteria-free larvae of *Artemia salina*. Two nonsymbiotic hydra species were maintained, but did not bud under similar conditions.

Stock cultures and bacteria-free cultures of hydra were grown in dim light, at about 600 lux and 20°C, in M solution, a buffered mixture of inorganic salts resembling pond water (*3*). Phenol red, a nontoxic *p*H indicator, was added at 1 mg/liter to give continuous visual monitoring of *p*H changes caused by bacterial contamination. In contaminated cultures, the color of the medium changes from pink to yellow as the *p*H drops (*4*).

Bacteria-free hydra were obtained as follows. Several hydra were taken from a stock culture, starved for 48 hours, and washed three times in fresh M solution and once in a sterile solution. The hydra were then placed in a drop of the sterile M solution on a microscope slide, and the basal part, to which many microorganisms adhere, was cut off immediately below the budding zone. The upper parts were then washed twice in sterile M solution and transferred with a Pasteur pipette into standard test tubes (22 by 160 mm) containing M solution and penicillin, streptomycin, neomycin, and chloramphenicol or rifampicin (100 μg/ml of each). Excess light was avoided when chloramphenicol was used (*5*). After 48 hours, the hydra were transferred into sterile test tubes containing M solution only. For sterility tests, we transferred one hydra from each prepared batch into a test tube with a sterile 2 percent proteose peptone solution and incubated it at 33°C. We took the absence of bacterial growth in this medium, after 3 or 4 days, as an indication that the hydra were bacteria-free.

We continuously verified the absence of bacteria in our cultures by watching the pink color of the phenol red in the medium. We also occasionally tested the spent medium for sterility. A sterile proteose peptone solution was added until a final concentration of 2 percent was attained and the medium was then incubated for 3 to 4 days at 33°C. This incubation time was found to be sufficient since no bacterial growth occurred when such a medium was incubated for longer periods.

Bacteria-free larvae of *Artemia salina* were obtained by decapsulation (*6*) of commercially available cysts (*7*). The cysts (200 to 300 mg) were hydrated by soaking for 1 hour in a 3 percent sodium chloride solution and agitated by air bubbling. Floating cysts were discarded and the rest were placed for 15 minutes in a 20 percent solution of sodium hypochlorite (~ 2 percent available chlorine), in a small crystallizing dish. Decapsulation was monitored by observing a gradual change in the color of the cysts from brown through white to orange, indicating complete decapsulation. Routine aseptic techniques were then applied. The cysts were collected with a Pasteur pipette and washed three times in 10 ml of sterile M solution in standard test

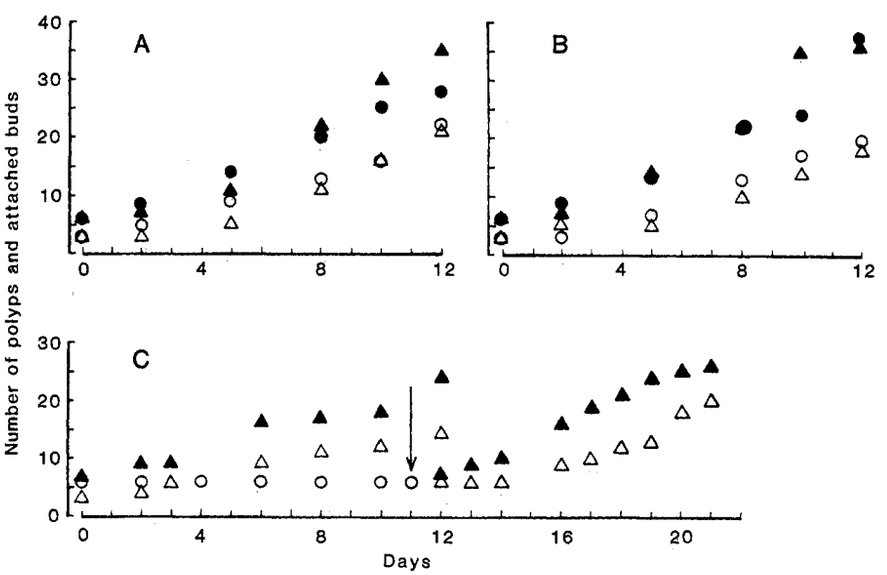

Fig. 1. Budding rates of nonsterile and bacteria-free hydra. (A) Green *H. viridis*, (B) aposymbiotic *H. viridis*, and (C) *H. vulgaris*. (○) Bacteria-free polyps, (●) bacteria-free polyps with their attached buds, (△) nonsterile polyps, and (▲) nonsterile polyps with their attached buds. Each experiment was done in three test tubes containing one budding polyp in 10 ml of M solution, except the bacteria-free *H. vulgaris* experiments (C), where each test tube contained two nonbudding polyps. Nonsterile animals were fed nonsterile *Artemia* five times a week and bacteria-free animals were similarly fed bacteria-free *Artemia*. In (C), no budding of bacteria-free *H. vulgaris* occurred up to the 11th day, at which time nonsterile *Artemia* were fed to these animals (arrow).

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