When a solution of the highly active oyster protein was mixed with an excess of palmitate for 30 minutes prior to testing, no diminution of activity was observed. It is presumed that (i) the protein does not bind palmitate and that (ii) free palmitate does not inhibit the feeding response. It should be remembered that palmitate (in similar concentrations) did significantly inhibit the activity of human serum albumin.

As previously mentioned, heating in a boiling water bath (30 to 45 minutes) had very little effect on the stimulatory activity of the oyster protein.

Since *N. obsoletus* is a facultative carrion-feeder, in addition to being a deposit feeder, it is not surprising that there is a nonspecificity for a wide variety of proteins which are stimulatory only at relatively high concentrations. In contrast, fatty acid–free human serum albumin and oyster fluid protein appear to have appropriate configurations and reactive sites which permit them to be remarkably stimulatory. Certainly other very potent proteins will ultimately be found. Hemolymph obtained from the blue crab, *Callinectes sapidus*, is for example, approximately twice as active as human plasma (on a volume basis). If, in addition to *N. obsoletus*, many other marine species respond similarly to highly stimulatory proteins present in blood, exudates of tissues, and so forth, such phenomena can explain why injured species are particularly vulnerable to attack in an aquatic environment.

This highly reactive "binding specificity" of certain proteins for receptor membranes is analogous to the adsorption of protein and peptide hormones by cell surfaces of target organs, and numerous other biological phenomena which suggest that unique proteins, in extremely low concentration, may play an important role as chemical and biological signals. Obviously, further work needs to be done on the chemistry as well as the functional groups and active sites of such proteins. Also, an extensive survey must be conducted to measure the effectiveness of these and other proteins on a variety of marine animals. Such information will be needed in order to appraise the true significance of proteins in chemoreception.

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11. The experimental conditions were identical with those previously described by Carr (*9*). The snails were placed in clean seawater for at least 6 hours prior to testing; the seawater was changed several times. Test solutions were made up with the same sample of seawater. Usually 40 ml of test solution was poured into a petri dish for the assay. Groups of ten snails each were tested at each concentration and the number of positive responses was recorded (four or more extensions of the proboscis within 60 seconds). Positive responses given in control solutions ranged from 0 to 10 percent. Reasonably consistent values for the concentration of proteins (micrograms of protein per milliliter of seawater) necessary to induce a 50 percent positive response (ED_{50}) were obtained. The protein estimations were done by the method of Lowry [see (*18*)].
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21 May 1971

Cyclic Adenosine Monophosphate: Function in Photoreceptors

Abstract. *Inactivation of adenylate cyclase in outer segments of retinal photoreceptor cells is proportional to the bleaching of rhodopsin. Membranes of the outer segments also contain a particulate, light-insensitive phosphodiesterase of high specific activity. In electrophysiological experiments, application of cyclic adenosine monophosphate along with a methylxanthine mimics the effects of illumination on the photoreceptor cell of the compound eye of* Limulus.

Adenylate cyclase (cyclase) is an enzyme usually associated with membranes which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic 3′,5′-adenosine monophosphate (cyclic AMP) in response to a variety of peptide hormones or catecholamines (*1*). Cyclic AMP in turn, acting through associated enzyme systems, mediates a number of specialized cellular functions such as melanosome dispersion, release of hormones, and regulation of certain synaptic activities (*2*).

Cyclase is present in retinal photoreceptor structures, where its specific activity is higher than that in any previously described tissue. The cyclase in outer segments of frog rods is inactivated by illumination (*3*). This observation implicates cyclase in photoreceptor function and raises the question of its precise role.

We have investigated this role by studying (i) the correlation between cyclase activity and bleaching of rhodopsin, (ii) phosphodiesterase activity and distribution, and (iii) physiological effects of cyclic AMP in photoreceptors.

Outer segments of bovine retinas adapted to dark were mixed with 45 percent sucrose, and the dense slurry was sonicated to produce a homogeneous suspension of rod disks (*3*). This suspension was pipetted into an ice-cooled Plexiglas chamber (with 1-mm light path) and exposed to tungsten illumination for 1 minute. The intensity of light was varied by adjustment with neutral density filters. After each exposure, 75 μl of the sample was mixed in 400 μl of 1 percent CTAB (cetrimonium bromide) for measurement of optical density at 500 nm, and three 10-μl portions were assayed for cyclase activity by measurement of the amount of labeled cyclic AMP generated from [8-^{14}C]ATP during a 10-

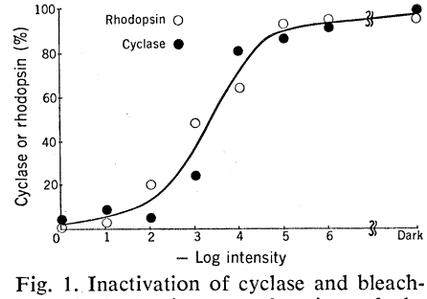

Fig. 1. Inactivation of cyclase and bleaching of rhodopsin as a function of the intensity of illumination. The points shown are the averages for six determinations.

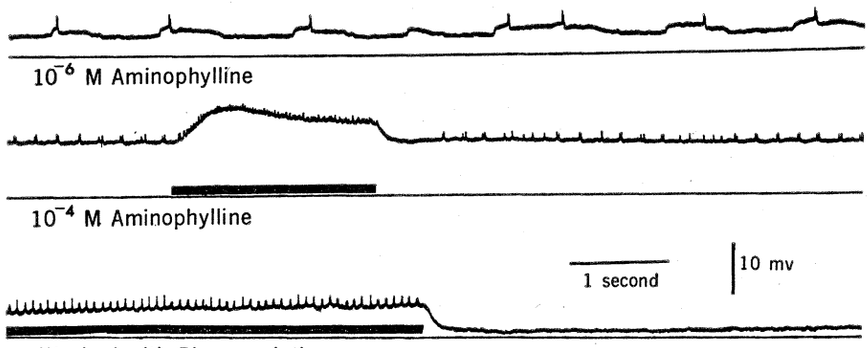

Fig. 2. Effects of cyclic AMP and methylxanthines, externally applied, on intracellular recordings from retinular cells in the compound eye of *Limulus*. Solutions were added within a few seconds before the start of the records. The thickened baseline indicates stimulation by light of equal intensity in middle and bottom records. Differences in spike height resulted from the presence of two eccentric cells in this ommatidium. Cyclic AMP alone and in combination with theophylline (not shown) depolarized the retinular cell in a manner similar to that for aminophylline. Results with cyclic AMP alone were occasionally erratic, which may be explained by the difficulty of cyclic AMP in penetrating the cell wall.

minute incubation at 30°C (*3*). The assays for different exposure intensities were all performed together, within the hour when all exposures were completed. These measurements (Fig. 1) show that the inactivation of cyclase is proportional to the bleaching of rhodopsin. We conclude that the photochemical state of rhodopsin and the activity of cyclase are closely coupled and probably causally linked.

Most of the protein in frog rod outer segments is rhodopsin (*4*). From what is known about opsin it appears unlikely that cyclase is a component of opsin, but it might be coupled to it. Without further information on this point we cannot say whether cyclase is located on disks or plasma membranes, although its relation to rhodopsin argues somewhat in favor of the disk.

In the above experiments, the cyclic AMP generated was protected from hydrolysis by phosphodiesterase by the presence of 6.67 m*M* aminophylline in the reaction mixture. Aminophylline and theophylline specifically inhibit phosphodiesterase, and thus allow the accumulation of cyclic AMP (*5*). When sonicated suspensions of disks, prepared from frog rod outer segments purified with Ficoll (*4*), were incubated with cyclic [8-^{14}C]AMP in the absence of aminophylline, cyclic AMP disappeared from the reaction mixture at a rate of 12 nmole per milligram of protein per minute. This rate was not affected by illumination. The enzyme activity responsible for the hydrolysis of cyclic AMP was associated with the particulate component of the sonicated rods. Extensive washing of the disk suspension removed less than 20 percent of the total phosphodiesterase activity. These data indicate that a particulate phosphodiesterase of high specific activity is present in rod outer segments, and that the activity of this enzyme is not regulated by illumination.

Electrophysiological studies of vertebrate photoreceptors have shown that illumination causes hyperpolarization of the receptor cell membranes and decrease in conductance to sodium ion (*6*). The inactivation of cyclase by illumination suggests that in the dark the production of cyclic AMP might be associated with increased conductance to sodium ion and depolarization of the receptor cell membrane. If cyclic AMP has a similar physiological role in systems like the *Limulus* eye, illumination would be expected to stimulate cyclic AMP formation, since illumination produces depolarization of the photoreceptor membrane in this and many other invertebrates.

We investigated the physiological effect of cyclic AMP on photoreceptor membrane potential by recording intracellularly from the retinular (photoreceptor) cells in the compound eye of the horseshoe crab, *Limulus*. Such recordings are suitable for this purpose even though there are activities other than photoreception in this system. The intracellular recordings from photoreceptor cells also show spike action potentials generated in an associated neuron, the eccentric cell. The frequency of these nerve impulses reflects an integration of excitatory and inhibitory stimuli, but the inhibitory interaction does not detectably alter measurements of the photoreceptor cell membrane potential (*7*).

We found (Fig. 2) that the effects of cyclic AMP, aminophylline, and theophylline were identical. Mimicking the effects of illumination, these agents caused depolarization of the photoreceptor cell and increased frequency of firing of the eccentric cell. The application of $10^{-6}M$ aminophylline (top record) to the *Limulus* eye maintained in Ringer solution in the dark caused abnormal prolongation of the spontaneous slow potential fluctuations (SPF's) (*8*). This observation suggests that SPF's could reflect accumulation of cyclic AMP resulting from normally occurring spontaneous bursts of synthesis in the dark. This cyclic AMP would be protected from hydrolysis by aminophylline, and its persistence could result in the abnormally prolonged depolarizations that we have observed in the presence of aminophylline. With higher concentrations of aminophylline or cyclic AMP the cell became progressively more depolarized within seconds after application, and the frequency of the eccentric cell impulses became correspondingly higher (middle record). These effects were rapidly reversed by washing the preparation with *Limulus* Ringer solution (bottom record).

Photoreceptor functions include excitation over a large dynamic range [in the rods it can occur with the absorption of one photon simultaneously in about ten rods (*9*)], and loss and recovery of sensitivity (light and dark adaptation). Since a single photon absorbed by rhodopsin can produce extensive changes in sodium ion conductance of photoreceptor cells, the molecular events following photon absorption must provide great amplification. The advantages for amplification provided by a series of enzymes have been pointed out (*10*). Amplification may result from the synthesis of cyclic AMP by cyclase and the effects of cyclic AMP on derivative enzyme systems. If the ratio of photosensitive rhodopsin molecules to cyclase molecules is high, this could materially aid the single photon detection problem.

The mechanisms by which cyclic AMP might act include activation of a protein kinase and changes in calcium ion concentration (*11*). The finding that cyclic AMP stimulates binding of calcium ion to sarcoplasmic reticulum in heart muscle (*12*) is of interest because of a recent report that external applica-

tion of calcium ion to cone photoreceptors mimics illumination (*13*). These facts call attention to anatomical and physiological similarities between sarcoplasmic reticulum, photoreceptor disks, and microvilli, and raise the possibility of related mechanisms for the various kinds of excitation mediated by these organelles.

Although the cyclase system offers an attractive model for explaining photoreceptor function, there can as yet be no definitive statement concerning its role. Our earlier finding that frog rod outer segments contain cyclase regulated by light has been extended. (i) Cyclase inactivation is directly proportional to rhodopsin bleaching. (ii) A particulate phophodiesterase of high specific activity accompanies the cyclase in photoreceptors. (iii) Cyclic AMP and methylxanthines mimic the physiological effects of illumination in the compound eye of *Limulus*.

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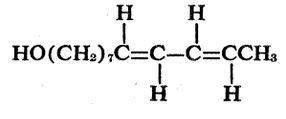
1 June 1971; revised 8 July 1971

Sex Attractant of the Codling Moth: Characterization with Electroantennogram Technique

Abstract. trans-*8*, trans-*10-Dodecadien-1-ol is a sex attractant of the codling moth,* Laspeyresia pomonella. *Antennal responses (electroantennograms) to a series of monounsaturated compounds were used in determining the location and configuration of the two double bonds. The synthetic compound is very attractive to male codling moths in the field.*

The codling moth, *Laspeyresia pomonella* (Lepidoptera: Tortricidae: Olethreutinae), has long been considered a major worldwide pest of apple. Insecticide spray schedules could be drastically modified in several apple-growing areas if alternative methods of control could be found. Sex pheromone traps have successfully been used with another tortricid moth (*1*) for monitoring the presence and abundance of moths and for insect suppression in apple orchards. Similar uses of an attractant may well be feasible with the codling moth. Studies on the female sex pheromone gland and its extract have been carried out (*2*, *3*), but the structure of the pheromone compound still remained unknown. We have developed a technique with electroantennograms (EAG) which uses a small number of female glands and male antennae and allowed us to propose a structure for the codling moth sex attractant. The attractant compound is *trans*-8, *trans*-10-dodecadien-1-ol (*1*).



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Abdominal tips from 50 female codling moths (*4*) were excised and placed in methylene chloride. Portions of this crude extract were injected on both polar (5 percent cyclohexanedimethanol succinate on chromosorb Q, 170°C) and nonpolar (10 percent JXR on chromosorb Q, 190°C) gas chromatographic (GC) columns, and the column effluent was collected in 30-cm glass capillary tubes at 1-minute intervals. The tubes were assayed for activity by connecting one end to a 5-ml glass syringe containing 1 ml of air and inserting the other end into an airstream passing across an antenna affixed for EAG measurements (*5*). The syringe plunger was quickly depressed to send a 30- to 35-msec "puff" of air through the capillary tubing and over the antenna. Collected fractions containing active material elicited responses up to 5 mv, whereas inactive fractions gave responses of approximately 0.5 mv. More precise retention times were obtained by taking 0.5-minute collections in the area of interest. The retention time of the active component relative to dodecyl alcohol was 2.3 : 1 to 2.6 : 1 on the polar column, and 1.15 : to 1.25 : on the nonpolar column. The nonpolar column retention time indicates a molecular weight similar to that of dodecyl alcohol, while the comparatively long retention time on the polar column indicates that the active compound contains more functionality than dodecyl alcohol.

Evidence that the active compound contains an alcohol moiety, as was previously reported (*3*), was obtained by assaying several chemical reactions of the crude extract with the GC-EAG technique mentioned above. Crude extract treated with 0.5*N* methanolic potassium hydroxide still contained active material with a GC retention time identical to the active component in untreated extract, whereas, treatment with acetyl chloride eliminated the active component at this retention time. The above data suggest that the pheromone is a C_{12} alcohol, too polar to be monounsaturated, but with a molecular weight that most likely excludes an additional oxygen atom. At least two sites of unsaturation are suggested, with a strong possibility that conjugation accounts for the polarity indicated by the polar GC column retention time (*6*).

A number of synthetic chemicals were tested for electroantennogram responses by puffing 1 ml of air through a disposable pipette containing 80 μg of test chemical on a piece of filter paper (*5*). Antennal responses of male *Argyrotaenia velutinana* to a series of monounsaturated acetates and alcohols had shown that the standard that is identical to the sex pheromone always elicits the