above 90 percent were found on 12 occasions when, on averaging a station series, more than six herring larvae and more than 90 ml of *Aurelia* were caught (Fig. 1). This can be interpreted as an effect of predation since the distribution of jellyfish in the fjord is independent of the localization of the herring spawning grounds (*1*).

During the 4 years of investigation, the first emergence of herring larvae in Kiel Fjord each year occurred between 20 April and 6 May (±4 days). An increase in the *Aurelia* biomass was observed about 3 weeks later, except in 1979. In 1978, 1980, and 1981 a decline of 50 percent or more in the larval yolk-sac herring population took place during those weeks when the biomass of *Aurelia* exceeded 75 ml per 100 m^3 for the first time. This decline was observed during 4 weeks in 1978, during 2 weeks in 1980, and during 5 weeks in 1981 after the first emergence of the larvae. This supports the hypothesis that the yolk-sac period of the larvae present in the plankton might be influenced by the growth of the jellyfish population.

The greatest number of herring larvae was caught in 1979, when the biomass of jellyfish was the smallest of the 4 years of the investigation (Fig. 2). Only about one-third of this larval quantity was caught in 1978 and 1980, the same 2 years during which the greatest biomass of *Aurelia* was present. The negative relation between the sizes of the larval herring and jellyfish populations seems to be strong, whereas larval abundance and abundance of the spawning parents (symbolized by an adult herring in Fig. 2) for the years indicated do not seem correlated.

Quantification and explanation of the high natural mortality among early larval stages of marine fish is one of the key problems in fisheries biology. Starvation after metamorphosis from the yolk-sac stage to an active feeder has been considered as the main contributive factor to this mortality. However, evidence has accumulated that shows that the effects of cannibalism (*9*) and predation by other planktivorous fish species (*10*) and invertebrates (*7*) might have been underestimated. The results of this study indicate that, at least in cases where fish spawn in small specific areas and where they are unable to avoid jellyfish blooms, predation might be a major regulating factor of the size of the surviving larval population.

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A Potential Second Messenger Role for Unsaturated Fatty Acids: Activation of Ca^{2+}-Dependent Protein Kinase

Abstract. *Arachidonate and other unsaturated long-chain fatty acids were found to activate protein kinase C from human neutrophils. Kinase activation by arachidonate required calcium and was enhanced by diolein but did not require exogenous phosphatidylserine. Submaximal levels of arachidonate also enhanced the affinity of the kinase for calcium during activation by phosphatidylserine. Thus the release of arachidonate, which is triggered in many cell types by ligand-receptor interactions, could play a second messenger role in the regulation of cellular function by activation of protein kinase C.*

A calcium- and phospholipid-dependent protein kinase (protein kinase C) may have an important role in the regulation of cellular function (*1*). Activation of protein kinase C in cells can be accomplished by an increase in intracellular calcium concentration or the release of diacylglycerol by stimulus-induced phosphatidylinositol turnover or both (*2*). The tumor promotor phorbol myristate acetate (PMA) also activates protein kinase C from rodent brain extracts (*3, 4*). PMA is a potent inducer of the respiratory burst in human neutrophils and other phagocytes (*5*), a response that is critical for the microbicidal, tumoricidal, and inflammatory functions of these cells. Physiological stimuli such as chemoattractants and opsonized particles also trigger the respiratory burst in phagocytes (*6*), as well as the remodeling of membrane phospholipids (*7*) and the release of arachidonate in leukocytes (*8*). Interestingly, arachidonate and other unsaturated fatty acids stimulate the respiratory burst in phagocytic cells (*9*). Moreover, arachidonate has been reported to directly activate the respiratory burst enzyme in disrupted cell preparations from macrophages (*10*). Taken together, these findings suggest that arachidonate or a metabolite could serve a second messenger function. We investigated the possibility that arachidonate could also affect the activity of protein kinase C and now report that unsaturated fatty acids, including arachidonate, directly activate human neutrophil protein kinase C in a dose- and calcium-dependent fashion.

Protein kinase C activity was measured in detergent extracts of isolated human neutrophils (*11*). Cells at a concentration of 5×10^7 per milliliter in 0.1 percent Triton X-100 extraction buffer (*12*) were sonicated (*11*) and stirred for 1 hour at 4°C, and insoluble debris was sedimented by centrifugation for 1 hour at 100,000*g*. Activity was assayed as the amount of incorporation of ^{32}P from [γ-^{32}P]ATP (New England Nuclear) into histone H1 (Sigma) for 10 minutes at 30°C (*1, 13*) and was expressed as picomoles of ^{32}P incorporated per minute per milligram of protein (*13*). Without addition of exogenous lipid, no calcium-dependent protein kinase activity was measurable (Fig. 1A), but the addition of arachidonate (100 μM) stimulated activity. At 2 mM added calcium, protein kinase C activity was 189 pmole of ^{32}P incorporated per minute per milligram of protein. When the arachidonate concentration was increased to 200 μM, higher protein kinase C activity was obtained (314 pmole of ^{32}P incorporated per minute per milligram of protein at 2 mM Ca^{2+}). Diolein alone did not activate neutrophil protein kinase C, similar to observations in other cell types (Fig. 1B) (*2*). However, 100 μM arachidonate plus diolein resulted in 1.6-fold more kinase activation than arachidonate alone (293 pmole of ^{32}P incorporated per minute per milligram of protein). This arachidonate concentration plus a normally optimal dose of phosphatidylserine also resulted in protein kinase C activity that was higher than that observed with either alone (Fig. 1C). Arachidonate had no further effect on kinase activation in the presence of optimal amounts of both phosphatidylserine and diolein, suggesting that arachidonate and phosphatidyl-

serine activate the same enzyme (Fig. 1D).

In the absence of other lipids, the dissociation constant (K_d) for Ca^{2+} with arachidonate as activator was approximately 17 μM (Table 1). A similar K_d (14 μM) was obtained with phosphatidylserine as activator. However, the mixture of both arachidonate and phosphatidylserine resulted in a 2.5- to 3-fold increase in Ca^{2+} affinity. Diolein had little effect on the K_d for Ca^{2+} with arachidonate as activator but caused an increase in Ca^{2+} affinity with either phosphatidylserine or arachidonate plus phosphatidylserine as activators. Thus, by increasing the affinity of the kinase for Ca^{2+}, arachidonate, like diolein (*2*), can trigger activation of protein kinase C in the presence of phosphatidylserine without a change in Ca^{2+} concentration.

Detergent extracts of cells could contain other lipids that affect the arachidonate-mediated activation of protein kinase C (Table 1). To decrease the level of contaminating membrane lipids, a cytosolic fraction obtained at 100,000*g* was prepared from neutrophils sonicated in the absence of detergent (plus EGTA). This fraction contained 90 to 95 percent of the cellular protein kinase C activity (not shown). Calcium-dependent activation of cytosolic protein kinase activity by arachidonate (100 μM) was 742 ± 66 pmole of ^{32}P incorporated per minute per milligram of protein, whereas maximum stimulation by phosphatidylserine and diolein was 962 ± 89 [mean ± standard error of the mean (S.E.M.), N = 4 to 5]. Also, arachidonate-mediated kinase activity in this fraction was unaffected by the presence of 50 μg/ml of leupeptin (not shown), suggesting that a Ca^{2+}-dependent protease was not involved in protein kinase C activation (*14*).

Arachidonate can be oxidized by air during storage as well as metabolized to oxidized products in the neutrophil (*15*), so it was important to determine the specificity of the arachidonate-mediated activation of protein kinase C. We compared the activity of fresh arachidonate and an arachidonate preparation allowed to air-oxidize overnight at room temperature. As shown in Fig. 2, A and B, oxidation caused a shift to the right in the dose-response curve for arachidonate and a decrease in maximum activity in the absence of diolein. The exposed arachidonate preparation was subjected to thin-layer chromatography (TLC) (*16*), and fatty acid spots were extracted in ethanol and tested for their ability to activate protein kinase C. Only the extract of the spot that migrated with the fresh arachidonate (a single spot on the plate) was capable of kinase activation (data not shown). Thus, the activity in the oxidized arachidonate preparation was apparently caused by the residual unoxidized arachidonate. Further, none of the extracts of the oxidized products had any inhibitory activity when mixed with fresh arachidonate.

Table 1. Effect of various lipids on the apparent dissociation constant (K_d) for Ca^{2+} of human neutrophil protein kinase C.

Lipid activator*	K_d for Ca^{2+} (μM)†	
	Without diolein	With diolein
Arachidonate	17.4 ± 2.4 (3)	14.3 ± 4.9 (4)
Phosphatidylserine	14.2 ± 1.0 (3)	2.2 ± 0.8 (3)
Arachidonate + phosphatidylserine	5.8 ± 1.9 (3)	1.6 ± 0.5 (3)

*Protein kinase C activity was measured in neutrophil extracts in the presence or absence of 2 μg per milliliter of diolein, with 100 μM arachidonate or 20 μg per milliliter of phosphatidylserine or both as indicated. Added Ca^{2+} concentration was varied from 0 to 2.0 mM as in Fig. 1. †The apparent K_d for Ca^{2+} was calculated by Lineweaver-Burk analysis of activity at the estimated true concentrations of Ca^{2+} (22) after basal activity at 0 added Ca^{2+} was subtracted. Values shown are the mean ± S.E.M. with the number of separate experiments given in parentheses.

We also tested the effect of two drugs which inhibit arachidonate metabolism—indomethacin, a cyclo-oxygenase inhibitor, and nordihydroguaiaretic acid (NDGA), a lipoxygenase inhibitor. Scant inhibition of the arachidonate-mediated or the arachidonate plus diolein–mediated activation of protein kinase C was observed with either NDGA at 10 μM or indomethacin at 100 μM [NDGA, 1.5 ± 1.5, N = 2; indomethacin, 11.3 ± 4.2, N = 4 (mean percent inhibition ± S.E.M.)]. To eliminate the possibility that added arachidonate was incorporated into more complex lipids, we incubated trace-labeled arachidonate with neutrophil detergent extracts under normal assay conditions and fractionated extracted lipids by TLC (*3*, *16*). No evidence was found for incorporation of arachidonate into diacylglycerol or phospholipid. These results indicate that arachidonate and not a product is responsible for protein kinase C activation.

We tested the ability of a variety of unsaturated or saturated long-chain fatty

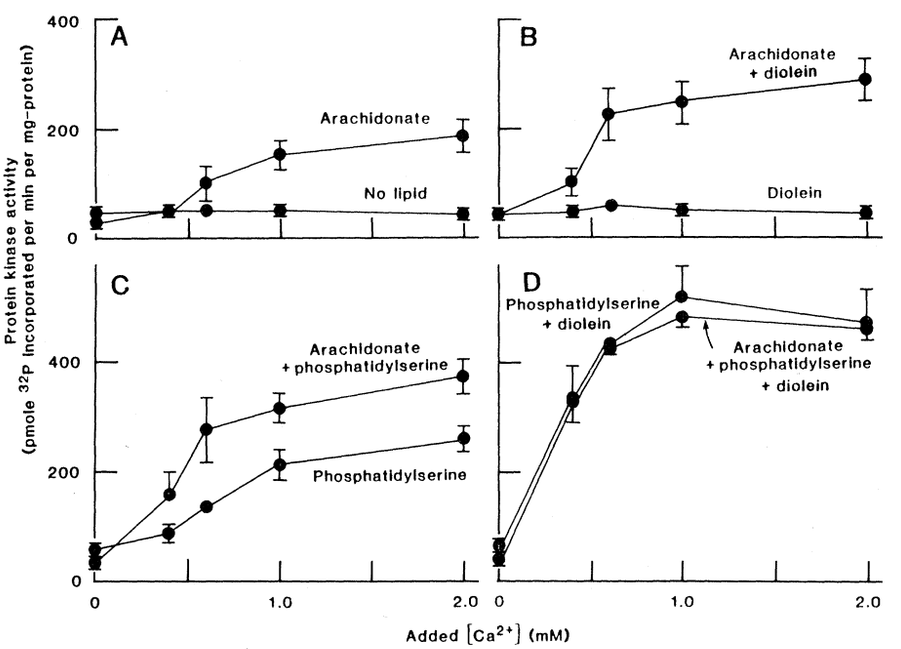

Fig. 1. Influence of arachidonate and other lipids on activation of human neutrophil protein kinase C. Neutrophil detergent extracts were stored at 4°C and were stable for at least 3 to 4 weeks. Assays used approximately 40 μg of extract protein; 0.4 mM EGTA; various amounts of added Ca^{2+}; 10 mM $MgCl_2$; 10 μM ATP; 0.2 mM phenylmethylsulfonyl fluoride; 40 μg of histone; 10 mM 2-mercaptoethanol; 35 mM tris-HCl, pH 7.5; various lipids as indicated; and approximately 1 × 10^6 count/min [γ-^{32}P]ATP in a final volume of 0.25 ml. Concentrations of lipids when present were 100 μM arachidonate (Nu-Chek Prep), 2 μg of 1,2-diolein (Sigma) per milliliter, and 20 μg of phosphatidylserine (Sigma) per milliliter. These concentrations of diolein and phosphatidylserine were optimal in preliminary experiments. Diolein and phosphatidylserine were stored at −20°C in chloroform, evaporated under N_2, and resuspended in buffer by sonication before use. Arachidonate was prepared fresh under N_2 before use as a 4.1 mM stock in 25 percent ethanol and adjusted to neutrality (*9*). Values plotted are mean ± S.E.M. of one to four experiments at each concentration of Ca^{2+}.

acids to activate protein kinase C. As shown in Fig. 2, C and D, other unsaturated fatty acids also activated protein kinase C in the presence or absence of diolein. The potency of the unsaturated fatty acids paralleled to some degree the number of *cis* double bonds, in that γ-linolenate (18:3) > linoleate (18:2) > oleate (18:1). In contrast, the saturated fatty acid palmitate (16:0) did not stimulate kinase activity. Concentrations of stearate (18:0) up to 100 μ*M* also failed to activate protein kinase C (data not shown). Higher concentrations were not tested because of insolubility.

Higher doses of unsaturated fatty acids resulted in a loss of protein kinase C activity, both in the presence and absence of diolein (Fig. 2). The loss of activity caused by arachidonate could not be reversed by the addition of an optimal concentration of phosphatidylserine (not shown). Thus, it is conceivable that, depending on its local concentration, arachidonate could either enhance or inhibit protein kinase C activity in the neutrophil. The actual local concentrations of unsaturated fatty acids achieved during neutrophil stimulation cannot be determined, but the concentrations we used were similar to the concentration of phosphatidylserine (40 μ*M*) required for maximal activation of purified heart protein kinase C (*17*).

Our results show that arachidonate and other unsaturated fatty acids can activate or, at higher concentrations, inhibit protein kinase C from human neutrophils. Thus, the release of arachidonate triggered by neutrophil stimuli such as chemoattractants, opsonized particles, and the calcium ionophore A23187 (*8*) may result in activation of protein kinase C, although observations of arachidonate release in human neutrophils mediated by chemoattractants have been challenged (*18*). Since arachidonate and other unsaturated long-chain fatty acids can directly stimulate phagocytic cell function including the respiratory burst (*9*), our results suggest that these effects are mediated by protein kinase C. The observations that PMA also activates this kinase (*3, 4*) further suggest a role for protein kinase C in neutrophil activation. We have also found that stimulation of human neutrophils by chemoattractants, A23187, or PMA induces a rapid three- to sixfold increase in membrane-associated protein kinase C activity (*19*).

A more general role for arachidonate as a modulator of stimulus-response coupling may exist. Many extracellular messengers, including muscarinic-cholinergic and α-adrenergic agonists, insulin and other peptide hormones, growth factors, histamine, and thrombin, stimulate phosphatidylinositol turnover and the release of arachidonate after binding to their receptors (*20*). Modulation of protein kinase C activity by diacylglycerol has been demonstrated (*2*); the ability of arachidonate to activate directly and regulate protein kinase C may play an equally important role in receptor function and cellular regulation.

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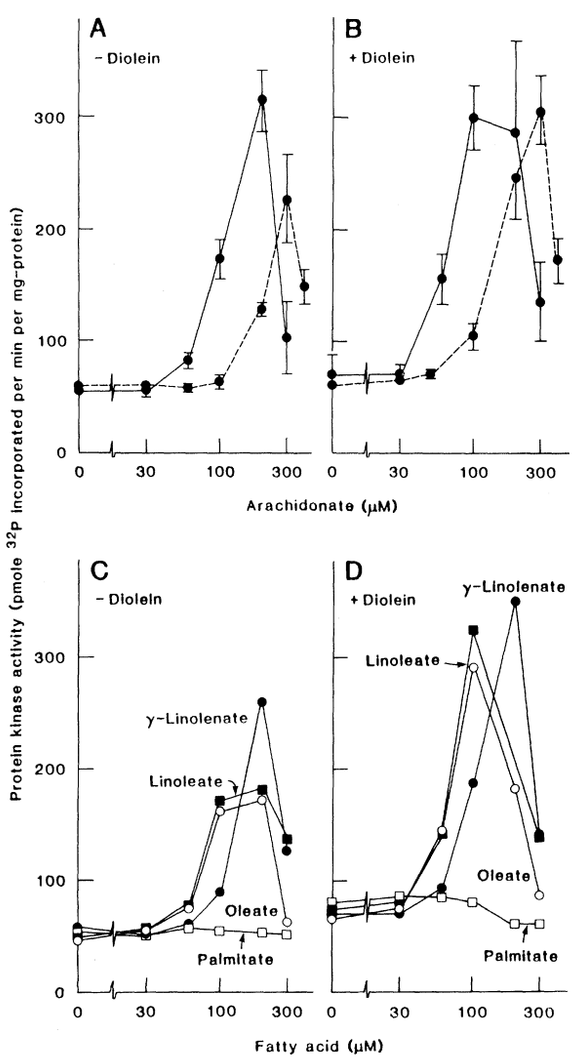

Fig. 2. Concentration-dependent effects of arachidonate and other long-chain fatty acids on activation of human neutrophil protein kinase C. Assays were performed with 2 m*M* added Ca^{2+} in the absence (A and C) or presence (B and D) of 2 μg/ml of diolein. (A and B) Comparison of fresh arachidonate (——) and an arachidonate preparation air-oxidized overnight (- - - -). Oxidation was shown by the appearance of an absorption peak at 236 nm (in two experiments, mean absorbances at 164 μ*M* concentration were 0.032 absorption units for fresh arachidonate and 0.759 absorption units for the oxidized arachidonate preparations). Values plotted are mean ± S.E.M. of three to six experiments with fresh arachidonate and one to three experiments with oxidized preparations. (C and D) Effect of other long-chain fatty acids on protein kinase C activity. *Cis* unsaturated fatty acids (γ-linolenate, linoleate, and oleate from Nu-Chek Prep) and palmitate (Sigma) were prepared fresh under N_2 in 25 percent ethanol. Values are means of two to four experiments performed with each fatty acid.

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Direct Excitatory Interactions Between Cones of Different Spectral Types in the Turtle Retina

Abstract. *Cone linear sensitivities to red and green stimuli were measured intracellularly in the dark- and light-adapted turtle retina. Test flashes of small diameter were used to minimize horizontal cell feedback. Light adaptation was achieved with either green or red background illumination. The ratio of cone sensitivities to red and green light depended on the color of the background light and differed from the ratio measured in the dark. Electron microscope studies of Golgi-stained turtle cones revealed direct synaptic connections between red and green cones mediated by cone telodendria. These data indicate that the red cone photoresponse is not univariant as has been previously supposed and suggest that mixing of signals from different spectral classes of cones can occur via direct excitatory connections between cones.*

The processing of color information by the vertebrate visual system has yet to be fully understood. A fundamental tenet in this processing is the principle of univariance; the isomerization of photopigment is solely a function of the number of quanta absorbed by the pigment and is independent of the wavelength of the incident illumination (*1*). It has generally been believed, and the evidence to date has supported the notion, that cone photoreceptors manifest univariant photoresponses when stimulated with stimuli of small diameter (*2–5*). This feature of the cone photoresponse was attributed to the univariance of the photochemistry in each class of cones and to the finding that cellular interactions between neighboring cones appeared to be limited to cones containing the same visual pigment (*2, 3, 6, 7*). If double cones are excluded (*8*), nonunivariant photoresponses have been observed in single cones only when they were stimulated by test flashes of large diameter, a stimulus condition that would produce negative feedback from horizontal cells onto cones (*3, 7, 9*). We describe in this report electrophysiological and anatomical evidence which suggests that the photoresponses of the "red" cones in the turtle retina are not univariant even when evoked by stimuli of small diameter.

Recordings were made in the cones of the red-eared turtle, *Pseudemys scripta elegans*, according to standard intracellular techniques (*10*). Cone identification was based upon previously published criteria of receptive field size (*11*) and cone spectral type was established by measuring sensitivities to red and green test flashes (*12*). The results described below show the effects of colored backgrounds on the kinetics and sensitivities of red cone "linear responses" (*13*) to red and green test flashes. Because our results run counter to previous published results, our experimental procedure (*14*) was designed to ensure reliable observations. To minimize the effects of horizontal cell feedback on cone sensitivity and yet fully stimulate the cone's excitatory receptive field, all test flashes were either 180 or 320 μm in diameter (*15*). Test flashes of these diameters, and of the intensities used in this study, evoked horizontal cell responses less than 1 mV in amplitude. The color and intensities of the test and background illumination were controlled with narrow-band interference filters and neutral density filters, each of which was calibrated at the wavelengths of the interference filters. The test flash duration was 50 msec. The colored background light illuminated a circular region approximately 3 mm in diameter.

Univariance of the cone photoresponse implies that (i) the intensities of two different colored test flashes can be adjusted such that they elicit linear range photoresponses that are indistinguishable, and (ii) the ratio of sensitivities measured with these two different colored stimuli is independent of the state of adaptation (*1, 3, 4*). Figure 1A shows dark-adapted photoresponses when the intensity of the green flash was adjusted to obtain a response as similar as possible to that evoked by the dim red flash. Although the peak amplitudes of the two responses can be perfectly matched, the kinetics of the responses differ. The differences in kinetics, although small, were observed in all ten red cones studied. These differences are inconsistent with the principle of univariant photoresponses.

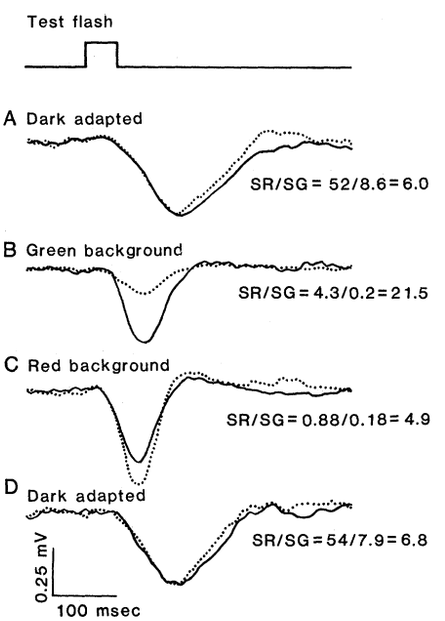

Fig. 1. Intracellular linear range responses measured in a red cone to red (694 nm, solid lines) and green (546 nm, dotted lines) test flashes. The numbers to the right of each set of responses are the red and green flash sensitivites (in microvolts of peak response per quanta per square micrometer on the retina) and the ratio of these cone sensitivities. The uppermost trace describes the timing and duration of the test flashes. From 30 to 40 responses were averaged in each of the displayed responses. (A) Responses recorded in the dark-adapted cone. The intensity of the green flash was adjusted to provide a response equal in amplitude to that evoked by the red flash. (B) Responses recorded in the same cone that had been light adapted with a green (500 nm) background light of an intensity of 1.4×10^{-6} quanta sec^{-1} μm^{-2}. The intensities of the red and green flashes have been increased by a factor of 12.1 over the values used in the dark-adapted retina. (C) Responses recorded in the same cone that had been light-adapted with a red (700 nm) background light of an intensity of 8.11×10^{4} quanta sec^{-1} μm^{-2}. The test flash intensities have been increased by a factor of 59 over the values used in the dark-adapted retina. (D) Control responses recorded in the dark-adapted retina after the periods of light adaptation.