with spinach fraction I protein. Gel electrophoresis [in the presence of sodium dodecyl sulfate (SDS)] of crystalline tobacco fraction I protein and spinach fraction I protein before and after crystallization reveal indistinguishable patterns (Fig. 2a) with only the two expected bands, corresponding to the large and small subunits. In addition, the protein crystals from spinach had identical immunological properties (Fig. 2b) to that of spinach fraction I protein before crystallization. Finally, the carboxylase and oxygenase activities of freshly prepared, dissolved protein crystals were comparable to those of noncrystalline spinach fraction I protein purified by zonal centrifugation (Table 1). The enzymatic activities were identical after the first and second crystallizations; thus the homogeneous enzyme was purified to constant specific activity.

Our measurements (Table 1) show that neither the carboxylase nor the oxygenase activities, which copurify after repeated crystallizations, are associated with a protein containing tightly bound copper. Furthermore, the oxygenase activity is inseparable from the carboxylase by crystallization. Although iron could also function as a prosthetic group to activate molecular oxygen in the oxygenase reaction, only trace amounts of iron are present in the crystalline protein (Table 1). These results are in agreement with earlier measurements of spinach (*9*) and crystalline tobacco (*10*) fraction I protein.

The crystallization of RuBP carboxylase-oxygenase from spinach and other plant species (*16*) provides the experimental material necessary to carry out comparative structural, enzymatic, and evolutionary analyses. Such studies are germane to elucidating the biological significance of the counterproductive enzymatic activities of this bifunctional protein.

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Both Photons and Fluoride Ions Excite *Limulus* Ventral Photoreceptors

Abstract. *Illuminating* Limulus *ventral photoreceptors with dim light results in a response composed of discrete waves. In the dark, adding 10 mM sodium fluoride to the artificial seawater that bathes the photoreceptor includes discrete waves. The fluoride-induced waves are similar to those evoked by light. These findings suggest that fluoride induces a process similar or identical to visual excitation of the photoreceptor.*

Hecht, Schlaer, and Pirenne (*1*) suggested, on the basis of psychophysical experiments, that rod photoreceptors are excited by the absorption of a single quantum of light. Subsequently, Yeandle (*2*) recorded discrete waves from *Limulus* lateral eye photoreceptors that behaved statistically as if they were the result of the absorption of single photons. Over the ensuing years discrete waves have been recorded from a variety of invertebrate photoreceptors (*3*). Recently, light-induced discrete fluctuations in membrane current, presumably the result of the absorption of single photons, have been observed in vertebrate rods (*4*).

Discrete waves are typically observed when a receptor is stimulated with dim light. Photoreceptors respond to brighter light with a change in membrane potential that is graded with stimulus intensity. Dodge *et al.* (*5*) have suggested that the response of *Limulus* photoreceptors, for all stimulus intensities, is the result of a summation of these discrete waves. Furthermore, they proposed that a change in the size of discrete waves is the primary mechanism of light and dark adaptation. Thus, the mechanism of discrete wave

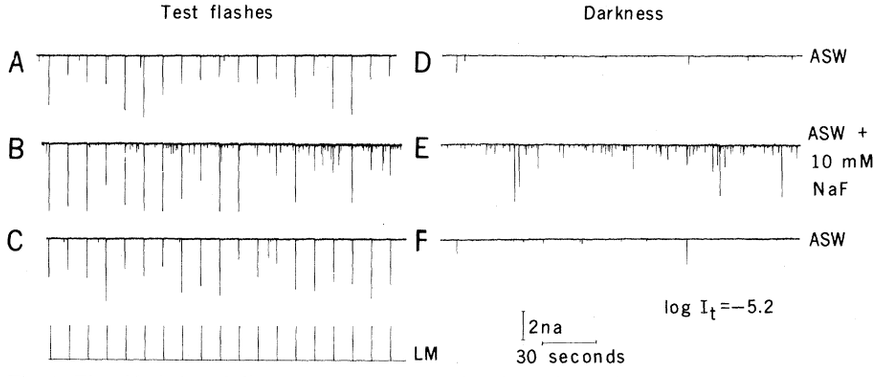

Fig. 1. Fluoride-induced discrete waves measured under voltage clamp. The cell was voltage-clamped to its resting dark potential throughout the experiment. Inward membrane current is shown as a downward deflection. I_t is the intensity of the 20-msec test flash, the occurrence of which is shown by the light monitor (*LM*).

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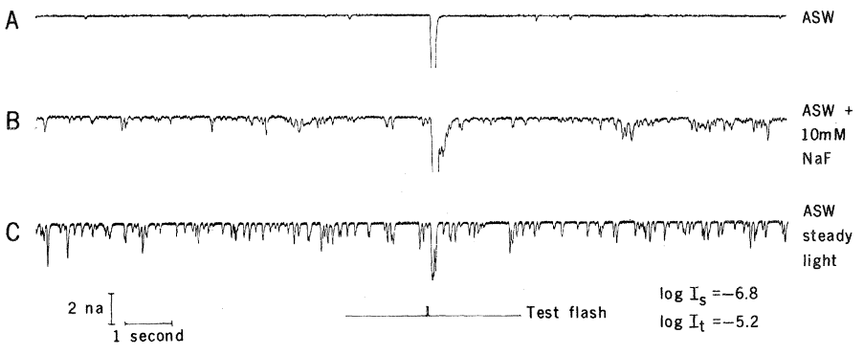

Fig. 2. Comparison of fluoride-induced discrete waves with light-induced discrete waves. I_s is the intensity of the steady light. In (A) and (B), the peak of the response to the test flash is off scale.

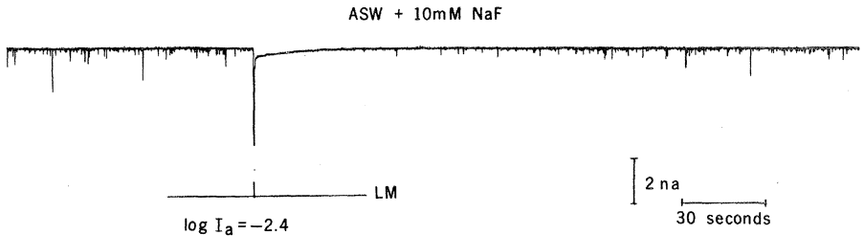

Fig. 3. The effect of light adaptation on fluoride-induced discrete waves. I_a is the intensity of the 20-msec adapting flash, the occurrence of which is indicated by *LM*. The peak of the response to the adapting flash is off scale.

production would be fundamental to the process of visual excitation. We report here that adding sodium fluoride to the artificial sea water (ASW) that bathes *Limulus* ventral photoreceptors results in a dramatic increase in the rate of discrete waves recorded in the dark. These fluoride-induced discrete waves appear to be similar to those evoked by light.

The detailed methods of dissecting, viewing, stimulating, and recording from the photoreceptor under voltage clamp have all been described (*6*). We have now continuously superfused the photoreceptors with ASW (*7*) at a rate of more than 6 chamber volumes per minute.

Figure 1 shows the effect of fluoride on these photoreceptors (*8*); similar results were obtained in all 15 cells studied. Figure 1A shows the recording from the cell before exposure to fluoride. After exposure to fluoride for 20 minutes the rate at which discrete waves occurred in the dark between stimulus flashes increased (Fig. 1B). The frequency increased without any appreciable change in the amplitude of the response to the test flashes, which were of constant intensity. Thirty minutes after the return to fluoride-free ASW, the effect of the fluoride was reversed (Fig. 1C).

This result suggested several questions. Was the production of fluoride-induced discrete waves dependent on the test stimulus? How similar were the fluoride-induced discrete waves to those evoked by light? Could the fluoride-induced discrete waves be light adapted? Did the waveform of the response to the test stimulus change in the presence of fluoride?

The increased rate of discrete waves (Fig. 1B) might result fully or partly from exposure to fluoride in the presence of light. We tested this possibility by exposing the cell to fluoride in the dark. Figure 1, D and F, show the recordings from the cell in the absence of fluoride (before and after exposure, respectively). The fluoride-induced discrete waves recorded from the cell in the dark are shown in Fig. 1E. The induction of discrete waves by fluoride thus does not require the presence of light (*9*).

There are no striking differences, if any, in the time courses of the discrete waves induced by fluoride and by light; they appear to have similar waveforms (Fig. 2). If the fluoride-induced discrete waves are analogous to those evoked by light, they should behave similarly; for example, it should be possible to adapt them to light. This was possible (Fig. 3). On the basis of the similarity of waveform (Fig. 2) and their susceptibility to light adaptation (Fig. 3) we tentatively conclude that the fluoride-induced discrete waves are similar to the light-evoked discrete waves.

Fluoride-induced discrete waves of membrane depolarization can also be observed in recordings of transmembrane potential. These are superimposed on a steady hyperpolarization of a few millivolts. In contrast, in *Balanus* photoreceptors, the substitution of fluoride for chloride irreversibly depolarized the preparation (*10*). This difference between the two preparations may result from the disparity in the concentrations of fluoride used.

Fluoride had other effects on the photoreceptor (Fig. 2). (i) The discrete waves induced by fluoride tended to be smaller than those induced by light. (ii) The duration of the response to the test flash was prolonged in fluoride. This increase in response duration occurred without any appreciable change in response amplitude (Fig. 1). (iii) The discrete waves induced by fluoride had a tendency to cluster together in time (Fig. 2, B and C).

We investigated the effect of changing extracellular calcium $[Ca^{2+}]_o$ (where o is outside) on the fluoride-induced discrete waves. The data of Fig. 2 were obtained from a cell bathed in ASW with $[Ca^{2+}]_o$ = 10 m*M*. The concentrations of calcium and fluoride constituted a supersaturated solution of CaF_2. Therefore, we measured the calcium activity in our test solutions with both a calcium electrode (Orion 92-20) and the calcium indicator dye murexide (Sigma). The calcium activity in our nominally 10 m*M* fluoride solution did not drop below 0.7 of the activity of 10 m*M* calcium in our ASW. When $[Ca^{2+}]_o$ was reduced to 1 m*M*, the fluoride-induced discrete waves tended to cluster together in time to a much greater extent than seen (Fig. 2). Changes in $[Ca^{2+}]_o$ appeared to modulate the timing of fluoride-induced discrete wave occurrence.

The data in Figs. 1 to 3 were obtained from cells bathed in ASW with a *p*H of 7.0. We found that the effect of fluoride on the photoreceptor depended on the *p*H of the ASW. For example, exposing a photoreceptor to ASW (*p*H 7.8) + 10 m*M* NaF for as long as 1 hour did not induce discrete waves, whereas exposing the same cell to ASW (*p*H 7.0) + 10 m*M* NaF induced discrete waves within 30 minutes. Thus, the mechanism by which fluoride induces discrete waves has a step that appears to be highly *p*H-dependent. It is well known that many enzymatic reactions are *p*H-dependent.

Our primary finding is that fluoride induces the occurrence of discrete waves within the photoreceptor in the absence

of light. These fluoride-induced discrete waves resemble those normally produced by light. We tentatively conclude that fluoride induces a process similar or perhaps identical to visual excitation of the photoreceptor.

One naturally wishes to speculate as to the mechanism by which fluoride may act. We tested the effect of fluoride on the photoreceptor to investigate the possible role of cyclic nucleotides in photoreceptor physiology and to use a pharmacological agent that would increase cyclic nucleotide concentrations by activating cyclase, the enzyme that synthesizes cyclic nucleotides. Fluoride activates brain adenylate cyclase apparently by acting directly on the catalytic subunit of the enzyme (*11*). However, it should be kept in mind that we have no evidence to indicate that changes in cyclic nucleotide concentrations mediate the effects of fluoride. Fluoride is known to have other effects; for example, it is an inhibitor of a variety of metallo enzymes (*12*). One obvious candidate for the site of action of fluoride is the visual pigment of the photoreceptor. Fluoride might induce spontaneous isomerizations of the pigment chromophore and thereby lead to visual excitation. Whatever the mechanism of action of fluoride on the photoreceptor, it seems clear that fluoride will be a useful agent for further studies of visual transduction.

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7. The ASW contained 435 m*M* NaCl, 10 m*M* KCl, 10 m*M* $CaCl_2$, 25 m*M* $MgSO_4$, 20 m*M* $MgCl_2$, and 10 m*M* Hepes; *p*H 7.0.
8. The addition of 10 mmole of NaF per liter of ASW that bathed the preparation resulted in the effects described in this paper. In control experiments the addition of 10 mmole of NaCl per liter of ASW was without effect. Therefore we conclude that the effects seen were the result of fluoride addition.
9. Inasmuch as the effect of fluoride occurs in the dark, we can rule out fluorescence as a source for fluoride-induced discrete waves. It is possible (although unlikely) that the fluoride might induce bioluminescence, which would give rise to discrete waves. If such luminescence existed, it should be possible to measure the light produced. We were unable to measure any luminescence of the tissue when exposed to fluoride. Our measurement system was sensitive enough to detect a light stimulus that gave rise to discrete waves of similar frequency to those induced by fluoride.
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Simultaneous Production of Q and R Bands After Staining with Chromomycin A_3 or Olivomycin

Abstract. *Human and mouse chromosomes, stained with either chromomycin A_3 or olivomycin, which bind preferentially to G · C-rich DNA (where G is guanosine and C is cytosine), exhibit a Q or a reverse banding pattern, depending on the wavelength used for excitation. The two complementary banding patterns can be observed in the same metaphase simply by changing the combination of excitation filters. These data suggest, therefore, that in addition to base composition, other factors are involved in the production of chromosome banding by chromomycin A_3 and olivomycin.*

Staining with fluorochromes that specifically bind to A · T-rich DNA (where A is adenine and T is thymine) produces a characteristic Q-banding pattern in human and other chromosomes (*1*). Some antibiotics, however, such as olivomycin, chromomycin A_3, and mitramycin, which bind preferentially to G · C-rich DNA (*2-4*), produce a reverse (R) banding pattern (*4-6*). Taken as a whole, these data support the hypothesis that variations in base composition along the chromosome are the major factors in Q and R banding.

We have investigated the cytochemical properties of chromomycin A_3 and olivomycin and have found that, depending on the wavelength used for excitation, these compounds can produce not only R banding but also a sharp Q-type banding pattern.

Standard chromosome preparations from short-term cultures of human leukocytes and from mouse fibroblasts (strain DBA_2) were stained for 20 minutes at room temperature with 5 μg (per milliliter) of either chromomycin A_3 (Boehringer) or olivomycin (Calbiochem) dissolved in phosphate buffer (0.15*M* NaCl, 0.03*M* KCl, and 0.01*M* Na_2HPO_4 adjusted to *p*H 7) containing $MgCl_2$ (2.5 m*M*). The slides were then washed and mounted in the same phosphate buffer at *p*H 7 and examined under a Zeiss fluorescence microscope equipped with incident illumination from a 200-W mercury light source. Two different combinations of dichroic mirrors and Zeiss filters were used for observation: (i) 2 × BG12 excitation filters, FT510 dichroic mirror and LP515 barrier filter (violet-blue excitation range); and (ii) UG5 plus BG3 excitation filters, FT460 dichroic mirror, and LP475 barrier filter (ultraviolet-violet excitation range). All observations were made with a Neofluar 100× Zeiss objective.

The observation of human metaphase chromosomes with combination (i) revealed that both chromomycin and olivomycin produce a specific green fluorescent banding pattern (Fig. 1a) that is very similar to the Q banding observed after staining with Hoechst 33258 (*7*). This was shown by the behavior of the secondary constrictions of chromosomes 1 and 16, which exhibited a bright fluorescence like that appearing after Hoechst staining. However, with combination (ii), we observed the yellow fluorescent R-banding pattern already described by van de Sande *et al.* (*4*). (Fig. 1b). The most surprising feature of this phenomenon was that the Q banding and the R banding could be observed and studied in the same metaphase simply by changing the combination of filters. Similar observations were made on mouse chromosomes stained with chromomycin A_3 or olivomycin (Fig. 2). With combination (i) the centromeric regions exhibited a bright fluorescence similar to that after Hoechst 33258 staining (*8*). With combination (ii), however, these regions were much less fluorescent than euchromatic arms.

We wish to point out that our staining technique, contrary to that described by van de Sande *et al.* (*4*), does not require a high concentration of the two antibiotics. The best results are obtained when the slides, stained with chromomycin A_3 or olivomycin (5 μg/ml), are kept for 24 hours in the dark at room temperature before observation. With this process of "maturation" a

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