action of colchicine on the alveolar membranes [compare also the increased electrical response of insect neurons as well as the inhibition of nucleoside transport in mammalian cells after colchicine treatment in (*18*) and (*19*)]. Possibly, colchicine, a lipophilic drug, "dissolves" in apolar membrane regions and decreases their overall fluidity by making the membrane lipids more rigid, for example, in a fashion similar to that of cholesterol (*20*). Alternatively, the drug may bind to a membrane-protein with similar colchicine-binding properties as the protein subunits of microtubules (*21*). In any case, one cannot attribute colchicine-induced alterations of membrane-phenomena uniquely to microtubule involvement.

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Control of Guanylate Cyclase Activity in the Rod Outer Segment

Abstract. *Mammalian photoreceptors contain a guanylate cyclase which has a high specific activity and is inhibited by exposure of the rod outer segment to light. Several minutes are required for this inhibition to take effect, indicating that it is not a step in visual excitation. The activity of the enzyme is sensitive to the concentration of calcium ion in the medium, suggesting that light-induced changes in calcium distribution in the photoreceptor could control guanylate cyclase activity.*

The rod outer segment is a highly specialized organelle, relatively poor in most enzymatic activities (*1*). The enzymes that synthesize and degrade guanosine 3′,5′-monophosphate (cyclic GMP) are an exception—they have exceptionally high specific activities in bovine rod outer segments (*2*, *3*). This report is concerned with the regulation of synthesis of cyclic GMP in this organelle.

Rod outer segments were prepared from dark-adapted cattle eyes. All operations were carried out in dim red light. Retinas were removed and swirled in a buffer consisting of 11 m*M* glucose, 0.18 m*M* $MgCl_2$, 0.18 m*M* $CaCl_2$, 3.5 m*M* KCl, 128 m*M* NaCl, and 12 m*M* sodium phosphate, *p*H 7.7. The resulting suspension was layered on a 41 percent sucrose solution and centrifuged at 5000 rev/min in a Spinco SW-25 rotor for 30 minutes. The sucrose solutions used in this and the following step were made up in the buffer described above. The material that collected at the top of the sucrose solution was centrifuged for 12 hours on a continuous sucrose density gradient (24 to 41 percent sucrose) at 25,000 rev/min. A band composed of rod outer segments was collected from the upper portion of the gradient. The ratio of the absorbance of this material at 280 nm to that at 500 nm was between 4 and 5, indicating that the rod outer segment preparation was reasonably pure and dark adapted (*4*).

When this preparation was incubated with [α-^{32}P]guanosine triphosphate (GTP), radioactivity was incorporated into cyclic GMP (Fig. 1). This reaction proceeded at a rate of about 1 nmole per minute per milligram of protein. Rabbit photoreceptors prepared in a similar manner had the same guanyl-

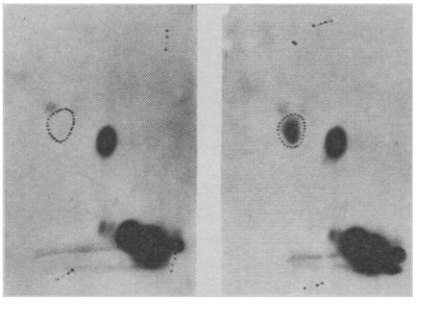

Fig. 1. Synthesis of cyclic GMP from [α-^{32}P]GTP. Rod outer segments (9.12 μg of protein) were incubated for 20 minutes at 37°C in 100 μl of 20 m*M* tris(hydroxymethyl)aminomethane (tris) buffer, *p*H 7.6, which contained NaF (2.5 m*M*), aminophylline (5 m*M*), $MgCl_2$ (5 m*M*), GTP (2.5 m*M*), and 2 μc of [α-^{32}P]GTP and 0.625 percent Lubrol PX. After the incubation, 20 μl of 10 m*M* cyclic GMP was added to each tube, and the reaction was stopped by boiling for 5 minutes. Portions (40 μl) were applied to polyethyleneimine-cellulose (PEI-cellulose) thin-layer plates, which were then developed in two dimensions (*11*). The chromatograms were dried and exposed to x-ray film for 1 week. (Left) Autoradiogram obtained with a control sample in which the rod outer segment material was omitted during incubation and added just prior to boiling. Protein was measured by the method of Lowry (*12*). (Right) Autoradiogram of the reaction products thus obtained. Cyclic GMP is found in the area that is outlined with dots.

ate cyclase activity. In both preparations, the rate of synthesis of adenosine 3′,5′-monophosphate (cyclic AMP) from [α-^{32}P]adenosine triphosphate (ATP) was variable and about an order of magnitude lower (0.03 to 0.22 nmole of cyclic AMP produced per minute per milligram of protein) than the rate of cyclic GMP synthesis. This rate of cyclic AMP synthesis was much lower than had been previously reported for mammalian photoreceptors (*5*) and was lower than the rate we had previously observed using [^{3}H]ATP as a substrate (*3*). Varying the method of preparation of rod outer segments (by homogenization or sonication) or the assay conditions did not affect the relative rates of cyclic GMP and cyclic AMP synthesis.

Exposure of the rod outer segment preparation to light reduced the activity of guanylate cyclase (Fig. 2). Several minutes were required for this inhibition to be expressed, indicating that this phenomenon was not a step in the much more rapid process of visual excitation (as had been suggested for light inhibition of adenylate cyclase of the photoreceptor) (*6*). We did not observe any reproducible effect of light on the synthesis of cyclic AMP by rod outer segments.

Processes that might occur at a rate similar to that observed for inhibition of guanylate cyclase include a slow chemical reaction such as the decay of metarhodopsin II (*7*) or a gradual change in the concentration of a small molecule as would occur with leakage of an ion from an intracellular pool. Hagins (*8*) has postulated that leakage of calcium ion from rod outer segment disks could mediate visual excitation. This led us to examine the influence of calcium ion on the activity of photoreceptor guanylate cyclase. The activity of this enzyme is affected by the concentration of calcium ion in the medium, with stimulation at concentrations between 2 and 3 m*M*, and inhibition above 4 m*M* (Fig. 3). In a separate experiment in which another photoreceptor preparation was used, doubling the GTP concentration did not alleviate the inhibition by 5 m*M* calcium ion, indicating that calcium was not exerting its effect through chelation of GTP. As a test of the relationship between the effect of calcium and the effect of light, rod outer segments were first incubated in 4 m*M* ethylenebis(oxyethylenenitrilo)tetraacetate (EGTA) (which chelates calcium ion) and were then assayed for enzyme activity with and without prior bleaching. There was a 30 percent reduction in activity, and the inhibitory effect of light was lost under these conditions. When untreated rod outer segments were assayed in the presence of 2 m*M* calcium ion, no effect of bleaching was observed. We propose that light inhibits guanylate cyclase activity through an alteration in the localization of calcium ion in the photoreceptor.

The disks of the rod outer segment arise through an infolding of the plasma membrane. If guanylate cyclase has the same orientation in the disk membrane as adenylate cyclase does in the plasma membrane of hormone-sensitive cells, then there would be a catalytic subunit of the enzyme exposed to the cytoplasmic side of the membrane and a regulatory subunit exposed to the other side, in this case, the intradisk side. If calcium exerted its effect on cyclase through binding to the regulatory subunit, then the enzyme activity would reflect the calcium ion concentration within the disk. Loss of calcium ion from this compartment through bleaching (*8*) would result in a decreased activity of the enzyme. In agreement with our results, both removal of calcium with EGTA or flooding the system with calcium would thus abolish the light effect.

While the onset of inhibition of this enzyme by light is too slow to be a step in visual excitation, it does occur at a rate comparable to that of a slow step in dark adaptation (*9*). Cyclic GMP could participate in this reaction through a cyclic nucleotide–dependent protein kinase which we have observed in the rod outer segment (*10*).

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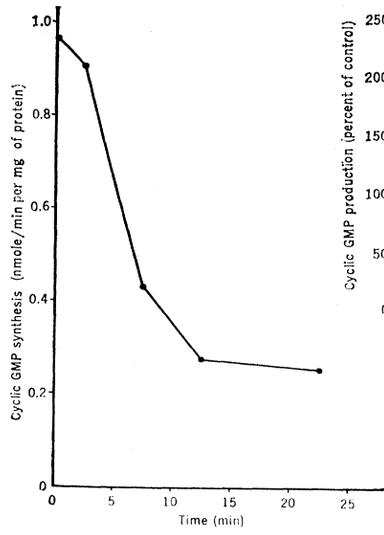

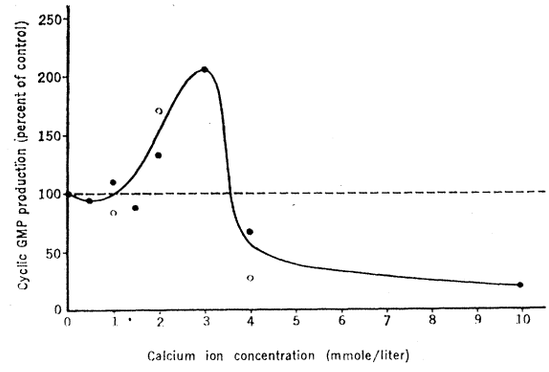

Fig. 2 (left). The inhibition of guanylate cyclase by light. A suspension of rod outer segments (1.23 mg of protein per milliliter) was exposed to the light of a 60-watt microscope lamp at a distance of 6 inches for 30 seconds. Then 36.9 μg of this material was incubated for 5 minutes at 37°C in 100 μl of 40 m*M* tris buffer, *p*H 7.6, which contained $MgCl_2$ (5 m*M*), KCl (25 m*M*), phosphoenolpyruvate (5 m*M*), 1-ethyl-4-(ethylthio)-1*H*-pyrazolo-(3,4b)pyridine-5-carboxylic acid ethyl ester (6.5×10^{-5}*M*) (Squibb SQ65442, an inhibitor of phosphodiesterase), cyclic GMP (2 m*M*), GTP (1 m*M*), and 2.5 μc of [α-^{32}P]GTP and 50 μg of rabbit skeletal muscle pyruvate kinase (Sigma). The reaction was stopped by boiling for 5 minutes. Portions (40 μl) were applied to plastic PEI-cellulose thin-layer plates and developed stepwise in one dimension, 5 to 8 cm in 0.2*M* NaCl and to the top of the chromatogram (20 cm) in 1.0*M* NaCl. The spots corresponding to cyclic GMP were located under ultraviolet light, cut out, and eluted in 1 ml of 0.02*M* tris, *p*H 7.6, containing 0.7*M* $MgCl_2$. Scintillation fluid (10 ml) (Aquasol, New England Nuclear) was added, and the samples were counted in a liquid scintillation spectrometer. The point at the far left of the figure was obtained with the rod outer segment preparation prior to bleaching. Subsequent points represent the enzyme activity assayed immediately after exposure to light, 5 minutes, 10 minutes, and 20 minutes after exposure. The samples were kept in darkness during the preliminary incubation and the incubation. Control tubes were incubated in the absence of rod outer segments, which were added just prior to boiling. Fig. 3 (right). The effect of calcium ion on guanylate cyclase activity. Rod outer segments were assayed for guanylate cyclase activity as described (Fig. 2), with the addition of calcium chloride to the assay mixture in the concentrations indicated. Two experiments are shown; the open and closed circles represent experiments in which different preparations of rod outer segment were used.

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Transovarial Transmission of LaCrosse Virus (California Encephalitis Group) in the Mosquito, Aedes triseriatus

Abstract. *LaCrosse virus (California encephalitis group) was recovered from F_1 eggs, larvae, and adults produced by experimentally infected* Aedes triseriatus. *The F_1 females transmitted the virus by bite to suckling mice and chipmunks. This, plus isolations of LaCrosse virus from larvae collected from their natural habitats in enzootic areas and from males and females reared from them, suggests that transovarial transmission is the overwintering mechanism for this arbovirus in northern United States.*

Epidemiological studies in Wisconsin have indicated that LaCrosse (LAC) virus is maintained in nature by an *Aedes triseriatus*–small mammal cycle (*1*). *Aedes triseriatus* has proved to be a very efficient laboratory transmitter of LAC virus (*2*) and considerably more efficient than nine other species of mosquitoes (*3*).

The method of survival of LAC virus during the winter months when adult mosquitoes are inactive has remained unknown; however, isolations of the virus from *A. triseriatus* larvae (*4*) in 1972 suggested overwintering of LAC virus in diapause eggs laid by *A. triseriatus*. In view of the implication of *A. triseriatus* as the major vector of LAC virus and the isolations from *A. triseriatus* larvae, this investigation was conducted to explore the possibility of transovarial transmission of LAC virus in this mosquito. In our experiments LAC virus was recovered from F_1 eggs, larvae, and adults produced by experimentally infected *A. triseriatus*. The F_1 females transmitted the virus by bite to suckling mice and chipmunks.

The LAC virus used was originally isolated from human brain tissue (*5*). It had undergone four serial intracerebral passages in suckling mice, one subcutaneous passage in chipmunks, and a fifth intracerebral passage in suckling mice.

Aedes triseriatus mosquitoes, 7 to 14 days of age and in their fifth generation of colonization, ingested LAC virus in guinea pig defibrinated blood. The virus content of the infectious bloodmeal was determined by the intracerebral inoculation into suckling mice of serial tenfold dilutions of the blood-virus mixture upon which the mosquitoes had fed. As a matter of convenience, control mosquitoes were fed on hamsters rather than through a membrane. Portions of blood from each animal were inoculated intracerebrally into suckling mice to show that mouse lethal agents were not present.

New engorged mosquitoes were transferred to screen cages or to ice-cream (about 500-ml size) containers. An oviposition site consisting of a moist strip of black cotton cloth inserted along the inner wall of a crystallizing dish (50 by 35 mm) was placed in each cage or container. A 5 percent solution of sucrose in water was provided the mosquitoes during the extrinsic incubation period. All mosquitoes, including developmental stages, were maintained at 27°C and 80 percent relative humidity under a 16-hour photoperiod. Controls, including eggs, larvae, and adult mosquitoes were maintained under similar environmental conditions. The possibility of inadvertent introduction of virus in the experiments was prevented by (i) maintaining the mosquitoes in an insectary in which no other experimental studies with LAC virus or other members of the California virus group were being conducted, (ii) sterilizing equipment used in maintaining mosquitoes, (iii) assaying specimens in a separate laboratory in which there had been no previous work with known arboviruses, and (iv) removing brain tissue from mice in a room in which there had been no previous work with viruses.

At various intervals during the extrinsic incubation period parent females were allowed to feed on adult hamsters or 1- to 3-day-old suckling mice, one mosquito per animal. This provided blood for egg development as well as providing an indicator of infection in parent mosquitoes. Immediately after each feeding the oviposition site was removed and replaced with a new one. After completing several ovarian cycles, mosquitoes were placed at −70°C for subsequent testing for the presence of virus. Approximately 25 percent of the eggs from each ovarian cycle were removed for virus assay while the remaining eggs were inundated in a nutrient solution of broth and water (1 : 1000). The first-instar larvae were transferred to tap water to which Tetramint was added as a food source. When larvae developed to the third or fourth instar, a pool of ten larvae from each ovarian cycle was stored at −70°C for virus assay. The F_1 females were retained for 7 to 14 days before they were allowed to feed on suckling mice. After feeding on mice, mosquitoes were placed at −70°C or retained for feeding on chipmunks, one mosquito per animal. Serial tenfold dilutions of blood specimens taken from the orbital sinus of each chipmunk were assayed for virus by a plaque test (*6*).

Adult mosquitoes were triturated individually in the U-shaped cavity of an 11-mm, sterile, rubber-style vaccine bottle stopper with a 1.0-ml glass syringe plunger. Eggs and larvae were triturated similarly, the eggs in pools of varying numbers and the larvae in pools of ten. Triturated specimens were suspended in 1.0 ml of medium similar to that employed for making dilutions of the infectious bloodmeal. Suspensions were assayed for virus by intracerebral inoculation of 1- to 3-day-old mice, 0.03 ml per mouse. All mice were observed for 10 days. The brains of moribund and dead mice were removed aseptically for use in neutralization tests. Recovery of LAC virus from mosquito suspensions, from brain tissue of mice, and from blood of chipmunks was confirmed by neutralization tests with the use of LAC hyperimmune mouse ascitic