In cerebellar samples taken within 30 seconds after decapitation, only a few (10 to 15 percent) of the P cells exhibited positive immunofluorescence (Fig. 1D). In contrast, fluorescence in the granule cell layer was only slightly reduced in intensity and showed essentially no differences in the distribution of positively stained cellular elements. These samples showed minimal positive staining in the molecular layer or in the white matter. If the histochemical staining pattern is correlated with biochemical estimations of cyclic AMP content 30 and 120 seconds after decapitation (*8*), the previously reported rise in cyclic AMP content is manifested by increased immunocytochemical staining detectable mainly in P neurons (Fig. 1, C and D).

Cerebellar biopsies from rats anesthetized with halothane showed staining patterns essentially identical to those obtained within 30 seconds after decapitation of unanesthetized rats; moderate staining was widely distributed in the granule cell layer and within a few (10 to 20 percent) of the P cells. On the other hand, cerebellar biopsies from animals anesthetized with chloral hydrate or pentobarbital showed even less neuronal immunoreactivity than did biopsies from halothane-anesthetized rats (*12*). The reduced staining in the rats treated with chloral hydrate or pentobarbital was exhibited by both the P cells and the granule cells. These results suggest that anesthetics alter factors that regulate cyclic AMP concentrations in P cells and granule cells.

The localization of cyclic AMP within the specific neurons of the rat cerebellar cortex implies that the cyclic nucleotide is not freely diffusible within the positively stained neurons under the conditions of the staining reaction (*13*). However, no precise quantitative relation between immunocytochemical staining and direct chemical estimation of cyclic AMP content has been established, and it is not known how much cyclic AMP was lost from tissue sections during processing and whether cyclic AMP was bound extracellularly or less firmly to other cells, such as glia (*14*). Nevertheless, with the use of the immunofluorescence technique for detecting localization of cyclic AMP, the cellular distribution of this important nucleotide can now be assayed. The rise in cerebellar content of cyclic AMP after decapitation thus can be correlated with elevated concentration within the P neurons. It remains to be determined whether the alterations in immunocytochemical staining of cyclic AMP shown by P cells after decapitation can be triggered in vivo by activation of specific cerebellar afferent synaptic pathways or by specific neurotransmitters.

F. E. BLOOM, B. J. HOFFER
E. R. BATTENBERG, G. R. SIGGINS
Laboratory of Neuropharmacology, Division of Special Mental Health Research, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, D.C. 20032
A. L. STEINER
Department of Medicine, Albany Medical College, Albany, New York
C. W. PARKER, H. J. WEDNER
Division of Immunology, Department of Medicine, Washington University Medical School, Saint Louis, Missouri 63110

References and Notes

1. S. Kakiuchi and T. W. Rall, *Mol. Pharmacol.* **4**, 367 (1968); H. Shimuzu, J. W. Daly, C. R. Creveling, *J. Neurochem.* **16**, 1609 (1969); E. C. Palmer, F. Sulser, G. A. Robison, *Pharmacologist* **11**, 157 (1969).
2. L. M. Klainer, Y.-M. Chi, S. L. Freidberg, T. W. Rall, E. W. Sutherland, *J. Biol. Chem.* **237**, 1239 (1962); B. Weiss and E. Costa, *Biochem. Pharmacol.* **17**, 2107 (1968); B. Weiss and A. D. Kidman, *Advan. Biochem. Psychopharmacol.* **1**, 131 (1969).
3. T. W. Rall, *Ann. N.Y. Acad. Sci.* **185**, 520 (1971).
4. H. J. Wedner, B. J. Hoffer, E. Battenberg, A. L. Steiner, C. W. Parker, F. E. Bloom, *J. Histochem. Cytochem.* **20**, 293 (1972).
5. A. L. Steiner, D. M. Kipnis, R. Utiger, C. W. Parker, *Proc. Nat. Acad. Sci. U.S.A.* **64**, 367 (1969); A. L. Steiner, C. W. Parker, D. M. Kipnis, *Advan. Biochem. Psychopharmacol.* **3**, 89 (1970).
6. Obtained from Miles Laboratories, item 64-173, lots 12 and 13; only this partially purified goat immunoglobulin prepared against purified rabbit IgG was found to be useful in the immunocytochemical detection of cyclic AMP.
7. B. Weiss and E. Costa, *Biochem. Pharmacol.* **17**, 2107 (1968); M. J. Schmidt, D. E. Schmidt, G. A. Robison, *Science* **173**, 1142 (1971).
8. B. Breckenridge, *Proc. Nat. Acad. Sci. U.S.A.* **52**, 1580 (1964); P. Uzunov and B. Weiss, *J. Neuropharmacol.* **10**, 697 (1971).
9. O. H. Lowry, J. V. Passonneau, F. X. Hasselberger, D. W. Schulz, *J. Biol. Chem.* **239**, 18 (1964).
10. The relatively high concentrations of cyclic AMP required to block immunocytochemical staining reflect the large binding capacity of the undiluted rabbit Ig against cyclic AMP used in the staining and blocking comparisons, and has no meaning in terms of a binding constant.
11. Cerebellar biopsies were obtained by mounting the anesthetized rats in a stereotactic apparatus. The cerebellar surface was exposed and then covered with thin slabs of warm agar (3 percent, in saline); small wedges of the outer folia were removed with a Hartman alligator forceps that had been chilled in liquid nitrogen before use. The excised sample was reimmersed into the liquid nitrogen within 5 seconds.
12. M. I. Paul, G. L. Pauk, B. R. Ditzion, *Pharmacology* **3**, 148 (1970); M. J. Schmidt, J. T. Hopkins, D. E. Schmidt, G. A. Robison, *Brain Res.*, in press. When comparisons were made between the effects of the anesthetics on the postdecapitation rise in immunocytochemical staining for cyclic AMP, six to eight stained sections from each biopsy sample were coded and examined by a double-blind protocol.
13. M. Ebadi, B. Weiss, E. Costa, *Arch. Neurol.* **24**, 353 (1971); G. N. Gill and L. D. Garren, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 786 (1971).
14. A. G. Gilman and M. Nirenberg, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 2165 (1971). Although we were unable to see neuroglia distinctly stained by the immunocytochemical procedure applied to cerebellum, we have seen such cells in unstimulated sympathetic ganglia from frog and calf (F. E. Bloom, F. F. Weight, J. Kebabian, unpublished results).
15. A.L.S. is an Established Investigator of the American Heart Association. Supported by NIH grants 1R01AM15676-01 to A.L.S. and A100219-10 to H.J.W. and C.W.P. We thank F. F. Weight, M. J. Schmidt, and A. G. Gilman for critical discussions.

13 April 1972

Photoreception in a Barnacle: Electrophysiology of the Shadow Reflex Pathway in Balanus cariosus

Abstract. *The photoreceptors in the median ocellus of the rock barnacle depolarize when illuminated. This depolarization spreads passively to the axon terminals in the supraesophageal ganglion. A small number of cells in the supraesophageal ganglion hyperpolarize when the median ocellus is illuminated and depolarize when it is shadowed. Nerve impulses are superimposed on the slow depolarization of the ganglion cells. Impulse activity in response to shadowing the median ocellus is recorded in a few fibers of the circumesophageal connectives. Picrotoxin blocks this shadow-induced activity. A model of the shadow reflex pathway is presented.*

The sensory pathway that mediates the shadow withdrawal reflex in the rock barnacle *Balanus cariosus* has been studied (*1*). When a shadow passes across an animal, the normal rhythmical behavior quickly stops, the cirri are withdrawn into the shell, and the opercular plates close tightly. We have never observed a response to an increase in the light intensity in these animals.

One group of the primary photoreceptors that initiate the shadow response in *B. cariosus* is in the median ocellus (*1*). The ocellus contains six to nine photoreceptors; each photoreceptor has multiple distal dendritic branches capped by microvilli and a large axon that runs in the median ocellar nerve to the supraesophageal ganglion (*2*).

A portion of the central nervous system containing the median ocellus,

ocellar nerve, supraesophageal ganglion, circumesophageal connectives (Fig. 1A), and ventral ganglion was removed from the animal and secured in a shallow dish filled with artificial seawater (Instant Ocean). Standard electrophysiological techniques were used to monitor the neural activity at different levels of the reflex pathway (*3*).

When stimulated with light, the receptors of the median ocellus give electrical responses typical of arthropod photoreceptors. The response to a step of light is a slow depolarization (receptor potential) having an initial large transient phase which decays to a somewhat smaller but maintained steady phase (lines 1 in Fig. 1, B and C). The amplitude of the receptor potential is graded with the intensity of the light, brighter lights producing larger responses. A cell with a resting potential of −40 mv may produce a receptor potential with a 50-mv transient phase and a 20-mv steady phase when stimulated by a moderately intense light (*4*).

When the stimulus is removed, the membrane potential returns to the resting level and in many cases, especially following a very bright stimulus, goes beyond—hyperpolarizing as much as 30 mv (*5*).

Propagated nerve impulses have never been seen in the axons of the median photoreceptor of *B. cariosus* (*6*). Intracellular recordings made in the median ocellar nerve show that the receptor potential, originating in the photoreceptor soma, spreads passively along the axon to the supraesophageal ganglion (lines 2 in Fig. 1, B and C) (*7*). At the level of the supraesophageal ganglion the amplitude of the steady phase of the receptor potential is about 70 percent of its value in the soma (*8*). The distance between the photoreceptor soma and the supraesophageal ganglion was about 2 mm in most of the experimental animals. This means that the characteristic length, λ, for these axons is approximately 2.2 mm (*9*). If we assume that the axoplasmic resistivity of the ocellar nerves is the same as that found in other crustacean nerves and that the nerves are bathed in a large volume of seawater, then the membrane resistance for a fiber 15 μm in diameter and with λ equal to 2.2 mm would be about 7800 ohm-cm^2 (*10*).

The activity of a small number of cells in the supraesophageal ganglion is altered by illuminating the median photoreceptors. The number or size of these cells, or both, must be very small, for no more than two cells of this type were found in any one preparation (with 50 to 100 penetrations per preparation). The cells are usually located on either side of the midline of the supraesophageal commissure and more often in the posterior half than in the anterior half. This is in the general region of the terminal branches of the photoreceptor axons. The branching pattern of the axon terminals was studied by injecting the fluorescent dye Procion Yellow into the axon. The injections were made by applying pressure to a micropipette filled with a 2 percent solution of the dye. The major finding was that each axon bifurcates shortly after entering the commissure of the supraesophageal ganglion. Each branch then sends out several small branches, spreading out very close to the midline. The major bifurcation suggests that each photoreceptor sends information to both halves of the central nervous system.

The response of the ganglion cells to a step of light on the photoreceptors is a slow hyperpolarization with respect to the resting potential in the dark (lines 3 in Fig. 1, B and C). If a shadow is presented to the receptors (a constant light is turned off, then on again) the cells slowly depolarize. The depolarization has a characteristic larger initial transient phase followed by a small maintained phase (lines 3 in Fig. 1, B and C). It should be noted that

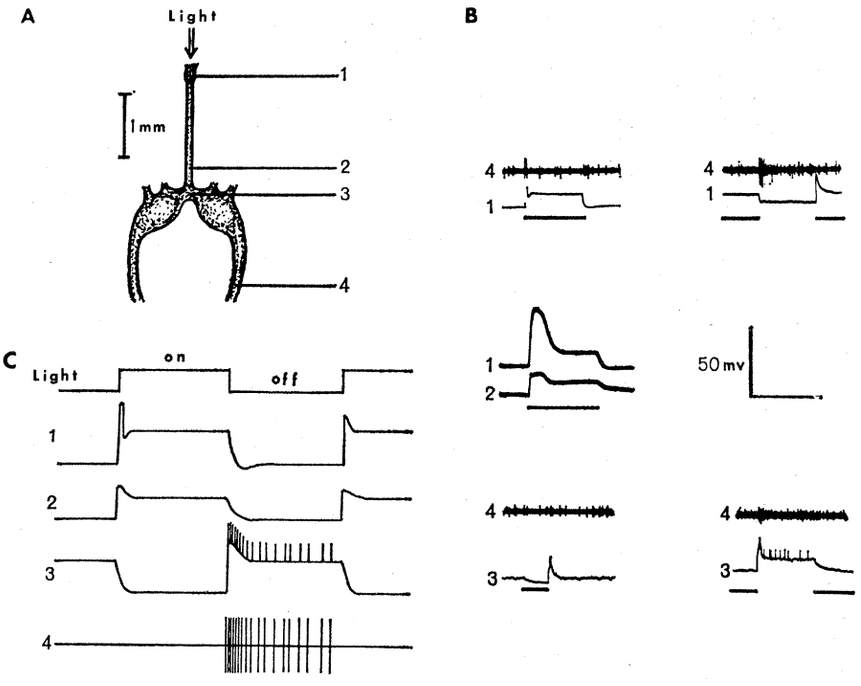

Fig. 1. (A) Schematic diagram of a portion of the central nervous system of *Balanus cariosus*. Micropipettes were used to record intracellular potentials from the photoreceptors in the median ocellus, 1; axons in the ocellar nerve, 2; and cells in the commissure of the supraesophageal ganglion, 3. A suction electrode recorded the impulse activity from the circumesophageal connective, 4. (B) Potential changes recorded from pairs of levels along the reflex pathway in response to steps of light (left column) or shadows (right column). The number preceding each record corresponds to the location of the recording electrode (see A). In the left column the solid bar beneath each pair of records indicates the duration of illumination (maximum intensity). In the right column the break in the line indicates the duration of shadow. The time scale (horizontal axis in the middle of right column) is 0.5 second for the middle pair of records in the left column and 2.5 seconds for all other pairs of records. The voltage scale of 50 mv is for all records from sites 1, 2, and 3. The zero baseline of the membrane potentials from sites 1 and 3 in the upper and lower pair of records in each column is the center of the trace from site 4. The zero baseline of the membrane potentials for the middle pair of records in the left column is not indicated. The spikes have been retouched in some records. (C) Summary of the potential changes at the four levels of the reflex pathway in response to step changes in illumination (top record). The number to the left of each record indicates the position of the recording electrode (see A). The unit activity of line 4 is for clarity and simplicity and does not mean that there is only one axon is each connective responding to the shadow. The one-to-one correspondence between the activity in the ganglion cell and that in the connective reflects only a tentative suggestion that there are no intervening synapses (see text). The amplitude and time courses of the responses are not drawn to scale, but the duration of light *on* is approximately 2 seconds.

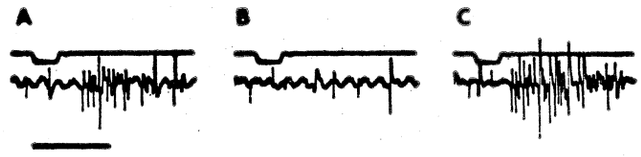

Fig. 2. Effects of picrotoxin on the shadow response. A series of 25-msec shadows was presented to the median ocellus (A) while the preparation was bathed in artificial seawater, (B) 5 minutes after picrotoxin was added to the bathing medium (final concentration 2×10^{-4} g/ml), and (C) 10 minutes after the preparation was returned to artificial seawater. The lower trace is the nerve impulse activity recorded with a suction electrode from a circumesophageal connective. The upper trace is a monitor of the illumination; a downward deflection is a decrease in the illumination from maximum intensity. The electrical artifact on the lower trace (trigger to shutter) precedes the response of the photocell monitor and is due to the slow response time of the shutter. The time scale is 50 msec (solid bar in A).

these depolarizations are with respect to the membrane potential during constant illumination, which for this cell was about −50 mv.

In most cases small nerve impulses are seen superimposed on the depolarizing responses of the ganglion cells. The amplitude of the depolarizations and the frequency of the nerve impulses are graded with the intensity of the constant illumination from which the shadow is cast. Shadowing a brightly illuminated preparation gives rise to a large depolarization and a high-frequency burst of impulses from the ganglion cell, whereas shadowing a dimly illuminated preparation gives only a small depolarization with no or only a few impulses.

Nerve impulse activity was recorded from a small number of fibers in the circumesophageal connectives when the median ocellus was shadowed (lines 4 in Fig. 1, B and C). Nerve impulse activity was also recorded from the circumesophageal connectives when a hyperpolarizing current was passed directly into an illuminated photoreceptor in the median ocellus or when a depolarizing current was removed in the dark. We did not perform the experiments necessary to demonstrate a one-to-one correspondence between the impulse activity in the ganglion cells and the activity in the nerve fibers. The time course and the amount of impulse activity in the ganglion cell and in the connective were quite similar. It is likely that the axons of the ganglion cells run in the circumesophageal connectives and give rise to the impulse activity, but further experiments are needed to establish this as fact.

The impulse activity in the connectives evoked by shadowing the median ocellus can be blocked by picrotoxin (Fig. 2). A few minutes after picrotoxin is added to the bathing medium (the final concentration of picrotoxin was 2×10^{-4} g per milliliter of seawater) the impulse activity in response to a shadow completely disappears (Fig. 2B). The background activity seems to be unaffected by the toxin. The effects are completely reversible, and the activity begins to return after a few minutes in the normal bathing solution (Fig. 2C). Picrotoxin has been shown to block the action of the inhibitory transmitter γ-aminobutyric acid (GABA) (*11*). The blocking action of this drug on the shadow-evoked activity (*12*) implies that an inhibitory synapse exists in the direct reflex pathway that extends from the photoreceptors in the median ocellus to the axons in the circumesophageal connectives. A likely place for this synapse is between the axons of the photoreceptors and the cells in the supraesophageal ganglion. We hope to establish this point with intracellular recordings in the ganglion cells.

A tentative model can be proposed for the overall shadow reflex pathway in the barnacle. The photoreceptors in the median ocellus depolarize when illuminated. This depolarization spreads passively to the axon terminals, which make synaptic connections with a few cells in the supraesophageal ganglion. The depolarized terminals liberate the inhibitory transmitter GABA, which hyperpolarizes the postsynaptic cells. The release of GABA stops when the median ocellus is shadowed, and this causes the ganglion cells to depolarize. The depolarization evokes nerve impulses that travel along the axons of the ganglion cells via the circumesophageal connectives to the ventral ganglion. The cells in the ventral ganglion innervate the muscles that mediate the shadow withdrawal reflex.

RONALD MILLECCHIA
Department of Physiology and Biophysics, West Virginia University Medical Center, Morgantown 26506
G. FRANK GWILLIAM
Biology Department, Reed College, Portland, Oregon 97202

References and Notes

1. G. F. Gwilliam, *Biol. Bull.* **125**, 470 (1963); *ibid.* **129**, 244 (1965).
2. W. H. Fahrenbach, *Z. Zellforsch.* **66**, 233 (1965).
3. Glass capillary micropipettes filled with 3*M* KCl (d-c resistance 10 to 40 megohms) were used to record the intracellular potentials of the photoreceptors, their axons, and cells in the supraesophageal ganglion. Polyethylene suction electrodes filled with artificial seawater were used to record the nerve impulse activity in the circumesophageal connectives. The median ocellus was illuminated with a 15-watt tungsten-filament lamp via a fiber-optic light guide. The light beam was interrupted with an electromagnetic shutter, which was used to present steps of light or "shadows" to the ocellus, with a minimum duration of 5 msec. The intensity of the light was attenuated with calibrated neutral density filters.
4. Voltage-clamp methods have been used to describe the major ionic events that are associated with the receptor potential and to analyze these events in terms of the underlying conductance changes for two photoreceptors, the ventral photoreceptor cells of *Limulus* [R. Millecchia and A. Mauro, *J. Gen. Physiol.* **54**, 331 (1969)] and the photoreceptors of the lateral ocelli of *Balanus eburneus* and *B. amphitrite* [H. M. Brown, S. Hagiwara, H. Koike, R. M. Meech, *J. Physiol. London* **208**, 385 (1970)].
5. An identical postillumination hyperpolarization that occurs in the cells of the lateral ocelli of *B. eburneus* has been shown to be the result of an increase in the activity of an electrogenic sodium pump [H. Koike, H. M. Brown, S. Hagiwara, *J. Gen. Physiol.* **57**, 723 (1970)].
6. Sensory axons that do not conduct nerve impulses have been found in a variety of invertebrates: in the retinular cells in the lateral eyes of *Limulus* [T. H. Waterman and C. A. G. Wiersma, *J. Exp. Zool.* **126**, 59 (1954); M. E. Behrens and V. J. Wulff, *J. Gen. Physiol.* **48**, 1081 (1965)], in the lateral and median ocelli of the barnacle (*1*), in the retinular cells of the blowfly [Y. Washizu, *Comp. Biochem. Physiol.* **12**, 369 (1964)], in the ventral photoreceptors of *Limulus* [R. Millecchia, J. Bradbury, A. Mauro, *Science* **154**, 1199 (19[illegible] R. Millecchia and A. Mauro, *J. Gen. Physiol.* **54**, 310 (1969)], in the retinular cells of the honey bee drone [F. Baumann, *J. Gen. Physiol.* **52**, 855 (1968)], and in the crab muscle receptors [B. M. H. Bush, A. Roberts, *Nature* **218**, 1171 (1968); S. H. Ripley, B. M. H. Bush, A. Roberts, *ibid.*, p. 1170].
7. An investigation of the passive cable properties of the lateral ocellar nerves in *B. eburneus* has been presented [S. Shaw, *J. Physiol. London* **220**, 145 (1972)]. The axons from the lateral ocelli appear to have large characteristic lengths, which enable the receptor potential produced in the photoreceptor of the ocelli to spread to the supraesophageal ganglion with very little decrement.
8. The 70 percent figure is an average value obtained from the steady-state responses to a maximum intensity light step recorded in a series of axons at about the level of the supraesophageal ganglion and in a series of photoreceptors. The mean value (plus or minus the standard error) of the steady-state response from 13 axons was 8.08 ± 1.20 mv and that of the response from 21 photoreceptors was 11.48 ± 1.21 mv. The ratio of the mean responses is 0.70 with 95 percent confidence limits 0.56 to 0.97.
9. The passive spread of current along the relatively short axons can be described by the equations worked out by Weidman [S. Weidman, *J. Physiol. London* **118**, 348 (1952)] for isolated Purkinje fibers. The potential, V_X, at a distance X from a steady current source is related to the potential at the source, V_0, by the following expression:

$$V_X = V_0 \{\cosh [(L - X)/\lambda]/\cosh(L/\lambda)\}$$

where λ is the characteristic length and L is the length of the fiber. The potential V_L at the end of the fiber ($X = L$) is given by the simpler expression:

$$V_L = V_0 \operatorname{sech} (L/\lambda)$$

In the present situation, at $L = 2$ mm, $V_L/V_0 = 0.70$; therefore, $\lambda \cong 2.2$ mm.

10. The characteristic length, λ, is a function of the internal and external resistances per unit length of axon, r_i and r_o, respectively, and of the membrane resistance of a unit length of axon, r_m. It is given by the following expression:

$$\lambda = [r_m/(r_i + r_o)]^{\frac{1}{2}}$$

[W. A. H. Rushton, *J. Physiol.* **82**, 332 (1934)]. If the axon is bathed in a large volume of conducting medium then r_o is essentially zero; and if we consider the axon to be a cylinder of radius a, then $r_i = \rho_i/\pi a^2$, where ρ_i is the resistivity of the axoplasm, and $r_m = R_m/2\pi a$, where R_m is the resistance of a unit area of axon surface. In the present study $\lambda = 0.22$ cm (*6*), $a = 0.75 \times 10^{-3}$ cm (*2*), and $\rho_i = 60$ ohm-cm² [A. L. Hodgkin and W. A. H. Rushton, *Proc. Roy. Soc. London Ser. B* **133**, 444 (1946)]; therefore, $R_m = 7800$ ohm-cm².

11. J. Robbins and W. G. Van Der Kloot, *J. Physiol. London* **143**, 541 (1958); J. Robbins, *ibid.* **148**, 39 (1959); A. Takeuchi and N. Takeuchi, *ibid.* **205**, 377 (1969); H. Grundfest, J. P. Reuben, W. H. Rickles, Jr., *J. Gen. Physiol.* **42**, 1301 (1959); S. Iwasaki and E. Florey, *ibid.* **53**, 666 (1969); P. N. R. Usherwood and H. Grundfest, *J. Neurophysiol.* **28**, 497 (1965).

12. The action of picrotoxin is to compete with GABA for sites on the postsynaptic membrane. In the presence of picrotoxin a reduction in the amount of GABA released by the presynaptic fiber due to shadowing the median ocellus would have little or no effect on the membrane potential of the postsynaptic cells; therefore, the shadow-evoked activity would be blocked.

13. R.M. was supported by a grant to Reed College from the Alfred P. Sloan Foundation. The research was supported by NIH-NINDS grant SR01 NS-08610-01 to G.F.G.

5 May 1972; revised 23 June 1972

Cuticle Deposition in Imaginal Disks: Effects of Juvenile Hormone and Fat Body in vitro

Abstract. *Wing disks from the last larval instar of the Indian meal moth,* Plodia interpunctella *(Hübner), were successfully cultured in modified Grace's medium. 20-Hydroxyecdysone induced cuticle deposition in these disks in vitro. This response was enhanced by treating the medium with larval fat body and was inhibited by application of juvenile hormone.*

Increasingly, attention has been focused on the action of insect hormones on target tissues which grow and metamorphose in vitro (*1*). After the first report of hormone-induced metamorphosis in insect tissues cultured in vitro (*2*), the action of the ecdysones, but not of other hormones or tissues, on in vitro cuticle deposition has been studied (*3*). Some effects of juvenile hormone in vitro have been reported, but none of these involved cuticle deposition (*4*). We report that 20-hydroxyecdysone (also known as β-ecdysone, crustecdysone, or ecdysterone) induced cuticle deposition in cultured wing disks of the Indian meal moth, *Plodia interpunctella* (Hübner). The deposition of cuticle in vitro was enhanced by fat body and inhibited by *Cecropia* juvenile hormone (*5*)—a mixture of isomers of methyl-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate. This is the first example of an effect of juvenile hormone in vitro on cuticle deposition in imaginal disks.

The insects were reared according to the methods of Silhacek and Miller (*6*). The culture and dissection procedures were the same as those described for *Galleria mellonella* (Linnaeus) disks (*7*, *8*). Mesothoracic wing disks were removed from final instar larvae and cultured in modified Grace's medium (Grand Island Biological) (*9*), which contains 10 percent whole egg ultrafiltrate, 7 percent fetal calf serum, and 1 percent albumin fraction 5. Unlike *Galleria* wing disks, *Plodia* disks did not survive in chemically defined Grace's medium, but remained healthy for about 1 month in the modified medium. Fat body was taken from the mesothoracic region of the same larvae as were the disks, except that fat body from 18- to 21-mg larvae was used with disks from the 8- to 11-mg larvae. Groups of ten disks were cultured with or without fat body for 24 hours before 20-hydroxyecdysone, in 10 percent ethanol, was added. Some of the control cultures received an equivalent amount of 10 percent ethanol. Because of the difficulty of solubilizing juvenile hormone in the culture medium, this hormone was first dissolved in dimethyl sulfoxide (Baker analyzed reagent containing 99.9 percent dimethyl sulfoxide), a method successfully employed in the investigation of *p,p'*-DDT effects on cultured tissues (*10*). A portion of the juvenile hormone–dimethyl sulfoxide solution (50 μg/μl) was mixed with the culture medium before addition of the disks. An equivalent amount of dimethyl sulfoxide was added to the control dishes. Thus, the disks were exposed to juvenile hormone or dimethyl sulfoxide alone for 24 hours before the addition of 20-hydroxyecdysone. The cultured disks were observed regularly over a period of 3 to 4 weeks. Our first experiments confirmed that 20-hydroxyecdysone without fat body stimulated tracheal migration and elongation of the disks as reported previously for cultured *Galleria* disks (*7*). A concentration of 0.05 μg of 20-hydroxyecdysone per milliliter of medium stimulated tracheal migration alone, and higher concentrations caused hypertrophy of the peripodal sac, elongation, and, in some cases, tracheal migration.

We first examined the effects of 20-hydroxyecdysone and fat body on wing disks taken from mature, feeding larvae (18 to 21 mg). None of the 70 disks cultured without hormone produced cuticle. Treatment with 0.5 μg of 20-hydroxyecdysone induced cuticle deposition in 13.3 percent of the disks. By contrast, 63.3 percent of the disks cultured with 0.5-μg hormone and fat body made cuticle. Similarly, at higher concentrations of hormone (2 μg/ml, 5 μg/ml, and 50 μg/ml), fat body increased the percentage of disks depositing cuticle (Table 1). The cuticle produced by the cultured disks was tanned, lacked scales or hairs, and looked like pupal cuticle. Typically, this cuticle was seen beneath the peripodal sac covering one side of the disk. This is similar to

Table 1. Effects of fat body on cuticle deposition in cultured imaginal disks of *Plodia interpunctella*. The cultures contained 10 percent ethanol.

Donor larvae (mg)	Disks examined (No.)	Fat body	20-Hydroxy-ecdysone (μg/ml)	Disks with tanned cuticle (%)
18-21	20	−	0 (no ethanol)	0
18-21	10	+	0 (no ethanol)	0
18-21	50	−	0	0
18-21	20	+	0	0
18-21	20	−	0.05	0
18-21	20	+	0.05	0
18-21	30	−	0.5	13.3
18-21	30	+	0.5	63.3
18-21	20	−	2.0	10.0
18-21	80	+	2.0	95.0
18-21	40	−	5.0	2.5
18-21	30	+	5.0	46.7
18-21	20	−	50.0	0
18-21	20	+	50.0	70.0