Diphasic Postsynaptic Potential: A Chemical Synapse Capable of Mediating Conjoint Excitation and Inhibition

Abstract. *Two identified interneurons in each buccal ganglion of* Aplysia *can mediate conjoined excitation and inhibition to a single follower cell. A single presynaptic action potential in one of these interneurons produces a diphasic, depolarizing-hyperpolarizing synaptic potential apparently as a result of a single transmitter acting on two types of postsynaptic receptors in the follower cell. These receptors produce synaptic potentials with differing reversal potentials, ionic conductances, time courses, rates of decrement with repetition, pharmacological properties, and functional consequences. The excitatory receptor controls a sodium conductance, the inhibitory receptor controls a chloride conductance. Both components of the synaptic potentials can be produced by iontophoretic application of acetylcholine on the cell body of the follower cell, and each component is differentially sensitive to different cholinergic blocking agents.*

Four identified, presumably cholinergic interneurons in the buccal ganglia of *Aplysia* each mediate both excitatory and inhibitory synaptic actions to different follower cells (*1*). We now report that these cells also mediate a novel type of dual chemical synaptic action to a single follower cell. A single action potential in one of these neurons produces a diphasic, depolarizing-hyperpolarizing, synaptic potential in the postsynaptic cell by the concomitant activation of excitatory and inhibitory receptors. The properties of the receptors and those of the receptors previously described for a dual synapse in the abdominal ganglion of *Aplysia* (*2*, *3*) indicate that similar excitatory and inhibitory receptor components can be combined in functionally different ways because of differences in their kinetics of activation and desensitization.

Each of the two symmetrical buccal ganglia of *Aplysia* contains two interneurons, BL_4 and BL_5 (left buccal ganglion) or BR_4 and BR_5 (right buccal ganglion), which mediate identical actions to a common, ipsilateral, follower cell population (*1*). Each interneuron mediates direct hyperpolarizing postsynaptic potentials (PSP's) to six identified follower cells (BL_3, BL_6, BL_8, BL_9, BL_{10}, BL_{11} or BR_3, BR_6, BR_8, BR_9, BR_{10}, BR_{11}) and depolarizing PSP's to two other identified follower cells (BL_7, BL_{13} or BR_7, BR_{13}) in each ganglion.

The hyperpolarizing PSP's in all six inhibitory follower cells are similar and behave as a single-component inhibitory postsynaptic potential (IPSP). When the membrane potential was decreased (depolarized) the IPSP became larger. When the membrane potential was increased (hyperpolarized) (Fig. 1A) the IPSP became progressively smaller and at a mean membrane potential ($n = 25$) of -74 ± 8 mv S.D. (14 mv hyperpolarized from an average resting potential of -60 mv) the IPSP was nullified. Further hyperpolarization caused the IPSP to invert to a depolarizing PSP (Fig. 1A). The IPSP has a Cl^- conductance component. When Cl^- was replaced by propionate, an impermeant anion, the IPSP inverted to a depolarizing potential at the resting membrane potential (Fig. 2, B1 and B2).

The depolarizing PSP in one of the two types of excitatory follower cells

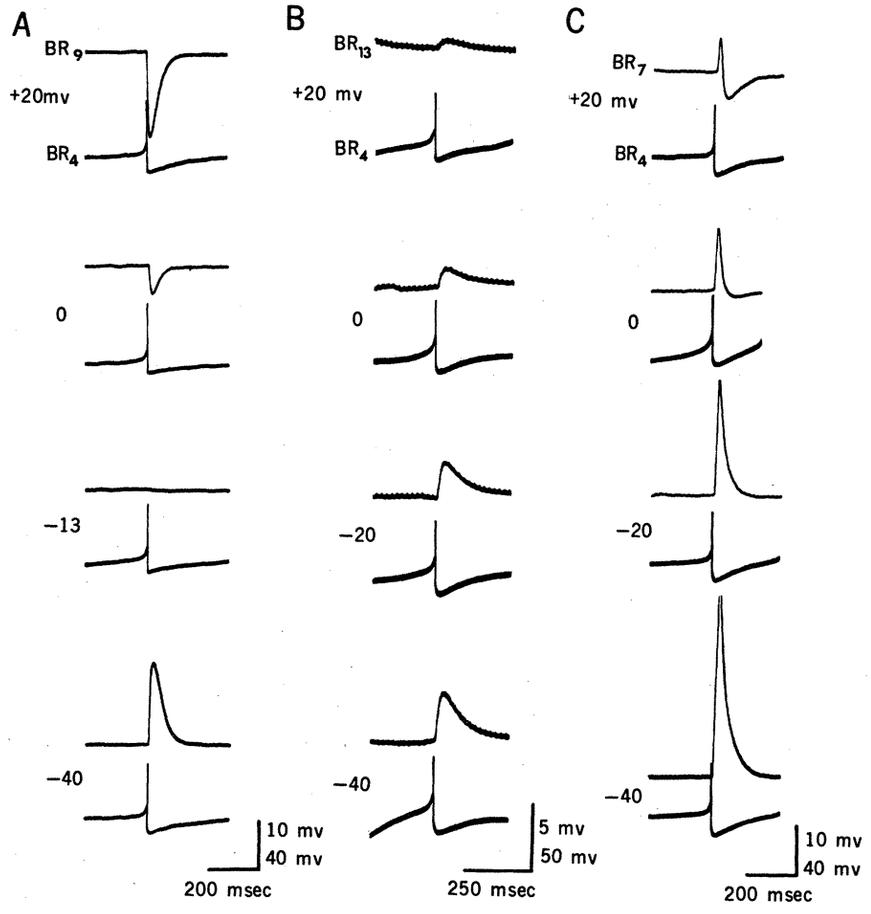

Fig. 1. Three types of synaptic actions produced by a single interneuron in three different follower cells in the buccal ganglia. (A) The IPSP's produced in cell BR_9 (upper traces) by action potentials in interneuron BR_4 (lower traces). The 0 mv represents resting membrane potential (absolute value, −63 mv). (B) The EPSP's in cell BR_{13} (upper traces) produced by action potentials in interneuron BR_4 (lower traces). The 0 mv represents resting membrane potential (absolute value, −52 mv). (C) Two-component PSP's produced in cell BR_7 (upper traces) produced by an action potential in interneuron BR_4 (lower traces). The 0 mv represents resting membrane potential (absolute value, −45 mv). As the membrane potential is progressively depolarized from the resting membrane potential, the presence of a second hyperpolarizing component becomes evident. This synaptic component is usually small or masked at the resting membrane potential. In (A), (B), and (C), the ganglia were bathed in seawater containing 60 m*M* Ca^{2+}. Positive numbers are millivolts depolarized, negative numbers are millivolts hyperpolarized from the resting membrane potential.

(BL_{13} and BR_{13}) also behaved as a single-component PSP (Fig. 1B). When the membrane was depolarized the excitatory postsynaptic potential (EPSP) became smaller. The extrapolated mean reversal potential was -14 ± 4 mv ($n = 3$). This PSP involved an increased conductance to Na^+ and was reduced or abolished in low Na^+ seawater. However, in the other type of excitatory follower cell (BL_7 and BR_7), the apparently depolarizing PSP proved to be diphasic (Fig. 1C). Changing the membrane potential revealed that this synaptic potential consisted of two different components that could be distinguished by their reversal potentials, pharmacological properties, and ionic mechanisms.

Near the resting membrane potential, the PSP in this follower cell often resembled an elementary, one-component EPSP (Fig. 1C). However, as the membrane potential was reduced, a late inhibitory component, which was small or absent at the resting membrane potential, became evident. The mean extrapolated reversal potential ($n = 5$) for the first component was -10 ± 7.3 mv (resembling the elementary EPSP in BL_{13}), whereas the mean reversal potential ($n = 10$) of the second component was -63 ± 6.4 mv [resembling the elementary IPSP in BL_3, see (*4*)]. The threshold for firing of cells BL_7 and BR_7 is about -40 mv. As a result the first component is excitatory, whereas the second component is inhibitory. The two actions appear to result from a direct connection. Presynaptic action potentials produced both PSP's in the follower cell with a constant and short latency at high frequencies of firing even in high Ca^{2+} seawater solutions that would tend to prevent the firing of an intercalated interneuron.

Each of the two components could be selectively blocked by different cholinergic blocking agents (*5*). Hexamethonium (10^{-4} g/ml), which blocks the elementary EPSP in the purely excitatory follower cells but does not affect the elementary IPSP in the purely inhibitory follower cells, also blocked the excitatory component in BL_7 and BR_7 without significantly affecting the inhibitory component (Fig. 2, A1 and A2). Curare (*d*-tubocurarine) (5×10^{-5} g/ml) preferentially reduced the hyperpolarizing component in BL_7 and BR_7, often leaving the depolarizing component relatively unaffected (Fig. 2, A3). Slightly higher concentrations of curare (2.5×10^{-4} g/ml) blocked both components in the dual follower cell, as well as the elementary IPSP and EPSP in BL_3 and BL_{13} (Fig. 2, B3). Selective blockade of one or the other of the two synaptic components revealed that the isolated EPSP (Fig. 2, A3) peaked earlier and decayed faster than the isolated IPSP (Fig. 2, A2). This difference in time course of the two components accounts for the diphasic configuration of the dual synaptic potential.

The synapse-free cell body of BL_7 and BR_7 contains receptors to chemical transmitters, the properties of which resemble those of the receptors found in the synaptic region of the neuron [see also (*1–3, 5*)]. The cells responded to iontophoretic applications of acetylcholine (ACh) with diphasic, depolarizing-hyperpolarizing, or, more frequently, hyperpolarizing-depolarizing responses that resembled the PSP's produced in these cells by presynaptic action potentials (Fig. 3, A1). In some experiments we obtained either a depolarizing-hyperpolarizing or a hyperpolarizing-depolarizing sequence by moving the electrode from one position on the cell body to another (Fig. 3, A1b and A1c). The variability in the sequence of the components may represent a differential distribution of the two types of receptors in different parts of the soma membrane (*6*). The two components of the ACh response persisted in high Mg^{2+} solution (six times normal) that blocks chemical transmission in *Aplysia* (*3*), indicating that both components were produced by ACh acting directly on the follower cell and not by way of other neurons (*7*).

The components of the ACh response resembled the corresponding components of the synaptic potential in their reversal potential (Fig. 3, A2) (*8*), sensitivity to cholinergic blocking agents, and response to changes in ionic concentrations (Fig. 3, A3). The depolarizing component of the ACh response was reduced by replacing Na^+ in the

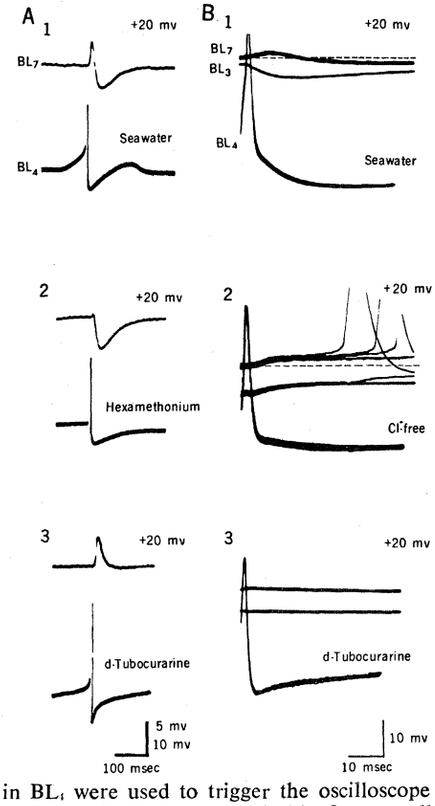

Fig. 2. Pharmacological properties and ionic mechanisms of the two components of the dual synaptic potential. (A) Differential sensitivity of the two synaptic components to cholinergic blocking agents. In all three parts of this figure the membrane potential of follower cell BL_7 was maintained at 20 mv depolarized from the resting membrane potential. (A1) In normal seawater an action potential in BL_4 (lower trace) produces a diphasic conjoint PSP in BL_7 (upper trace). (A2) With the ganglia bathed in seawater containing hexamethonium (10^{-4} g/ml), the depolarizing component of the dual PSP is reversibly blocked and an action potential in BL_4 produces only a hyperpolarizing response in BL_7. The ganglia were perfused with control seawater solutions to wash out the hexamethonium and then bathed in seawater containing *d*-tubocurarine (7.5×10^{-5} g/ml) (A3). In seawater containing this concentration of *d*-tubocurarine (see B3) the hyperpolarizing synaptic component was reversibly blocked and an action potential in BL_4 produced a depolarizing PSP in BL_7. (B) Comparison of the two components of the dual synaptic potential in cell BL_7 to the purely hyperpolarizing synaptic potential in BL_3. Simultaneous intracellular recordings from interneuron BL_4 (lower traces) and two of its follower cells, BL_7 (upper traces) and BL_3 (middle traces). Action potentials in BL_4 were used to trigger the oscilloscope sweep, and several sweeps were superimposed. In all three parts of this figure, cell BL_7 was depolarized by approximately 20 mv, whereas BL_3 was kept at its resting membrane potential. (B_1) In normal seawater an action potential in cell BL_4 produced a characteristic diphasic synaptic potential in BL_7. (B2). Substituting propionate for Cl^- in the seawater bathing medium produced an inversion of both the second hyperpolarizing synaptic component in BL_7 and the elementary hyperpolarizing IPSP in BL_3 to a depolarizing synaptic potential. (B3) Seawater solutions containing a high concentration of *d*-tubocurarine (5×10^{-4} g/ml) abolished nonselectively both synaptic components of the BL_7 PSP as well as the IPSP in BL_3 (see A3). A small brief residual coupling sometimes remained after treatment with *d*-tubocurarine. This potential was due to variable amounts of electrotonic coupling between the interneuron and its various follower cells. All solutions contained 60 m*M* Ca^{2+}.

seawater bathing solution with tris(hydroxymethyl)aminomethane and the hyperpolarizing component of the ACh response was inverted by replacing external Cl^- with propionate (Fig. 3, A3).

The two components of the dual synapse also differed in their response to repeated presynaptic stimulation. Although both components decreased with repeated stimulation, the decrease was greater for the hyperpolarizing component (Fig. 3, B1). This preferential decrease in inhibitory synaptic efficacy appears to be due to a preferential desensitization of the inhibitory receptor. A similar decrease in the inhibitory component was noted when responses to consecutive iontophoretic ACh pulses were examined (Fig. 3, B2). The first ACh pulse produced a diphasic response, but a second pulse applied within the next several seconds produced only a pure depolarizing response. These changes in response amplitude were not associated with changes in membrane potential. Moreover, the rate of decrement for the hyperpolarizing component was the same whether or not the depolarizing component of the PSP or the ACh response was blocked with hexamethonium. Thus here, as in the dual abdominal ganglion synapse (*3*), receptor desensitization (*9*) may serve a physiological role in regulating synaptic function.

It is interesting to compare our re-

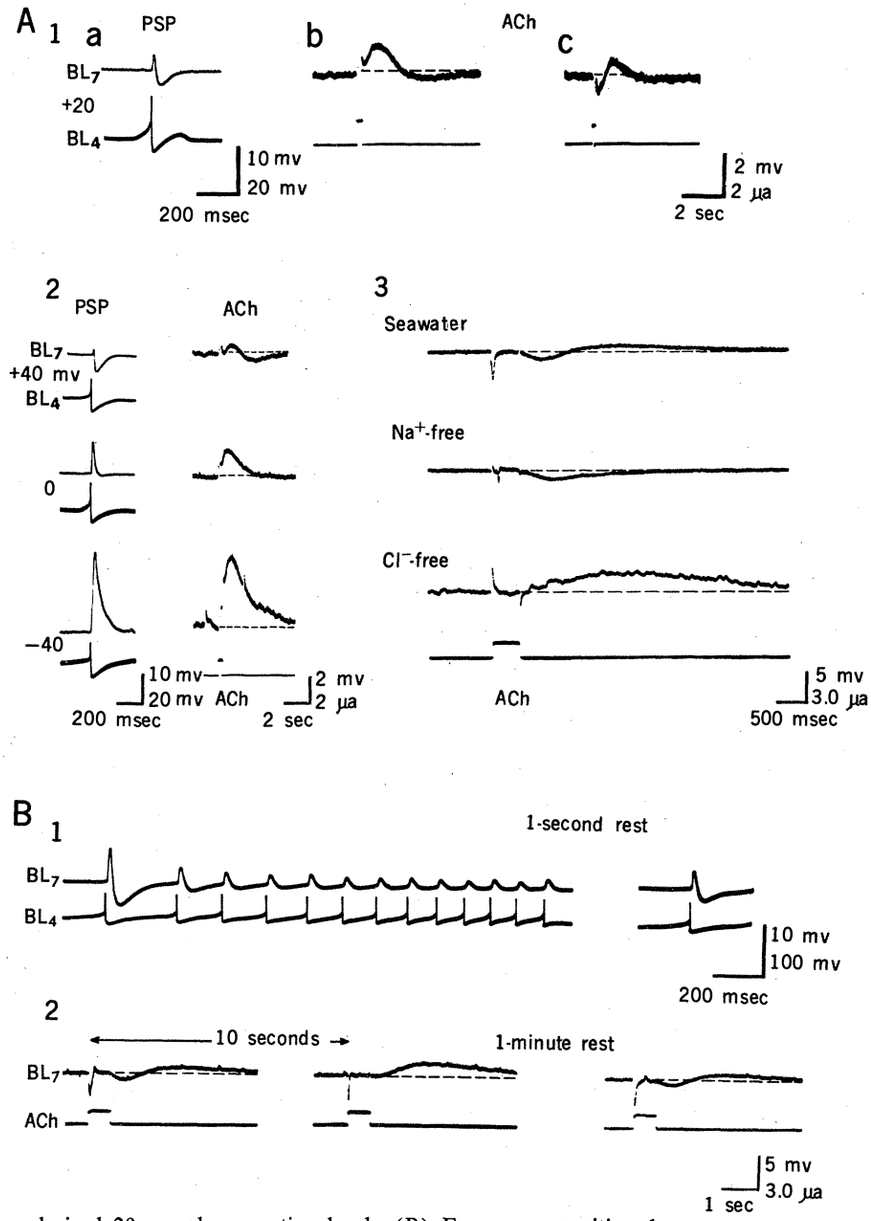

Fig. 3. (A) Comparison of diphasic synaptic potential and ACh responses in cell BL_7. (A1) and (A2) were obtained in the same experiment; cell BL_7 depolarized 20 mv above the resting membrane potential. (A1a) Simultaneous intracellular recordings showing conjoint diphasic depolarizing-hyperpolarizing PSP produced in BL_7 (upper trace) by a spike in interneuron BL_4 (lower trace). (A1b) Diphasic depolarizing-hyperpolarizing ACh response produced in the same cell by iontophoretic application of ACh (monitored on lower trace). (A1c) By altering the position, pressure, and pulse parameters of the ACh electrode, the response changed from depolarizing-hyperpolarizing to hyperpolarizing-depolarizing. Ganglia were bathed in solutions containing 60 m*M* Ca^{2+}. (A2) Comparisons of the dual synaptic potential and depolarizing-hyperpolarizing ACh responses in cell BL_7 at different membrane potentials. Same experiment as in (A). (*PSP*) Simultaneous intracellular recordings of dual PSP produced in BL_7 (upper traces) by spikes in interneuron BL_4 (lower traces) at different membrane potentials. (*ACh*) Response produced in BL_7 by iontophoretic application of ACh. Iontophoretic current magnitude and duration shown on lowest trace. The 0 represents resting membrane potential (absolute value, −58 mv). Note that the second component of both the PSP and the ACh response is prominent at 40 mv above the resting membrane potential, is reduced and largely nullified at the resting membrane potential, and is inverted at 40 mv below the resting membrane potential. (A3) Ionic mechanisms of the diphasic ACh response in BR_7. In each case, constant current and duration iontophoretic pulses of ACh were delivered to the cell body of BR_7. Upper trace, seawater. Middle trace, in Na-free seawater the depolarizing component was abolished and the ACh pulse produced a purely hyperpolarizing response. Lower trace, in Cl-free water, the hyperpolarizing component inverted, producing a purely depolarizing response (see also Fig. 2, B2). Cell BR_7 depolarized 20 mv above resting level. (B) Frequency sensitive decrease of the diphasic postsynaptic potential and ACh responses in follower cell BL_7. (B1) Simultaneous recordings from interneuron BL_4 and follower cell BL_7. Repetitive firing of BL_4 causes a decrement of the amplitude of both components of the dual PSP, but the hyperpolarizing component decreased more than did the depolarizing component. (B2) Desensitization of the dual follower cell response to ACh. Intracellular recordings from dual follower cell BL_7 (upper traces). The lower traces monitor iontophoretic current magnitude and duration. Three identical pulses of ACh were applied iontophoretically onto the cell body of the dual follower. The first two pulses were spaced 10 seconds apart, whereas the second and third pulses were separated by 1 minute. With BL_7 depolarized, the first pulse produces a diphasic hyperpolarizing-depolarizing response. The second pulse yields only a depolarization. A 1-minute rest restored the diphasic response. In both (B1) and (B2) the follower cell was depolarized 15 mv above the resting membrane potential to show both components of the dual synaptic response.

sults on multiaction cells in the buccal ganglia to those obtained on cell L10, a multiactioned cholinergic neuron in the abdominal ganglion of *Aplysia* (*2, 3*). In both ganglia a single presynaptic neuron can act on inhibitory and excitatory receptors in different follower cells to produce opposite synaptic actions. In addition, some follower cells in each ganglion have both types of receptors to ACh so that the presynaptic cell can mediate opposite synaptic actions to a single follower cell. In the dual follower cell of the abdominal ganglion the two types of receptors have different kinetic properties so that an action potential in the presynaptic cell produces depolarizing PSP's at low rates of firing and hyperpolarizing PSP's at high rates of firing (*3*). The excitatory receptors have a low threshold for activation but are desensitized at high rates of stimulation; the inhibitory receptors have a higher threshold for activation and their action becomes most apparent at high rates of stimulation. By contrast, in the dual follower cells of the buccal ganglia the two receptor types have similar thresholds to ACh so that a single presynaptic action potential activates both sets of postsynaptic receptors concomitantly, producing a diphasic PSP. As a result of these differences, the sign of the abdominal ganglion dual synapse is frequency dependent, excitatory at low frequencies and inhibitory at high frequencies, whereas the sign of the buccal ganglion dual synapse is dependent on membrane potential as well as frequency. The buccal ganglion dual synapse tends to be primarily excitatory near the resting membrane potential and to become progressively more inhibitory as the membrane is depolarized, by other inputs or by injected current, and the threshold is raised by accommodation. The buccal ganglion synapse is also sensitive to the frequency of firing of the presynaptic neuron. At high rates of firing both components decrease but the second component is more affected because the inhibitory receptors appear to desensitize more rapidly than the excitatory receptors. It therefore appears possible for a nervous system to employ otherwise similar receptor components in very different ways by varying the sequence of their activation and their kinetics for desensitization.

One of the striking features to emerge from studies of these several cholinergic neurons in *Aplysia* (*2, 3, 5, 10*) is that a large variety of synaptic actions can be triggered by a single chemical transmitter compound. In principle it therefore appears possible to construct a ganglionic mass or even a whole nervous system by using only one transmitter substance and by simply varying the types of receptor, the combination of receptors, and their sequence of activation in the postsynaptic cells. That this is not generally the case suggests that different transmitters may be necessary for other purposes, such as cellular recognition or trophic maintenance of synaptic contacts, than for providing different types of synaptic actions.

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4. To obtain a more reliable measure of the reversal potential the two components were examined separately by using hexamethonium (10^{-4} g/ml) to block the excitatory component and *d*-tubocurarine (5×10^{-5} g/ml) to block the inhibitory component.
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7. In addition to having been described in the abdominal ganglion of *Aplysia* (*3*) diphasic ACh responses have been noted in neurons of *Navanax* (H. Levitan and L. Tauc, personal communications) and in dissociated mouse neuroblastoma cells [P. G. Nelson, J. H. Peacock, T. Amano, *J. Cell Physiol.* **77**, 353 (1971)]. Diphasic responses to *l*-glutamate are seen in neurons of *Anisodoris* [A. L. F. Gorman and M. F. Marmor, *Fed. Proc.* **30**, 323 (1971)]. Finally, biphasic PSP's have been found in the left pleural ganglion of Aplysia [G. M. Hughes and L. Tauc, *J. Physiol. London* **197**, 511 (1968)]. These PSP's are mediated by electrotonic coupling between cells, and not by the action of a chemical transmitter [M. Biedebach, J. M. Meunier, L. Tauc, *J. Physiol. Paris* **60**, 220 (1968)].
8. In five paired experiments the reversal potentials for both PSP and ACh response were directly compared. With hexamethonium in the seawater the reversal potential for the inhibitory component was -62.8 ± 6.3 mv for the PSP and -61.0 ± 6.3 mv for the ACh response. With *d*-tubocurarine in the seawater the extrapolated reversal potential for the excitatory component was -12.4 ± 5.9 mv for the PSP and -18 ± 6.1 mv for the ACh response. The values for the PSP and ACh responses were not significantly different ($P > .1$). In some experiments, desensitization rather than *d*-tubocurarine was employed to isolate the ACh excitatory component.
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11. Supported by PHS grant NS 09361, by a fellowship to D.G. from a PHS predoctoral training grant GM 00920 to the Department of Physiology, New York University Medical School, and by a Career Scientist Award 5K5-MH 18558-03 to E.R.K. We thank M. V. L. Bennett, V. F. Castellucci, J. Koester, H. Koike, and I. Kupfermann for their comments on an earlier draft of this manuscript and K. Hilten for help in preparing the illustrations.
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29 October 1971; revised 20 December 1971 ■

Cholesteric Liquid Crystal-Like Structure of the Cuticle of Plusiotis gloriosa

Abstract. *The toroidal but parallel array of planes of unidirectionally oriented molecules believed to characterize cholesteric liquid crystals also gives rise to certain geometrical patterns. The reality of this structure is demonstrated by micrographic evidence.*

Michelson (*1*) was first to note that the shell of the beetle *Plusiotis resplendens* reflects circularly polarized yellow light when white light is incident on the shells. Later, Friedel and others (*2*) noted this phenomenon in cholesteric crystals. Shortly afterwards, Gaubert (*3*) studied the phenomenon as represented by a variety of beetles.

Robinson (*4*) has treated and related the combined phenomena, as shown by polypeptide solutions, beetles, and cholesteric liquid crystals, and concluded that "further research into the nature and origin of these irridescent [beetles'] elytra would be repaying." More extensive optical data of Neville and Caveney (*5, 6*) seem to confirm the conclusion.

Thus the concept that cholesteric liquid crystals effect the selective reflection of light of limited color is now even more convincing as being due to their layered structure whose periodicity approximates the wavelength of the band reflection maximum (for a medium of the average refractive index of the liquid crystal). However, the selective reflection of this light of limited color as circularly polarized light of a spe-