bution of the peripheral nervous system by inhibition and excitation. Tonic inhibition of the peripheral pathway, via the branchial nerve, is reflected in the reflex amplitude and latency; additionally, electrical stimulation of the branchial nerve depresses the gill response to electrical stimulation of the ctenidial nerve. Excitatory regulation is apparent when very weak tactile stimuli are used; also, gill response to electrical stimulation of the branchial nerve is enhanced by electrical stimulation of the ctenidial nerve.

In addition to its established role as an initiator and organizer of patterned rhythmic behavior (*14*), the central nervous system regulates the activity of the peripheral nervous system. This finding supports the proposal (*15*) that in mollusks the central nervous system has a regulatory influence on the peripheral system. We conclude from our work reported here and that reported previously that habituation of the gill withdrawal reflex is an expression of adaptive change in both the peripheral and central nervous systems (*16*).

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12. With the PVG removed, the gill withdrawal reflex was most reliably evoked by stimulation of the basal area of the siphon near the anus and only poorly elicited by stimulation of the frilly outer siphon. These sites on the siphon overlap those fields innervated by central sensory neurons [see Byrne *et al.*, in (*5*)]. After PVG removal, the reflex is a more stereotyped movement and lacks the variability contributed, in part, by secondary withdrawal movements [see (*1, 4, 7*)] as choreographed by the PVG.
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Apparent Modification of Forces Between Lecithin Bilayers

Abstract. *Small sugar solutes effect variation in the equilibrium separation of lecithin bilayers in aqueous solution. Since sugars have negligible influence on bilayer structure, they probably act by modifying interbilayer forces. The observed widening and narrowing of the bilayer separation is correlated with the predicted weakening and strengthening of the attractive van der Waals forces between lipid bilayers that occurs with increasing sugar concentrations.*

When immersed in excess water, lecithin forms a separate lamellar phase—alternating layers of lipid and water in equilibrium with a pure water phase. The lecithin interbilayer separation of about 28 Å reflects a balance between attractive and repulsive forces whose existence has been recognized as contributing to the interaction between biological cell membranes (*1*). Although long-range attractive van der Waals forces are not strong enough to confer the mechanical stability that has been observed between cell membranes in tissues, such forces are probably felt as membranes approach to make initial contact. They clearly prevent lecithin bilayers, in lamellar arrays, from separating beyond 28 Å. In these systems there is only water or aqueous solution in the region between the lipid bilayers.

According to Dzyaloshinskii *et al.* (*2*), van der Waals or electrodynamic forces between large bodies depend on differences in the material polarizability of the interacting bodies and the intervening medium. On this basis, the van der Waals attraction between two bodies may be different in a solution from what it is in a pure solvent. We report here changes in the lamellar spacing which might reflect modification of attractive forces between lecithin bilayers caused by the addition of solute to the suspending medium.

To do this, we have deliberately varied

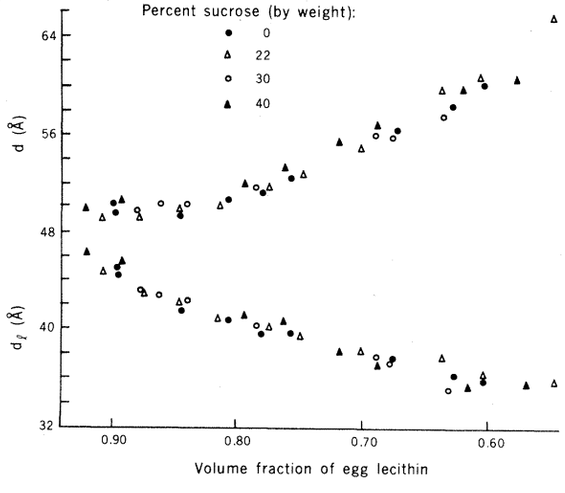

Fig. 1. Structural parameters of the lamellar phase formed by egg lecithin in pure water and in 22, 30, and 40 percent sucrose solutions (*5*). The abscissa gives the volume fraction ϕ of egg lecithin in the total mixture. The ordinate gives d and d_l. In the single-phase system formed with fixed ratios of water solution and lipid, $d_l = \phi d$. The presence of up to 40 percent sucrose has a negligible effect on the lipid layer thickness. Chromatographically pure egg lecithin was prepared as described elsewhere (*6*); x-ray diffraction analysis was carried out as in earlier work (*3*).

the polarizability of the water between lipid bilayers by adding sucrose or glucose. There is a predicted successive weakening and strengthening of the attractive force with an increasing concentration of sugar. Observation of concomitant changes in lattice spacing during the addition of small sugars reveals a waxing and waning of the bilayer separation.

X-ray diffraction techniques (*3–5*) were used to measure the bilayer thickness d_l and bilayer separation d_w of the multilayer system formed by chromatographically pure egg lecithin (*6*) in aqueous solutions. Figure 1 gives the total lamellar repeat distance d ($=d_l + d_w$) and d_l formed by egg lecithin in water and in 22, 30, and 40 percent (by weight) sucrose solutions for the single phase that occurs in the absence of excess water. Over the range of separations of the bilayers where d_l and d_w can be calculated (that is, up to near maximum swelling), these results show that, at any given lipid volume fraction, substitution of as much as 40 percent sucrose solution for the water between the bilayers does not affect the bilayer thickness. Linear regression analysis of data from similar studies done at a constant volume fraction of lipid (0.72) with increasing sucrose concentrations shows that substitution of as much as 40 percent sucrose for water between bilayers increases the 39-Å-thick bilayer by only 1 Å.

Figure 2a shows the d spacing for egg lecithin suspended in excess sucrose or glucose solutions. As the concentration of each sugar increases, the d spacing first increases, reaches a maximum at approximately 22 percent sugar, and then decreases. From the results shown in Fig. 1, it is clear that these changes in d are due to changes in d_w since d_l probably remains constant.

In order to find out whether the sugar concentration of the interbilayer compartment was the same as that of the external excess solution, we measured the relative uptake of water and sucrose by dry lecithin as it swells in the various sugar solutions. This was done by comparing the index of refraction, and thus the sugar concentration, of the suspending solution with that of the solution left in excess with the swelled lamellar phase. The concentration of any of the sugar solutions between bilayers was found to be lower than that in the bathing medium. The concentration difference is such that there are 13 more water molecules per lecithin molecule in a given volume of the interbilayer solution than in the same volume of the bathing medium. This difference is roughly constant over the entire range of sugar concentrations up to 40 percent. [By linear regression fit, the number of water molecules is equal to 13.4 − 0.05 × (the percentage of sugar).] The difference may represent water that does not freeze near 0°C (*7*), or the apparent exclusion of sugar may simply reflect the fact that sucrose molecules are too big to come as close to the lipid as water molecules can. These results are consistent with similar determinations of nonsolvent water in dimyristoyl lecithin liposomes (*8*). If these 13 molecules are located around the head groups of the phospholipid molecules, then only the middle two-thirds of the interbilayer space is occupied by a sugar solution in equilibrium with the excess solution.

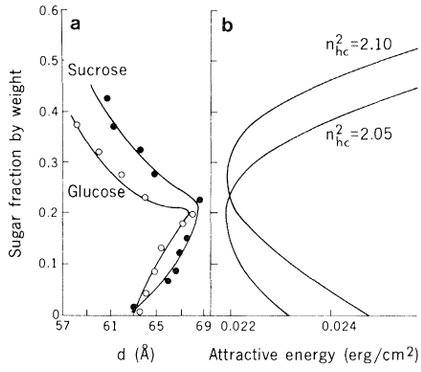

Fig. 2. (a) The d spacing formed by egg lecithin when suspended in effectively infinite volumes of sucrose or glucose solutions having various concentrations. Because sugars do not affect bilayer thickness, the changes in d represent changes in d_w. Solid lines are drawn to help make clear systematic changes in d. (b) Correlation of changes in computed van der Waals attraction with variation in the d spacing. The computation of the attractive energy between lipid layers across the sugar solution (at 20-Å separation) was carried out by standard methods (*9*) based on the macroscopic theory of van der Waals forces (*2*). The quantity n^2_{hc} is the refractive index assumed for lipid hydrocarbon.

To compare these results with expected changes in attractive forces, we have computed (Fig. 2b) the van der Waals energy between lipid layers as a function of the sugar concentration in an intervening aqueous solution (*9*). Although details of the energy variation depend on assumed absorption spectra for sugar solutions, there is always a predicted successive weakening and strengthening of the van der Waals forces between lamellae.

This behavior occurs because the strength of the electrodynamic force depends on the square of the differences in the material polarizability over the entire spectrum. At visible frequencies the refractive index of water is less than that of the lipid bilayer. Added sugar increases the index of the aqueous layer, decreases the difference in polarizability, and thereby decreases the contribution from these frequencies to the total attractive energy. As the sugar concentration continues to increase, the difference in polarizabilities, summed over the whole spectrum, passes through a minimum. Hence the attractive energy goes through a minimum.

On the basis of the correlation between the energy and spacing curves (Fig. 2), we suggest that the observed variation in bilayer separation might reflect concomitant changes in the van der Waals attractive energy. Observed d spacing changes are larger than would be expected from the 10 percent variation in the van der Waals attractive force seen in Fig. 2b when acting against an exponential repulsion (*10*). If one considers a bilayer model which includes a separate layer for polar groups plus bound water, a much larger variation in attractive forces is predicted; reasonable agreement with the observed variation in spacing is achieved. Since the interpretation we present here is based only on correlation, we believe alternative explanations for this observation of changing interlamellar forces should be pursued, although no alternative is as yet apparent to us. A detailed description of the experimental procedure and force computation will appear elsewhere (*11*).

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