that isorhodopsin, although not normally present in vivo in significant quantity, possesses physiological activity. Thus, the mechanism in the photoreceptor that relates sensitivity and the content of visual pigment appears to "accept" isorhodopsin, as well as rhodopsin.

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15. A decrease in visual sensitivity of more than 4 log units upon bleaching about 90 percent of the rhodopsin in the skate eye is consistent with earlier studies, which have compared b-wave sensitivity with levels of rhodopsin during dark adaptation (*6*).
16. The sensitizing effect of 11-*cis* retinal did not depend on prior treatment of the partially bleached isolated retina with all-*trans* retinal, since in other experiments (not illustrated) the application of 11-*cis* retinal alone was observed to promote a substantial increase in receptor sensitivity.
17. In most experiments, complete recovery of sensitivity did not occur. Usually, as here, more exhaustively bleached or older preparations displayed a higher final threshold. In the range of bleaching that we examined (about 34 to 97 percent of the rhodopsin bleached), increases in receptor sensitivity induced by 11-*cis* or 9-*cis* retinal did not appear to depend critically on the time of application of the retinal.
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Habituation of Reflexes in *Aplysia*: Contribution of the Peripheral and Central Nervous Systems

Abstract. *We studied the contribution of the* Aplysia *peripheral nervous system, in the siphon and gill, to habituation of the gill withdrawal reflex. After removal of one central ganglion, the parietovisceral, repeated stimulation of the siphon caused habituation of the reflex as it had with the ganglion intact, showing that there is a peripheral pathway between the siphon and gill with competence to mediate habituation. Repeated electrical stimulation of two efferent nerves to the gill, after removal of the parietovisceral ganglion, resulted in habituation of withdrawal movements, which shows that the terminals of the ganglion neurons in the gill are a site of habituation. Also, stimulation of one nerve dishabituates the withdrawal movements elicited by the other. These results identify two sites of habituation in the gill in addition to sites in the parietovisceral ganglion.*

The peripheral nervous system in *Aplysia*, a marine gastropod, is competent to mediate habituation and dishabituation of the siphon or gill responses to direct tactile stimulation in the absence of the central nervous system (*1, 2*). In contrast, central neurons have been thought to mediate habituation of the reflex withdrawal of the gill in response to tactile stimulation of the siphon (*3, 4*). The demonstrated competence of the peripheral nervous system to mediate habituation (*1, 2*) suggests that, in addition to central sites, peripheral sites in the reflex pathway for gill withdrawal in response to siphon stimulation are also contributing to habituation. We tested this hypothesis by (i) observing if the amplitude of the gill withdrawal changed with repeated electrical stimulation of the nerves to the gill after removal of the central nervous system and (ii) observing habituation of the gill withdrawal to tactile stimulation of the siphon before and after central nervous system removal. The results show that: the peripheral nervous system is competent to mediate habituation of the gill withdrawal reflex; the terminations of central motor neurons in the gill are sites of habituation; and the central nervous system exerts excitatory and inhibitory influences on the peripheral system.

We used 50 *Aplysia californica* (100 to 500 g; Pacific Biomarine, Venice, Calif.) kept in artificial seawater (Instant Ocean) at 15° to 16°C. The preparation consisting of the parietovisceral ganglion (PVG or abdominal ganglion), the gill, mantle, and siphon was pinned out in a chamber containing 500 or 1500 ml of seawater at 15°C. The PVG, the only part of the central nervous system remaining, innervates the gill, mantle, and siphon by the intact branchial, ctenidial, and siphon nerves (Fig. 1A). A punctate stimulus was applied to the siphon by a stiff wire with a plastic sleeve at the tip which had a total diameter of 1.5 mm. The wire was attached to a solenoid which was activated by square-wave pulses. By varying the voltage and the duration of the pulse to the solenoid the force of the tactile stimulus could be varied in discrete steps from 200 mg to 20 g (*5*). This stimulus applied to the siphon produced a reflex withdrawal of the gill (Fig. 1A). The reflex withdrawal is the same as that studied previously [figure 1A in (*4*); figure 1A in (*6*); and figure 3C in (*7*)]. The amplitude of the reflex was measured by recording the tension (Grass FT.03 force transducer) developed by the gill (Fig. 1A). Microelectrodes, filled with 0.6*M* K_2SO_4, were used for intracellular recordings. Suction electrodes were used to electrically stimulate specific nerves after PVG removal.

We first determined the effect of repeated electrical stimulation of the branchial and ctenidial nerves, which are motor pathways from the PVG to the gill, on gill movements after the PVG was removed (Fig. 1B). Others reported that habituation of gill movements does not occur with repeated electrical stimulation (*3*). Single pulses (0.5 to 0.7 msec in duration, about 25 volts), were applied to the nerves at the rate of one every 30 seconds. This interstimulus interval is commonly used in habituation paradigms (*1–4*). Repeated branchial nerve stimulation caused the amplitude of the gill movement to decrease (Fig. 1B). In 13 out of 18 preparations the response amplitude decreased (Fig. 1B, curve a) at a rate comparable to habituation elicited by tactile siphon stimulation when the PVG is present (*6–8*). In the other five cases the response amplitude increased initially and then decreased (curve c; see legend). A train of pulses applied to the ctenidial nerve, interposed between two trials of branchial nerve stimulation, resulted in dishabituation (curve a). The recovery, after rest, of the habituated withdrawal had the same time course as that from habituation elicited by tactile siphon stimulation (*4*). With repeated ctenidial nerve stimulation, the amplitude of the gill movement was more variable, decreased more slowly (curve b), and often resulted in sensitization-like responses in some preparations (curve d). Branchial nerve stimulation interposed between two stimuli ap-

plied to the ctenidial nerve resulted in prolonged suppression of the movement (curve b). These results show that stimulation of one nerve affects the response amplitude elicited by the other. The interaction between the two presumably occurs in neurons located in the gill and not at junctions between PVG neurons and gill muscles (*1, 9*). Ctenidial nerve dishabituatory effect on branchial nerve–elicited movements can be explained by dual excitatory innervation of muscles [see (*10*)], but such innervation cannot explain the branchial nerve suppressive effect on ctenidial nerve–elicited movements. These results establish that central efferent pathways in the gill are sites of habituation.

We then determined the contribution of the peripheral nervous system to habituation of the gill withdrawal reflex in response to tactile stimulation of the siphon. Habituation was measured before and after removal of the PVG (Fig. 1C), with each preparation serving as its own control (*11*). Punctate stimuli of 4 g were applied to the inner siphon surface (*12*) once every 30 seconds; these conditions were the same as those used previously (*6, 7*). Before PVG removal, the reflex amplitude habituated (Fig. 1C) with the same time course as shown earlier [figure 3A in (*4*)]. Dishabituation of the reflex was caused by stimulation of the gill with the same punctate stimulus (Fig. 1C). The PVG was then removed and 3 hours of rest followed [see (*11*)]. Siphon stimulation still elicited the gill withdrawal reflex of comparable amplitude to that before removal [but sometimes the amplitude was greater or smaller at the same intensity (see Fig. 2A)]. The reflex habituated to repeated stimulation with the same time course as that observed before removal. Gill stimulation caused dishabituation of the reflex (Fig. 1C). These results show that the peripheral nervous system mediates habituation between the siphon and the gill in the absence of the central nervous system. Yet, as shown below, the central nervous system definitely regulates the reflex.

Our finding that the amplitude of the withdrawal response after removal of the PVG was equal to or greater than the response with the PVG attached was intriguing. We investigated this further by sequentially cutting nerves to the PVG. The response was tested with the three nerves intact and then again 40 minutes after the siphon nerve was cut. The response amplitude to a 10-g stimulus ($N = 4$) was reduced to an average of 14 percent of the response with the nerves uncut. We then cut the branchial and ctenidial nerves, thus removing the PVG, waited 40 minutes, and tested the response again. The average amplitude was 280 percent of that after the siphon nerve was cut. Cutting the nerves in all possible sequences showed that cutting the branchial nerve alone was responsible for the enhancement of the response (Fig. 1D), which was still observed the next day. The branchial nerve apparently mediates a tonic suppressive influence on the amplitude of the peripherally mediated gill reflex to siphon stimulation, as it does to direct gill stimulation (*11*).

We further investigated the reflex amplitude by perfusing the PVG with isosmotic $MgCl_2$ (inset, Fig. 2B). It was reported that the amplitude of the response was reduced to about 15 percent of control by perfusing

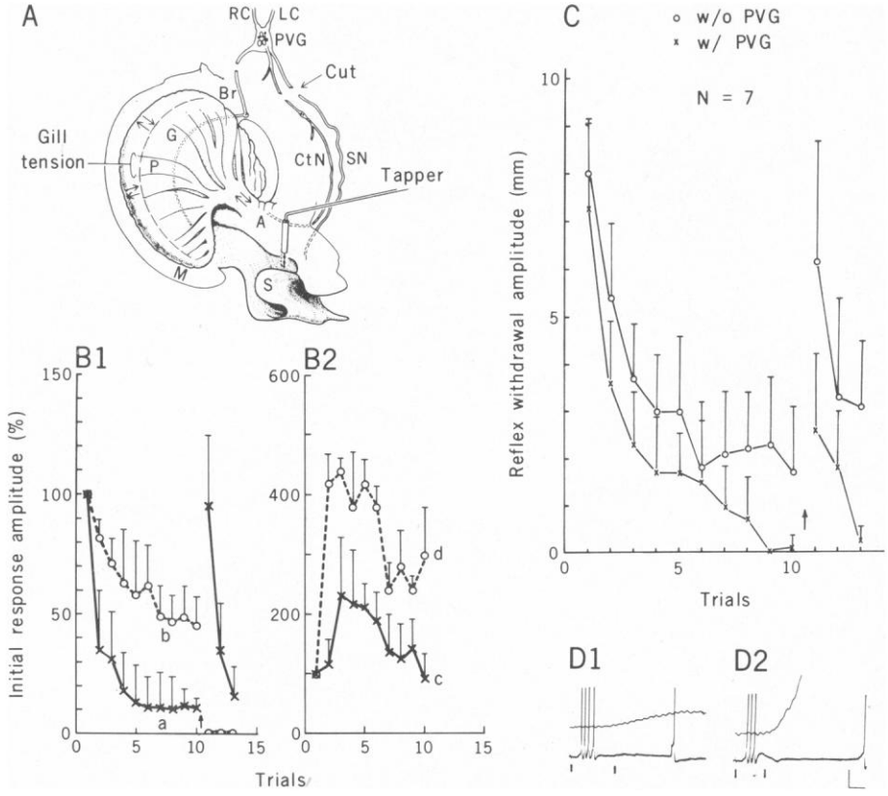

Fig. 1. (A) Ventral view of the parietovisceral ganglion (*PVG*), siphon, mantle (*M*), and gill preparation. The *PVG* innervates the gill (*G*) by way of the branchial (*Br*) and ctenidial (*CtN*) nerves and innervates the siphon (*S*) via the siphon nerve (*SN*). The left (*LC*) and right (*RC*) connectives connect the *PVG* to the pleural ganglia. Surgical thread was tied around the pinnules (*P*) of the gill and led to a force transducer to record gill tension in response to tactile stimulation of the siphon or to electrical stimulation of *Br* or *CtN*. The tactile stimulator (*Tapper*) applied punctate stimuli to the siphon or to the gill. Bidirectional arrows on the afferent vessel (*A*) and *P* of the gill show the movements evoked by siphon stimulation before and after *PVG* removal. The *CtN* mediates contraction of *A* and *P*, and the *Br* mediates anterior rotation of the gill and contraction of the efferent vessel (*1*), not shown. The *PVG* was removed by cutting (*Cut*) the nerves as shown. (B) Habituation of the gill movements elicited by stimulation of *Br* and *CtN*, at 30-second intervals. Stimulation of each nerve caused a characteristic movement described in (A). The amplitude of the first response in a session was taken as 100 percent. (B1) Curve a shows the mean response ($N = 13$) to *Br* stimulation. Curve b shows the results for *CtN* stimulation ($N = 4$). (Arrow) Interposition of a stimulus to the other nerve between trials 10 and 11: in curve a *CtN* was stimulated, 8 pulses per second for 1 second, and resulted in dishabituation; in curve b, *Br* stimulation with a single pulse caused suppression of *CtN*-elicited movement. (B2) In 5 of 18 preparations *Br* stimulation elicited gill withdrawal that increased in amplitude [sensitization (*17*)] prior to decrease in amplitude (curve c). In three of seven preparations *CtN* stimulation elicited similar responses (curve d). Standard deviation is given at each point. (C) Habituation of the gill withdrawal reflex in response to siphon stimulation (4 g) before (x) and after (○) removal of the *PVG*. The reflex withdrawal measured here was a whole gill withdrawal and not simply a pinnule response. The measurements were made from polygraph records and the mean amplitude in millimeters is shown ($N = 7$). Tactile stimulation (4 g) of the gill (arrow), applied between trials 10 and 11, dishabituated the reflex withdrawal to siphon stimulation, before and after *PVG* removal. (D) Gill withdrawal and the response in neuron L_7 to a 4-g stimulus applied to the siphon. (D1) The response with all three nerves (*Br, CtN*, and *SN*) intact, after a 1-hour rest. The latency of gill reflex (note vertical bars) was 240 msec and that of L_7's first spike was 60 msec. (D2) After the branchial nerve was cut and after a 1-hour rest, the gill withdrawal amplitude increased and the latency decreased to 160 msec. L_7 response latency was the same as in (D1). Note that although the withdrawal amplitude increased the number of spikes evoked in L_7 [a gill motor neuron (*3, 6*)] decreased, showing that L_7's activity did not contribute to the increase. The photograph of the oscilloscope trace was retouched to show L_7's spike amplitude. Scale: 20 mv and 100 msec.

the PVG with isosmotic $MgCl_2$ which blocks synaptic activity in the PVG evoked by siphon stimulation (*6*). Exposing the PVG to isosmotic $MgCl_2$ ($N = 4$), or to isosmotic sucrose, gave results similar to removal of the PVG (Fig. 2A).

The amplitude of the reflex withdrawal was dependent upon stimulus intensity. The response amplitudes before and after PVG removal were compared by forming a ratio of the amplitudes (without PVG/with PVG) over a range of intensities of siphon stimulation (Fig. 2A). A 200-mg stimulus elicited a gill response with the PVG present but not after its removal; therefore, the ratio was zero. Stimuli of 2 g and greater reliably elicited the reflex withdrawal before and after removal of the PVG, and the median ratio approached 1 (Fig. 2A) (see inset, Fig. 2B).

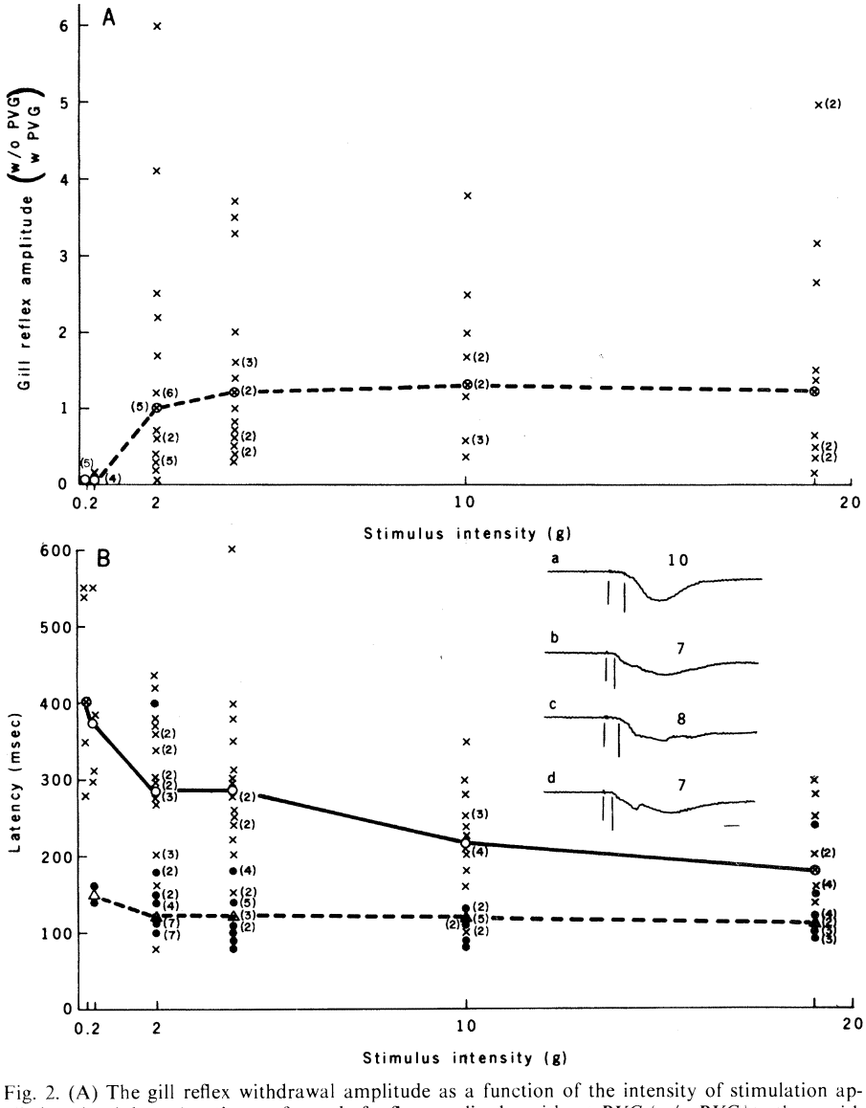

Fig. 2. (A) The gill reflex withdrawal amplitude as a function of the intensity of stimulation applied to the siphon. A ratio was formed of reflex amplitudes without *PVG (w/o PVG)* to those with *PVG* (*w PVG*). A ratio of 1 denotes no change in amplitude before and after *PVG* removal; a ratio less than 1 denotes a larger amplitude with the *PVG* present; a ratio greater than 1 denotes a larger amplitude without *PVG*. The dashed line is drawn through the median ratio at each intensity. Rest intervals of 30 to 60 minutes were interposed between tests to avoid decrement of the reflex amplitude resulting from carry-over effects from previous stimuli. The numbers in parentheses are the number of preparations that gave the same ratio. One of the striking findings was the greater variability of the response amplitude with the *PVG* present, which accounts for the great range of ratios. Without the *PVG*, the amplitude was more stable. (B) Latency of the gill reflex withdrawal as a function of stimulus intensity applied to the siphon. The latency for each preparation denoted by (x) before and (●) after *PVG* removal. Numbers in parentheses are the number of preparations giving the same latency. The solid line connects median latency at each intensity with the *PVG* present and the dashed line connects median latencies after *PVG* removal. The *PVG* regulation of the gill withdrawal reflex appears to depend on the intensity of peripheral stimulation [see (*5*)]. (Inset) Recordings of the gill withdrawal reflex to a siphon stimulation of 4 g. The first vertical line is the stimulus onset and the second is the response onset. (a) Latency of 240 msec with the *PVG* intact; (b) latency of 120 msec with the *PVG* perfused with 370 m*M* $MgCl_2$; (c) latency of 220 msec after $MgCl_2$ wash-out; and (d) latency of 140 msec after *PVG* removal. The *PVG* was perfused with $MgCl_2$ for 30 minutes before siphon stimulation, and 30- to 45-minute rest intervals were interposed between each test. These results show the equivalence of *PVG* removal and blocking with $MgCl_2$ with respect to withdrawal amplitude and its latency. Perfusing the *PVG* with isosmotic (710 m*M*) sucrose gave similar results. Time scale, 220 msec.

The latency of the gill reflex markedly decreased at each stimulus intensity after removal of the PVG (Fig. 2B). This decrease was most apparent at lower intensities (Fig. 1D) (inset, Fig. 2B). At higher intensities the latency with the PVG present approached that obtained after PVG removal. Decreases in latency, of the same magnitude as those produced by PVG removal, were also obtained by exposing only the PVG to isosmotic $MgCl_2$ (inset, Fig. 2B), or isosmotic sucrose. Thus, the results are not attributable to residual excitability changes in peripheral neurons as a result of cutting nerves, but are due to removal of a tonic PVG influence (compare Fig. 1D and inset, Fig. 2B).

These experiments show the following. (i) The terminations of the two major nerves in the gill from the PVG are sites of habituation since they show decrement, recovery with rest, and dishabituation (*13*). Repeated activation of the terminations leads to response decrement, with a time course comparable to habituation produced by siphon stimulation with the PVG present. This suggests that habituation occurs in these terminals as well as at sites in the PVG when the gill reflex is activated through the PVG. Thus adaptive change in the terminals contributes to habituation even when the siphon is isolated from the rest of the preparation [figure 1C in (*6*)] or when the siphon nerve is electrically stimulated in a habituation paradigm (*8*). (ii) We have also demonstrated an interaction between the terminals of the branchial and ctenidial nerves in the gill. This interaction in the gill presumably occurs in neuron-neuron junctions involving gill neurons (*1*, *9*), although its occurrence in neuromuscular junctions cannot be excluded (*6*, *10*). (iii) A peripheral pathway that mediates habituation exists between the siphon and the gill. The gill withdrawal reflex elicited by stimulating appropriate receptive fields on the siphon (*12*) after removal of the PVG can be comparable in amplitude to that before removal; the time course of habituation is comparable. (iv) The habituated reflex is dishabituated by tactile gill stimulation, thus demonstrating an interaction in the periphery between the gill and the siphon. (v) The PVG has a lower threshold for initiating reflexes to siphon stimulation than the peripheral nervous system. (vi) The PVG regulates the contri-

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

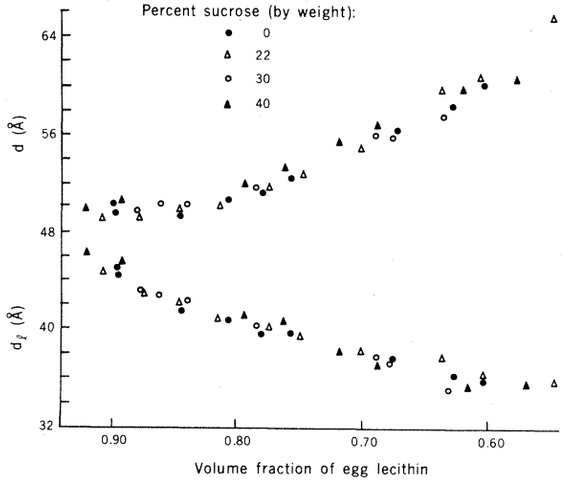

Fig. 1. Structural parameters of the lamellar phase formed by egg lecithin in pure water and in 22, 30, and 40 percent sucrose solutions (*5*). The abscissa gives the volume fraction ϕ of egg lecithin in the total mixture. The ordinate gives d and d_l. In the single-phase system formed with fixed ratios 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*).