ment levels (C_{sat}) off southern California were considerably lower than on 19 April 1982 (Fig. 1E) (*8*). The 1983 C_{sat} values along the coast were generally less than half the 1982 values. In 1982, waters with $C_{sat} > 0.8$ mg m^{-3} extended more than 100 km offshore in plumes associated with upwelling near Point Conception. This represents typical early spring conditions in the Southern California Bight. In the 1983 image, only tenuous upwelling plumes can be seen at Point Conception, with high C_{sat} values only in nearshore waters. Monthly ship transects off southern California since March 1983 have shown increasing departures from normal: the maximum layer of chlorophyll deepened along with the mixed layer, and integrated chlorophyll values in the water column declined to one-quarter of the normal by August (*2, 9*). The satellite images suggest that the initial reduction of nearshore phytoplankton productivity during El Niño was associated with weakened upwelling.

These satellite data corroborate the results of two routine data analyses providing more complete spatiotemporal coverage of El Niño (summarized in Fig. 2). Along the coast from 27° to 49°N, markedly anomalous warm temperatures and weak upwelling indices began in December 1982 and peaked in February and March 1983 (Fig. 2A). The slope of SST anomaly contours in December and January indicates poleward propagation at 105 km day^{-1}, compared to a theoretical phase speed of 100 km day^{-1} for coastal trapped internal Kelvin waves. In theory, poleward geostrophic flow induced by a Kelvin wave may transmit El Niño SST and sea-level anomalies from the equator, although Simpson shows that the 1982–1983 California anomalies were more consistent with onshore transport induced by large-scale atmospheric forcing in the north Pacific (*10*). In fact, the anomalies in the upwelling index in Fig. 2B, which indicate positive onshore transport anomalies, appear to propagate equatorward at 150 km day^{-1}. For several months beginning in April, upwelling indices returned to near or above normal and SST anomalies declined to 0.5° to 1.5°C. This moderation of El Niño is also evident in time series of sea level along the California coast (*11*) and follows a transient decline in equatorial SST anomalies that began in January and February (*12*).

The view of El Niño provided by these satellite images, especially the direct evidence of weakened coastal upwelling, could not be readily obtained from conventional ship and buoy data. One of the primary benefits of oceanographic sensors on satellites is that they provide extensive and synoptic coverage of localized mesoscale changes at the sea surface.

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8. Crosses in Fig. 1E mark surface pigment stations. Pigment algorithms are from R. C. Smith and K. A. Baker [*Mar. Biol.* **66**, 269 (1982)] for $C_{sat} < 1.5$ mg m^{-3}, and from (*7*) for $C_{sat} > 1.5$ mg m^{-3}. The C_{sat} values on 15 March 1983 matched ship values from 17 to 18 March (all < 0.5 mg m^{-3}, J. A. McGowan, Scripps Institution of Oceanography) to ±42 percent, with a bias of +23 percent. The CZCS algorithms were less successful in matching higher ship values on 19 April 1982 (up to 8 mg m^{-3}, R. W. Owen, National Marine Fisheries Service), with a root-mean-square error of ±66 percent and a bias of −35 percent. The biases reduce the apparent difference between 1982 and 1983 C_{sat} values.
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Ca^{2+}-Dependent Protein Kinase Injection in a Photoreceptor Mimics Biophysical Effects of Associative Learning

Abstract. *Iontophoretic injection of phosphorylase kinase, a Ca^{2+}-calmodulin–dependent protein kinase, increased input resistance, enhanced the long-lasting depolarization component of the light response, and reduced the early transient outward K^+ current, I_A, and the late K^+ currents, I_B, in type B photoreceptors of* Hermissenda crassicornis *in a Ca^{2+}-dependent manner. Since behavioral and biophysical studies have shown that similar membrane changes persist after associative conditioning, these results suggest that Ca^{2+}-dependent protein phosphorylation could mediate the long-term modulation of specific K^+ channels as a step in the generation of a conditioned behavioral change.*

A learned behavioral change is acquired by the nudibranch mollusk *Hermissenda crassicornis* when trained with paired light and rotation but not with randomized stimuli (*1*). Biophysical changes intrinsic to the soma membrane of the type B photoreceptors have repeatedly been found only in conditioned animals during the acquisition and retention of the learned behavior. These changes, which have been implicated in the generation of conditioning in *Hermissenda*, consist of an increase in the dark membrane input resistance (R_{in}), an enhancement of the light response, notably the long-lasting depolarization (LLD) component that follows a light step (*2, 3*), and reduction of the early transient K^+ current (I_A) (*4*). Preliminary studies suggest that other membrane currents also change with conditioning (*5*), as has recently been proposed (*6, 7*).

A number of studies taken together have suggested how these biophysical changes may occur during acquisition of the learning and how Ca^{2+} might be involved in this process. Synaptic and light-induced depolarization of the type B cells is enhanced and more prolonged when light and rotation are paired (*6*). With repeated light and rotation pairings membrane depolarization persists (*2, 8*). Light- and voltage-dependent depolarization of the type B cell is accompanied by a rise in intracellular Ca^{2+} ($[Ca^{2+}]_i$) (*9*). Elevation of $[Ca^{2+}]_i$ in turn causes long-lasting inactivation of I_A (*10*) and possibly a Ca^{2+}-activated K^+ current (*11*). During retention of the conditioned response, the type B cell does not remain depolarized, but the I_A reduction persists and the LLD remains enhanced.

What biochemical process might underlie the long-term biophysical effects of conditioning, which seem to be related to elevated $[Ca^{2+}]_i$? Previously, we found that phosphorylation in a 20,000-dalton protein was altered in the eyes of conditioned animals (*12*) and that iontophoretic injection of the catalytic subunit of adenosine 3′,5′-monophosphate (cyclic AMP)–dependent protein kinase into

type B cells reduced the late voltage-dependent K^+ current or currents (I_B) more than I_A (*13*). Because elevated $[Ca^{2+}]_i$ was related to the biophysical properties (such as reduction of K^+ currents) that were affected by conditioning, we hypothesized that conditioning induces a rise in $[Ca^{2+}]_i$ that can activate Ca^{2+}-calmodulin–dependent protein kinases, which in turn phosphorylates channel (or channel-associated) proteins either directly or through a biochemical cascade, leading to long-lasting inactivation of K^+ currents. To test this hypothesis, we have injected a species of Ca^{2+}-calmodulin–dependent protein kinase, phosphorylase kinase, into type B photoreceptors under conditions that allow for the control of Ca^{2+} influx and have measured biophysical properties known to change after associative conditioning. We now report that injection of phosphorylase kinase under conditions resulting in a rise in $[Ca^{2+}]_i$ simulates biophysical effects of conditioning (*14*).

All experiments were performed on the isolated type B photoreceptor preparation in untrained animals (*13*). Data were evaluated by one-tailed *t*-tests for correlated samples. Membrane responses were compared before and after iontophoretic injection of phosphorylase kinase (*15*) in the same cell, so that each cell could serve as its own control. In the dark ($N = 7$), phosphorylase kinase injection did not significantly affect R_{in} (*16*) [$\bar{X} \pm$ standard error of the mean (S.E.M.): before, 38.32 ± 5.29 megohm; after, 42.11 ± 4.29 megohm]. However, when followed by one or more light steps (30 seconds long, 10^4 erg cm^{-2} sec^{-1}, every 2.5 minutes), a single phosphorylase kinase injection significantly ($P < 0.001$, $N = 7$) increased R_{in} ($\bar{Y} \pm$ S.E.M. before, 39.64 ± 5.21; after, 56.4 ± 5.93). In some cases, a second phosphorylase kinase injection, followed by additional light steps, further increased R_{in} (Fig. 1A). The effect on R_{in} was apparent within seconds and persisted for the duration of the recording period (30 to 45 minutes). No significant or consistent change in membrane resting potential (E_r) (−55 to −60 mV) followed phosphorylase kinase injection. Control injections of the carrier solution (*15*) (without phosphorylase kinase) did not significantly increase R_{in} (table 1 in *13*). The phosphorylase kinase–induced increase in R_{in} after light steps was not statistically significant in Ca^{2+}-free artificial seawater (ASW) ($N = 6$) or in Ca^{2+}-free ASW plus 1 m*M* EGTA ($N = 3$). These results indicate that a substantial increase in $[Ca^{2+}]_i$ was necessary to produce the phosphorylase kinase–induced increase in R_{in}, which is consistent with the Ca^{2+}-dependent activation of phosphorylase kinase (*17*).

An increased R_{in} could result from a decreased resting K^+ conductance (*18*). In type B cells, I_A constitutes approximately 60 percent of the dark membrane resting conductance (*19*). Since 4-aminopyridine (3 to 10 m*M*) preferentially blocks I_A in type B cells (*11*), we examined its effects on the R_{in}. External 4-aminopyridine (5 m*M*) also significantly ($P < 0.005$, $N = 18$) increased (78 ± 7 percent) R_{in} over the whole voltage-current range (Fig. 1B), in agreement with earlier findings (*20*).

The effects of phosphorylase kinase injection on the light response were also examined. All components of the light response, notably LLD, were significantly enhanced by phosphorylase kinase in a Ca^{2+}-dependent manner (Fig. 2A and Table 1). External 4-aminopyridine (5 m*M*) also significantly ($P < 0.01$, $N = 5$) enhanced all components of the light response, notably LLD (Fig. 2B). Blocking of I_A by 4-aminopyridine would be expected to enhance LLD by unmasking $I_{Ca^{2+}}$ (*21*). [4-Aminopyridine also enhances the sustained depolarization (steady-state component) of the light response in *Limulus* photoreceptors (*22*).] The results of these 4-aminopyridine experiments suggested that the

Table 1. Effects of phosphorylase kinase on the type B photoreceptor light response. Three components of the light response were measured in normal and Ca^{2+}-free ASW: peak, steady state (measured 15 seconds after light onset—SS_{15}), and LLD (measured 30 seconds after light offset—LLD_{30}). Values are means ± S.E.M. for the third light response after a single phosphorylase kinase injection (36 to 54 nC); 30-second light steps (10^4 erg cm^{-2} sec^{-1}) were presented every 2.5 minutes after 10 minutes of initial dark adaptation; N.S., not significant.

Condition	Light response (mV)			*P**
	Before injection	After injection	Difference	
Normal Ca^{2+}-ASW ($N = 5$)				
Peak	28.03 ± 2.15	35.18 ± 1.32	7.15 ± 2.26	< 0.025
SS_{15}	26.17 ± 3.27	32.60 ± 2.27	6.43 ± 2.57	< 0.05
LLD_{30}	0.715 ± 0.71	5.29 ± 0.83	4.58 ± 0.73	< 0.005
Ca^{2+}-free ASW ($N = 6$)				
Peak	38.48 ± 3.14	39.80 ± 3.16	1.32 ± 0.70	N.S.
SS_{15}	30.74 ± 3.53	32.28 ± 4.12	1.54 ± 0.86	N.S.
LLD_{30}	0.86 ± 0.60	0.74 ± 0.66	−0.12 ± 0.84	N.S.

*One-tailed *t*-tests for paired correlated samples.

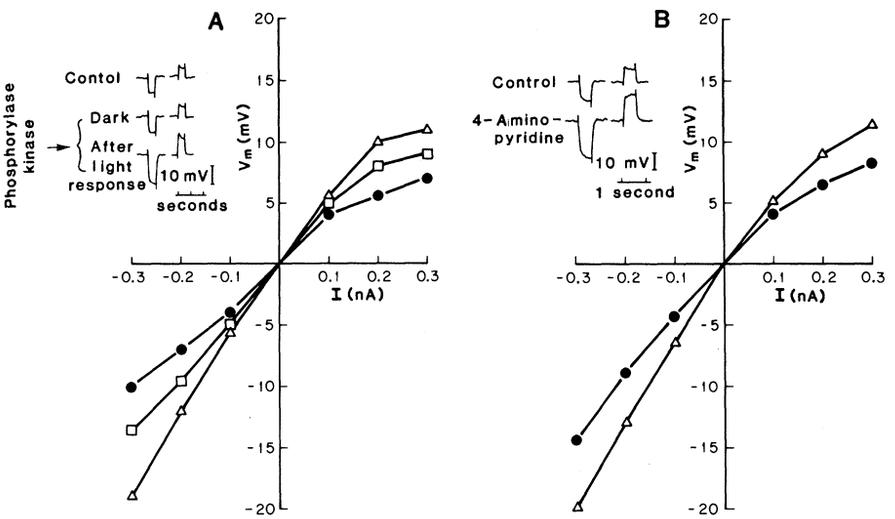


Fig. 1. Effects of phosphorylase kinase (A) and 4-aminopyridine (B) on input resistance (R_{in}) in axotomized type B photoreceptor in normal (10 m*M*) Ca^{2+}-ASW. Each point is a mean of five cells; standard errors are smaller than the symbols. (A) Steady-state voltage-current plots before (control, ●) and after phosphorylase kinase injections. A single injection (□, 36 nC) followed by three light steps increased R_{in} over the entire range. A second injection (△, 54 nC) followed by two additional light steps further increased R_{in} · R_{in} (±0.2-nA, 800-msec pulses) did not increase after an injection given in the dark (inset). (B) 4-Aminopyridine (△) (5 m*M*), which preferentially blocks I_A, mimicked the increase in R_{in} induced by phosphorylase kinase.

Table 2. Effects of phosphorylase kinase on early (I_A) and late K^+ currents (I_B) in type B photoreceptor in darkness and after pairing a light step with a command depolarization to give a Ca^{2+} load in normal Ca^{2+}-ASW; N.S., not significant.

Condition	K^+ currents (nA)			P*
	Before injection	After injection	Difference	
I_A dark (N = 16)	33.26 ± 2.97	34.01 ± 3.07	0.75 ± 0.47	N.S.
Pair 1† (N = 7)	42.70 ± 6.25	38.06 ± 5.69	−4.61 ± 0.87	$P < 0.005$
Pair 2‡ (N = 5)	36.91 ± 6.21	26.79 ± 6.41	−10.12 ± 1.36	$P < 0.005$
I_B dark (N = 16)	16.69 ± 1.42	16.90 ± 1.34	0.21 ± 0.52	N.S.
Pair 1† (N = 6)	18.34 ± 2.29	17.0 ± 1.86	−1.34 ± 0.71	N.S.
Pair 2‡ (N = 5)	13.49 ± 2.09	8.50 ± 2.41	−4.99 ± 1.54	$P < 0.025$

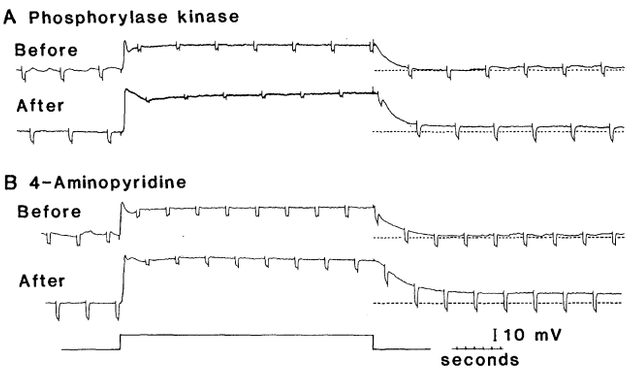
*One-tailed t-test for paired correlated samples. †Single pairing of a light step with command depolarization. ‡Repeated pairings (maximum phosphorylase kinase effect).

phosphorylase kinase–induced increase in the R_{in} and the light response could also be attributed at least in part to a reduction of I_A.

Voltage-clamp experiments (*23*) revealed that a single phosphorylase kinase injection significantly reduced I_A only when the injection was followed by a light step paired with a command depolarization; repeated pairings reduced both I_A and I_B (Fig. 3 and Table 2). In the dark (and with no conditioning depolarization) phosphorylase kinase injection had no statistically significant effect on either I_A or I_B (Table 2). Pairing light with depolarization was necessary because light alone probably caused only a slight rise in $[Ca^{2+}]_i$ under voltage clamp (at V_H = 60 mV). Depolarization alone raises $[Ca^{2+}]_i$ by activating $I_{Ca^{2+}}$, but light paired with depolarization gives rise to a larger increase in $[Ca^{2+}]_i$ (*9*). These results indicate that a substantial increase in $[Ca^{2+}]_i$ is required for the phosphorylase kinase–induced reduction of K^+ currents, in agreement with the aforementioned Ca^{2+}-dependent phosphorylase kinase–induced increase in R_{in}. Identical iontophoretic injections of a control carrier solution were without statistically significant effects on I_A and I_B (table 1 in *13*).

We conclude from the voltage-clamp results that intrasomatic injection of a species of Ca^{2+}-calmodulin–dependent protein kinase, phosphorylase kinase, can reduce K^+ currents in a type B photoreceptor in *Hermissenda*. It is likely that the reduction of I_A by phosphorylase kinase can account for much of the observed increase in R_{in} and the light response. This interpretation is reinforced by the 4-aminopyridine results, which show that an agent that pharmacologically blocks I_A mimics the phosphorylase kinase effects on the R_{in} and the light response. Phosphorylase kinase injection also potentiates the Ca^{2+}-mediated inactivation of I_A and reduces late K^+ currents in giant identifiable *Hermissenda* neurons (*24*). Although the enzyme is capable of reducing K^+ currents and phosphorylating endogenous substrates in *Hermissenda* nervous system homogenates (*25*), we do not imply that endogenous phosphorylase kinase is the main catalytic agent that reduces K^+ currents. We used phosphorylase kinase as a model enzyme for Ca^{2+}-calmodulin–regulated protein phosphorylation because it has not been possible to obtain a purified and stable form of neuronal Ca^{2+}-calmodulin–dependent protein kinase in quantities needed for these experiments.

We have focused on the possible role of Ca^{2+}-calmodulin–dependent protein kinase in the regulation of K^+ currents, whereas a number of recent reports have implicated cyclic AMP–dependent protein kinase in the regulation (both decrease and increase) of various species of K^+ currents in a variety of molluscan neurons (*26*). While it remains to be shown whether specific K^+ channels (or channel-associated proteins) exhibit preferential substrate specificity for a Ca^{2+}-calmodulin–dependent or cyclic AMP–dependent protein kinase, the greater reduction of I_B versus I_A by cyclic AMP–dependent protein kinase injection (*13*) suggests that late K^+ channels have a preferential substrate specificity for cyclic AMP–dependent phosphorylation. However, since I_A and I_B are reduced by both phosphorylase kinase and the catalytic subunit of cyclic AMP–dependent protein kinase, early and late K^+ channels (or channel-associated proteins) may be substrates for both types of protein kinases. Evidence that cyclic AMP–dependent and Ca^{2+}-calmodulin–dependent protein kinases can phosphorylate the same protein substrate has been found in mammalian brain, cardiac muscle, and *Aplysia* and *Hermissenda* neural tissue (*25*, *27*). Interactive regulatory effects of Ca^{2+} and



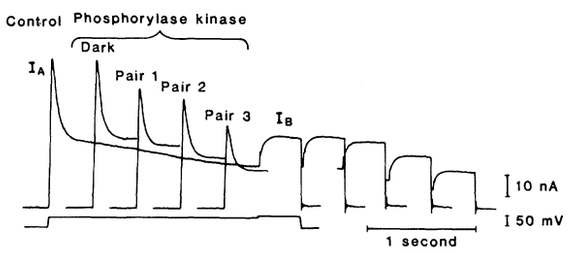


Fig. 2 (left). Effects of phosphorylase kinase and 4-aminopyridine on the light response in axotomized type B photoreceptors in normal Ca^{2+}-ASW. (A) Before (control) and 10 minutes after single injections (36 nC, third light response). (B) 4-Aminopyridine (5 m*M*) mimicked the enhancement of all components of the light response by phosphorylase kinase. Constant current pulses (−0.2 nA, 400 msec) monitored R_{in} before, during, and after the light response. The dotted baseline shows the magnitude of LLD after each treatment. Fig. 3 (right). Effects of a single injection of phosphorylase kinase (60 nC) on axotomized type B photoreceptor K^+ currents in normal Ca^{2+}-ASW. From a V_H of −60 mV, I_A (left) was elicited by the first command step to −10 mV and decayed within approximately 1 second to the steady state. A second superimposed step to 0 mV, applied 1.9 seconds after the first step, elicited I_B (*31*). The family of K^+ currents (I_A and corresponding I_B) show that phosphorylase kinase had no effect on I_A or I_B in darkness, but reduced I_A after a 20-second pairing of light with depolarization to give a Ca^{2+} load (pair 1, to −10 mV). Further reduction of I_A and also I_B was evident after two additional pairings associated with increasing command steps (pair 2, to 0 mV; pair 3, to 15 mV). A full record is shown only for the control condition before the phosphorylase kinase injection.

cyclic AMP (*28*) are also conceivable since an increase in $[Ca^{2+}]_i$ could activate both endogenous Ca^{2+}-calmodulin– and cyclic AMP–dependent protein kinase, the latter by way of Ca^{2+}-calmodulin regulation of cyclic AMP production. It is also possible that the native Ca^{2+}-calmodulin–dependent protein kinase could itself become activated by cyclic AMP–mediated phosphorylation in analogy with the activation of phosphorylase kinase in skeletal muscle (*29*).

We suggest that Ca^{2+}-calmodulin–dependent protein phosphorylation might amplify the Ca^{2+}-mediated inactivation of I_A to produce the long-term reduction of I_A in type B photoreceptors after associative conditioning. We propose that repeated stimulus pairings (light and rotation) during acquisition of the learned behavior can elevate $[Ca^{2+}]_i$, which then activates Ca^{2+}-calmodulin–dependent protein kinases capable of phosphorylating I_A channels (or channel-associated proteins) to produce the long-lasting Ca^{2+}-mediated inactivation of I_A. Reduction of I_A (and possibly late K^+ currents) on retention days produces an enhanced depolarizing light response resulting in increased impulse activity during and after a light stimulus. This increased activity, through known synaptic interactions among type A photoreceptors, the statocyst, and identified interneurons, can reduce the motoneuron output to the foot musculature and thereby could reduce the phototactic locomotor behavioral response (*30*).

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23. Phosphorylase kinase was iontophoretically injected (60 nC) through the voltage-recording electrode while equal and opposite current was passed through the current-delivery electrode to prevent any net change in membrane potential (E_m) during phosphorylase kinase injection. The following criteria for acceptability of results were used: (i) E_m recorded by the voltage-recording and current-passing electrodes should be approximately equal and ≥ −40 mV; (ii) the light response recorded by each electrode should be the same; and (iii) the holding current at a holding potential (V_H) of −60 mV should be ≥5 nA and should not change after phosphorylase kinase injection. Leakage current correction for I_A and I_B was obtained by extrapolation from the linear portion of the current-voltage relation.
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31. I_A was measured as the peak component of outward current from baseline (after leak correction). From the staircase command voltage procedure used (20° to 22°C), I_A peaks within 25 msec, whereas I_B peaks between 400 and 500 msec and is made up of two components: a voltage-dependent late K^+ current ($I_{K(V)}$, delayed rectifier), and a Ca^{2+}-activated K^+ current ($I_{K(Ca^{2+})}$ or I_C. Although there is variability in the relative contribution of $I_{K(V)}$ and I_C to I_B for each cell, a 2-second preliminary pulse to −10 to 0 mV causes substantial inactivation of I_C (*11*). The remaining I_B after the preliminary pulse consists therefore of a partially activated $I_{K(V)}$ and markedly inactivated I_C.
32. We thank J. Kuzirian for helping to prepare the manuscript, B. and L. Golder for their help with the illustrations, H. Rasmussen for initially suggesting the use of phosphorylase kinase, E. G. Krebs for providing highly purified phosphorylase kinase, and J. Oschman for total support. J.A.U. is a Fogarty International Visiting Fellow, National Institutes of Health.

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Do Bacteria-Sized Marine Eukaryotes Consume Significant Bacterial Production?

Abstract. *Up to 60 percent of the total marine primary production (or about one-fourth of the total global carbon dioxide fixation) passes through the free-living bacterioplankton. Grazing by bacteriovores is probably the predominant fate of the bacteria, although data are scarce. Evidence is presented that previously uncharacterized, small eukaryotes that are able to pass even 0.6-micrometer filters may be responsible for a large fraction (more than 50 percent) of the total grazing in coastal waters. These organisms have not yet been observed microscopically.*

Approximately 40 percent of the global primary production is marine (*1*), and measurements show that 20 to 60 percent of the marine production passes through the free-living, heterotrophic bacterioplankton (prokaryotes, mostly 0.2 to 1 μm in length), with about half of this appearing in the form of bacterial biomass (*2–4*). The importance of production in this size class is even greater since the observation was made that 20 to 80 percent of the autotrophic production in tropical waters can be attributed to organisms (mostly prokaryotes) passing 1-μm filters (*5*). The subsequent fate of these prokaryotes remains uncertain, although studies indicate the importance of grazing by small flagellate protozoa, observed to be mostly 2 to 10 μm in diameter (*6, 7*). Knowledge of this fate is important because different potential pathways can lead to different patterns of nutrient regeneration and of carbon and nitrogen cycling in the sea (*2, 8*). We now describe our study of eukaryotic grazing on natural bacteria; with the use of antibiotic inhibitors we have found that at times more than 50 percent of the total apparent grazing activity in coastal waters can pass through even 0.6-μm filters.

Earlier studies of marine bactivory