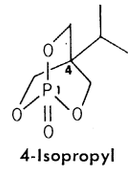
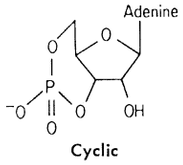
of DFP, 3 mg/kg intraperitoneally, show signs of excessive cholinergic stimulation and a 54 percent inhibition of AChE activity in whole blood and an 87 percent inhibition in whole brain. While this enzyme assay might not detect reversible AChE inhibition, the symptoms of poisoning rule out the possibility that the isopropyl bicyclic phosphate acts in this manner. Barbiturates may be useful antidotes in combatting acute convulsions produced by the bicyclic phosphorus esters, as judged by preliminary studies involving intraperitoneal administration of phenobarbital to mice poisoned with the ethyl bicyclic phosphate.

Those who work with bicyclic phosphorus esters should use suitable precautions to avoid poisoning since some of these compounds, in mouse tests, are 33 times more toxic than DFP or parathion. They are not only highly toxic materials but they appear to poison by a mechanism different from that of any other known organophosphorus toxicants. While the mode of action of the bicyclic phosphorus esters is not as yet defined, their structural similarity to the cyclic phosphate adenosine 3′,5′-monophosphate (cyclic AMP) is possibly relevant. These compounds may be useful probes in pharmacological and biochemical studies.



4-Isopropyl bicyclic phosphate



Cyclic AMP

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Direct Evidence for a Colchicine-Induced Impairment in the Mobility of Membrane Components

Abstract. *Freeze-etch electron microscopy was used to show that colchicine interacts with membranes of the ciliate protozoan* Tetrahymena pyriformis. *Colchicine impairs the temperature-induced translational and vertical mobility of the membrane-intercalating particles of the freeze-fractured alveolar membranes lying just below the plasma membranes.*

Recent studies suggest that membranes may be envisaged as two-dimensional "fluid" or "plastic" entities that are assemblies of noncovalently bonded lipids and proteins (*1*). This would explain findings suggesting that diverse membrane components can move translationally and normally to the membrane plane (*2–4*). Such motion of membrane components has been recently implicated in the lectin-induced agglutination of various neoplastically transformed cells (*5*), as well as to the "cap" formations and redistributions of surface antigens in different cell types (*6*).

However, these membrane properties can be inhibited by colchicine, Colcemid, or vinblastine (*7*). Since these alkaloids disaggregate microtubules (*8*), microtubular proteins are now thought to influence the mobility of membrane components. On the other hand, a direct action of these agents on membranes, possibly causing a decreased motion of membrane components, cannot be excluded, particularly since colchicine also binds to isolated membrane fractions (*9*).

We have accordingly examined the possible direct action of colchicine on biomembranes by freeze-etch electron microscopy, since only this method can directly detect movements of membrane components at high resolution (about 20 Å). At fracturing, membranes are internally split, exposing two faces of their apolar membrane cores (*10*). Such membrane faces are generally studded with randomly distributed 85-Å particles, composed at least partially of proteins or glycoproteins (or both) (*11*) and bearing diverse antigenic specificities (*12*).

These membrane-intercalated particles can move translationally at least in some membranes as a function of *p*H (*3*) or temperature (*4*). We now analyze the influence of colchicine on particle movement induced by variations in temperature, using the membranes of the alveolar sacs lying just below the plasma membrane of the ciliate eukaryote *Tetrahymena pyriformis* (*4, 13*).

Early log-phase *Tetrahymena* cultures were grown at the optimal temperature of 28°C in a medium of 2 percent proteose peptone plus 0.4 percent liver extract. Cultures were divided into two portions; one was incubated with colchicine (5 mg/ml) and one used as control. The relatively high dose is not lethal for the cells; cell division is first blocked, but cell growth continues, and about 4 hours after incubation the cells even "adapt" to the drug (*14*). About 30 minutes after incubation with colchicine, both control and colchicinized cells were simultaneously cooled (cooling rate, about 6°C/min) from the optimum growth temperature of 28° to 5°C, kept at 5°C for 4 minutes, and then reheated to 28°C within 30 seconds, under the same conditions. For freeze-etch electron microscopy, cell samples were taken at 28°C just prior to chilling and immediately after reheating, and, during chilling, at 20°, 15°, 10°, and 5°C. The cells were fixed with 3 percent glutaraldehyde in 0.05*M* sodium cacodylate, *p*H 7.2 (about 10 minutes), washed, glycerinated in steps up to about 25 percent during a period of 2 to 3 hours, and frozen in Freon 22 on cardboard disks. Fracturing, etching (1 minute at −100°C), and replicating were performed in a Balzers machine (model BA 360 M; Balzers, Liechtenstein). Replicas were studied in a Siemens Elmiskop Ia.

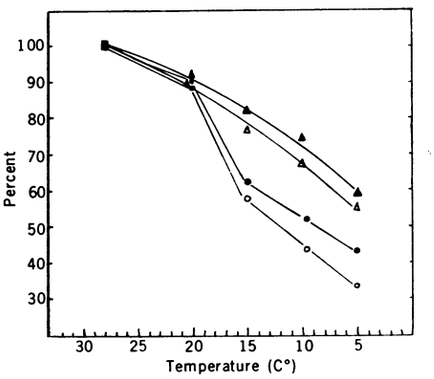
At 28°C, in untreated cells the face of the outer fractured alveolar membranes oriented toward the plasma membrane [for closer description see (*4, 13*)] reveals in two experiments about 480 ± 75 particles and 640 ± 65 particles per square micrometer, respectively. The average distance between neighboring particles amounts to 44.9 ± 2.1 and 39.6 ± 1.9 nm, respectively (means of at least 100 measurements with standard error). These particles nearly always showed diameters larger than 100 Å. The average distance between the particles, as well as the frequency of the particles, diminish on cooling (Figs. 1 and 2). This phenomenon suggests a critical

transition temperature between 20° and 15°C. At 5°C, the remaining particles have aggregated, leaving "smooth-faced" areas extended laterally parallel to the membrane plane. Reheating these cells from 5° to 28°C within 30 seconds eliminates these smooth areas, resulting in about 91 percent of the original average particle distance and particle frequency. We have suggested that this reversible segregation of membrane core components arises from reversible disorder ⇌ order transitions of the membrane lipids (*4*). Upon cooling, lipids "crystallize out" two dimensionally inducing the "protein" particles to move translationally or normally (or both) to the membrane plane. When the crystallized lipids become again fluid, for example, reheating, the particles again redistribute normally. This interpretation is in accord with the findings of Verkleij *et al.* (*15*), who observed similar particle aggregations on the fracture faces of *Acholeplasma* and *Escherichia coli* plasma membranes after chilling below the phase transition point of the membrane lipids.

The outer alveolar membranes of *Tetrahymena* cells incubated with colchicine at 28°C reveal about the same particle distribution as untreated cells on their fracture faces (Figs. 1 and 2). In the two experiments, the particle frequencies were 520 ± 80 particle/μm^2 (corresponding to the above 480 ± 75) and 610 ± 70 particle/μm^2 (corresponding to the above 640 ± 65), and the particle distances 44.1 ± 2.0 nm and 40.9 ± 2.1 nm, respectively. However, in the presence of the drug the critical transition temperature range for particle distribution is "smeared" as compared to the controls (Fig. 1). Moreover, reheating of colchicine-treated cells from 5° to 28°C within 30 seconds does not affect complete disappearance of the smooth-faced areas as compared to controls, leaving many particles still aggregated (Fig. 2). Then their average separation and their frequency amount to only 72 percent of the original values.

No one has observed contact between the outer alveolar membranes of *Tetrahymena* and microtubules, although such structures abound at the cells' peripheries (*13, 16*). Moreover, colchicine in the concentration we use does not disaggregate these peripheral microtubules (*17*). We therefore assume that the different mobility of the membrane-intercalated particles in colchicinized cells reflects the direct

Fig. 1. The percentage decrease of the particle frequencies per square micrometer (open circles and triangles) and the average distances between the particles (solid circles and triangles) of freeze-fractured alveolar membranes in normal (circles) and colchicine-treated *Tetrahymena* cells (triangles). Probes were taken at different temperatures during cooling from 28° to 5°C. Symbols are means of two experiments (see text), which are normalized to 28°C. In each experiment, at least eight different fracture faces of at least two different replicas were evaluated per temperature degree.



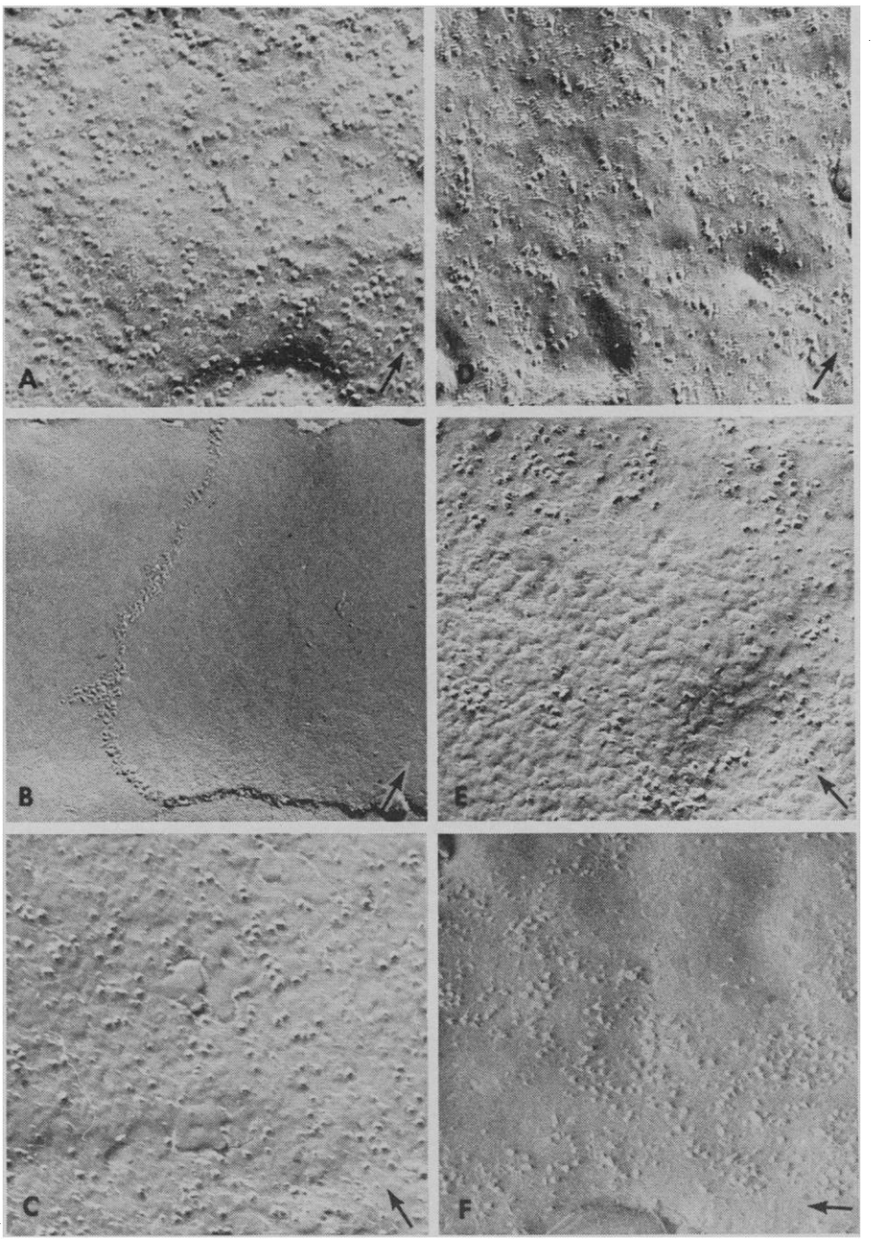


Fig. 2. Fracture faces of the outer alveolar membranes of *Tetrahymena* with colchicine incubation. (A to C) Without colchicine; (D to F) with colchicine (5 mg/ml). At 28°C, membrane-intercalating particles are uniformly distributed in normal (A) and colchicinized cells (D). Upon cooling to 5°C, the particles are more strongly aggregated in normal cells (B) than in colchicinized cells (E). Reheating to 28°C causes a normal redistribution of the particles in untreated cells (C), while in colchicinized cells the particles are still aggregated (F). Arrows indicate shadowing direction (× 80,000).

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-

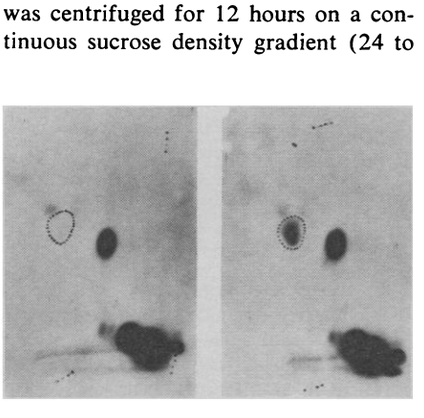

Fig. 1. Synthesis of cyclic GMP from [α-^{32}P]GTP. Rod outer segments (9.12 μg of protein) were incubated for 20 minutes at 37°C in 100 μl of 20 m*M* tris(hydroxymethyl)aminomethane (tris) buffer, *p*H 7.6, which contained NaF (2.5 m*M*), aminophylline (5 m*M*), $MgCl_2$ (5 m*M*), GTP (2.5 m*M*), and 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.