ning zodiacal light obtained by one of us (J.L.W.) at 3052 m on Mount Haleakala, Hawaii, on 16 nights in February, March, and April 1966 and March 1967 (*4*) were used to determine the symmetry plane at elongations, ϵ, of 32° to 50° from the sun. Details of the data reduction were given earlier (*5*). Figure 1 shows an example of zodiacal light brightness contours in ecliptic coordinates after discrete starlight, background starlight, and airglow continuum emission are subtracted out and corrections are made for atmospheric extinction and scattering. The photometric axis (dashed line) was located by a least-squares fit to the contour peaks. The position of each peak was obtained by connecting the midpoints between intersections of the contour with lines at 1° intervals of differential ecliptic longitude. The position is relatively insensitive to the method used; that is, the position corresponding to the actual contour peak was generally the same as the position obtained from the midpoints of that contour.

Figure 2a shows the displacements from the ecliptic of the observed planes of maximum brightness on four representative nights in March 1966 and March 1967. The predicted displacements for the invariable plane and for the orbital plane of Venus correspond to what would be observed if the dust that contributes most to the maximum brightness were in these planes (*3*). Figure 2b shows the positions of the same three planes as a function of day of year for three different elongations. By the method used in (*3*), the mean inclination and ascending node of the planes of maximum brightness observed on 16 nights are found to be $i = 2.7° \pm 0.3°$ and $\Omega = 85° \pm 5°$, respectively. For comparison, $i = 3.4°$ and $\Omega \simeq 78°$ for the orbital plane of Venus and $i = 1.6°$ and $\Omega = 107°$ for the invariable plane. These results show beyond doubt that the observed photometric axis of the inner zodiacal light lies closer to the orbital plane of Venus than to the invariable plane.

Interplanetary dust must eventually spiral into the sun by the action of solar radiation (Poynting-Robertson) drag (*6*). During the Poynting-Robertson lifetime, gravitational perturbations by the planets on the dust near their orbital planes may bring the orbital elements of the dust closer to those of the planets' orbital planes. This effect depends primarily on the mass of the planet and on the orbital inclination and eccentricity of the dust (*7*). If the dust particles are in circular orbits, the chances for planet-particle encounters are increased (*8*). Although small in magnitude, this effect could be appreciable over expected time scales of thousands of years. We suggest that Venus is in the process of concentrating nearby dust toward its orbital plane.

The observational results reported here, which were obtained in the limited elongation range 32° to 50°, indicate that the dynamic influence of Venus on the dust extends to 0.2 A.U. inside and perhaps to the same amount outside its orbit. This was found by calculating the distance between the line of sight at $\epsilon = 32°$ and the tangent to the orbital path of Venus at the point where the brightness contribution along the line of sight is 50 percent (*3*). More information on the extent of Venus' influence could be obtained from analyses of similar observations at $\epsilon < 32°$ and at $50° < \epsilon \leq 80°$; suitable observations are not now available. These data could provide information on average inclination and average eccentricity, which are directly related to the source and evolution of the interplanetary dust.

Similar gravitational effects can be expected for Jupiter, Earth, Mars, and perhaps Mercury. Observations are needed at $\epsilon < 32°$ from space (especially for Mercury) and at $50° < \epsilon \leq 180°$ from the ground or from space to verify the presence of these effects. In a recent analysis of zodiacal light observations at ϵ from 110° to 140° from Skylab and from Mount Haleakala, the photometric axis was found to lie between the ecliptic and the orbital plane of Mars (*9*). Whether Mercury has an appreciable influence on the dust despite its small mass (0.053 of Earth's mass) remains to be seen. The inclinations of the solar equatorial plane, the orbital plane of Mercury, and the orbital plane of Venus are 7.3°, 7.0°, and 3.4°, respectively. Therefore, it should be possible to separate the effects of Mercury from those of Venus. Possible effects of the solar equatorial plane and the orbital plane of Mercury can be separated by using the difference in their ascending nodes ($\Omega = 49°$ and 73°, respectively).

Future studies should examine the dynamics of gravitational perturbations that might lead to the concentration of dust toward the orbital planes of the planets. They should include the question raised by Whipple (*10*) of whether the planets can concentrate dust from Comet Encke toward their orbital planes in spite of the high inclination (12°) of Encke's orbit. Finally, additional ground-based observations of the zodiacal light should be made to determine the position of the photometric axis at $\epsilon > 50°$ and $\epsilon < 32°$.

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16 March 1978

Initiation of Light Adaptation in Barnacle Photoreceptors

Abstract. *Intracellular recordings were used to measure the action spectrum of light adaptation in barnacle photoreceptors. The action spectrum closely resembles the absorption spectrum of rhodopsin (λ_{max} at 530 nanometers) and is clearly different from that of metarhodopsin (λ_{max} at 495 nanometers). These results suggest that absorption of light by rhodopsin initiates both excitation and light adaptation. The previously reported antagonistic process initiated by metarhodopsin does not appear to play a role at moderate light intensities.*

Photoreceptors exhibit two principal light-dependent processes, excitation and light adaptation. Excitation is the process by which light brings about a change in transmembrane potential; light adaptation is the process by which light lowers the cell's sensitivity (the amount of voltage change per incident photon). Absorption of light by the cell's visual pigment or pigments initiates both excitation and light adaptation, but it is unclear whether a single pigment initiates both processes or whether cells contain two visual pigments (or pigment states),

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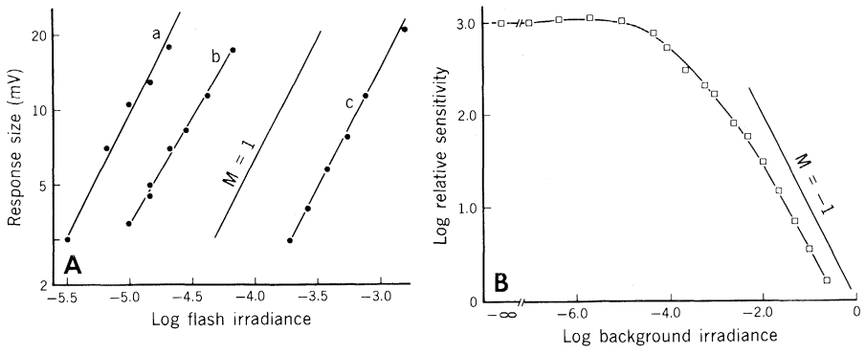

Fig. 1. Light adaptation in barnacle photoreceptors. (A) Response size as a function of log flash irradiance in several different adaptation states. Curve a, dark-adapted; curve b, during a dim (3×10^{-8} W/cm^2 at 530 nm) background light; curve c, during a background light 2 log units brighter than that used in curve b. Best-fit lines were drawn by eye through points in the 0- to 12-mV range. A line of slope (M) = 1 is included for comparison. A slope of 1 on log-log coordinates indicates a linear relationship between the variables. (B) Relative sensitivity as a function of background irradiance. The 0 background = 9×10^{-5} W/cm^2 at 530 nm. Data are from the same cell as those in Fig. 1.

one of which initiates light adaptation and the other excitation.

A two-pigment hypothesis is suggested by previous studies on barnacle photoreceptors. Barnacle visual pigment exists in two thermally stable states, rhodopsin and metarhodopsin (λ_{max} at 532 nm and 495 nm, respectively), which are interconverted by light (*1*). The spectral sensitivity of the excitatory, depolarizing response is the same as the absorption spectrum of rhodopsin (*2, 3*). Under certain conditions, a very bright light causes a membrane depolarization that persists long after the light is terminated. The action spectrum inducing these prolonged depolarizing afterpotentials also resembles the absorption spectrum of rhodopsin (*3, 4*). The afterpotential can be terminated by another flash of light whose action spectrum resembles the absorption spectrum of metarhodopsin (λ_{max} at 495 nm). This evidence from the barnacle and similar evidence from other invertebrates (*5*) suggests that a process initiated by metarhodopsin can antagonize an excitatory process initiated by rhodopsin. Since light adaptation is antagonistic to excitation, it seemed plausible to us that light adaptation might also be initiated by metarhodopsin. To test this hypothesis we have measured the action spectrum of light adaptation. If light adaptation is initiated by metarhodopsin, its action spectrum should coincide with the absorption spectrum of metarhodopsin.

The ocelli of *Balanus eburneus* were mounted corneal-side down in a chamber containing barnacle artificial salt water (*6*), and the reflecting tapetum and surrounding pigment were removed. Cells were impaled with a 3*M* KCl microelectrode. At the beginning of each experiment, a bright 530-nm light was used to establish a rhodopsin-metarhodopsin photoequilibrium; at this wavelength, both pigment states were present in substantial fractions as indicated by the biphasic waveform of the early receptor potential (*1*). Cells were subsequently dark-adapted until the absolute sensitivity had been stable for 15 to 20 minutes.

Figure 1A demonstrates how light adaptation was measured. In the dark-adapted cell, there was a range of response size (3 to 10 mV) over which the voltage change produced by a brief flash varied linearly with the flash irradiance.

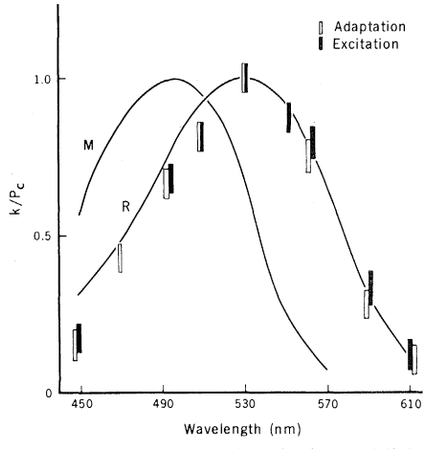

Fig. 2. Action spectra of excitation and light adaptation determined in the same cell. The ordinate is proportional by k to the reciprocal of the number of photons (P) required to produce a criterion (C) (5-mV) response (excitation) or to produce a criterion (0.6 log unit) desensitization (adaptation). Both sets of values data are plotted relative to their maxima, which coincided at 530 nm. Bar height represents our estimate of the experimental error. Theoretical absorption spectra for metarhodopsin (M), with a peak at 495 nm, and rhodopsin (R), with a peak at 530 nm, are included for comparison (*12*).

In the presence of a background light, the responses to superimposed light flashes still varied linearly with flash irradiance over the range from 3 to 10 mV, but, because of the desensitization induced by the background light, a brighter flash was required to elicit any given response. The ratio of the voltage change to the flash irradiance was used as a measure of sensitivity. Figure 1B shows the relationship between the relative sensitivity and the background light. Sensitivity is approximately inversely proportional to background irradiance at higher background levels.

To find the action spectrum of light adaptation, we determined the effectiveness of various monochromatic background lights in desensitizing the cell (*7, 8*). Once a steady plateau depolarization had been reached, 530-nm test flashes were superimposed on the background light. The background was then adjusted until the average response to the test flashes was reduced to a criterion value (*9*). The procedure was repeated for monochromatic backgrounds of eight different wavelengths. The action spectrum of adaptation has a peak near 530 nm (Fig. 2). The action spectrum of excitation (*8, 10*), measured in the same cell in the absence of a background light, also peaked near 530 nm as previously reported (*2, 3*). These results indicate that light adaptation, in the intensity range examined, is not initiated by metarhodopsin, which has a λ_{max} at 495 nm.

The antagonistic process initiated by metarhodopsin was demonstrated by Hochstein *et al.* (*3*), who used the very bright lights required to produce and terminate depolarizing afterpotentials. Since depolarizing afterpotentials do not occur for dim or moderately intense stimuli (*10*), it was not possible, with their paradigm, to determine if metarhodopsin also initiated an antagonistic process at moderate light intensities. The entirely different experimental approach described here can be applied to the moderate intensity range, and the results show that absorption of light by metarhodopsin does not significantly antagonize the excitation process. Thus, the antagonistic process described by Hochstein *et al.* (*3*) is important only at high intensities and does not contribute to light adaptation at moderate ones.

Our results cannot definitively disprove other variants of the two-pigment hypothesis since two separate pigments with almost identical relative absorbance spectra could conceivably control light adaptation and excitation. However, the simplest explanation of the results is that

a single pigment, rhodopsin, initiates both excitation and light adaptation at moderate light intensities.

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8. The following procedure was used to find the absolute intensities at each wavelength. The cells were placed in a chamber, mounted on the stage of a compound microscope, and illuminated from beneath through the condenser. We incorporated a photodiode, calibrated against a United Detector Technology PIN-10 photodiode, into the head of the microscope. Light transmitted through a region 75 μm in diameter and centered on the cell was focused onto the photodiode. At the end of each experiment, the unattenuated intensity provided by the beam at each wavelength was measured with the photodiode.
9. In order to have adapting lights that were too dim to significantly alter the pigment concentration, but which were still bright enough to have a desensitizing effect, it was necessary to use sensitive cells. None of the cells used showed any signs of facilitation. Some cells never became sensitive enough to be used; one of these did show an increase in sensitivity when exposed to a dim background light. M. Hanani and P. Hillman [*J. Gen. Physiol.* **67**, 235 (1976)] report that such facilitation is seen more often in less sensitive cells, while light adaptation as we have described it is seen more often in sensitive cells. The background lights desensitized the cell by about a 0.6 log unit but did not significantly alter the pigment photoequilibrium established at the beginning of the experiment. We verified this point at the end of the experiment by demonstrating that exposing the cell to ten times as much monochromatic light as had been used during the experiment did not, at any wavelength, measurably alter the pigment concentration as assayed by the early receptor potential. It was necessary to maintain an essentially constant pigment concentration; only under this condition does an action spectrum approximate the relative absorbance spectrum of the pigment initiating the process in question. The monochromatic background lights used to produce a criterion desensitization evoked responses with a plateau phase of approximately 10 mV amplitude. This potential was independent of wavelength within $\pm$0.5 mV.
10. The action spectrum of excitation was determined by finding, at each wavelength, the intensity of a brief monochromatic flash that would elicit a criterion response in a dark-adapted cell. The relative attenuation of the different monochromatic flashes and background lights used was readily determined since calibrated neutral-density filters were used to attenuate the light beam.
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Nitrite and Nitrate Are Formed by Endogenous Synthesis in the Human Intestine

Abstract. *Studies of nitrate balance in humans and analyses of fecal and ileostomy samples indicate that nitrite and nitrate are formed de novo in the intestine, possibly by heterotrophic nitrification. These findings significantly alter our previous conceptions of human exposure to nitrite and suggest an even wider role for nitrite in the etiology of human cancer.*

Nitrite is a precursor of the putatively carcinogenic *N*-nitroso compounds that may be formed in situ in humans (*1*). These compounds are thought to form in the stomach (*2*), the bladder (*3*), and, as recent evidence suggests, in the lower intestinal tract (*4*). The origin of this nitrite is significant, since it might be related to the etiology of several types of cancer in humans (*5*).

An earlier investigation of nitrate metabolism (*6*) indicated that bacterial reduction of nitrate in saliva was the major mode of nitrite formation in humans (*7*). Further studies with vegetables and vegetable juices rich in nitrate indicated that levels of salivary nitrite could reach hundreds of milligrams per liter in a process of recirculation of unmetabolized nitrate through the blood via the salivary glands (*8, 9*). Thus, to assess the extent of nitrate intake in a population through measurements of nitrate in urine and saliva (*10*), nitrate balance studies were undertaken in humans. The results of these studies, and of subsequent studies of effluent samples from the intestinal tract, led to the discovery that nitrite and nitrate are formed by endogenous synthesis in humans.

Six Caucasian males, 68 to 72 years old, weighing 57 to 105 kg, participated in the nitrate balance study. All were judged to be healthy on the basis of a medical history, physical examination, and clinical and laboratory tests. Procedures for the selection of subjects and their initial characteristics have been described (*11*).

The subjects were studied as inpatients at the Clinical Research Center (CRC), Massachusetts Institute of Technology (MIT). After having a diet of their own choosing for at least 1 week, the subjects were given a protein-free diet (6 mg of nitrogen per kilogram of body weight per day) for 10 days and then a diet containing 0.8 g of egg protein per kilogram of body weight per day (based on a liquid formula) for 10 days. The compositions of these diets have been described (*11–13*). The diets were consumed under the supervision of the CRC research dietician to ensure adherence to the experimental protocol. Only minor fluctuations in body weight occurred during the diet periods. Urine and dietary components were analyzed for nitrate and nitrite by a modified Griess microprocedure (*9*). Complete daily urine collections were made throughout the diet periods and the samples were preserved with HCl (*14*).

Since nitrate is known to be absorbed from the proximal small intestine and cleared from plasma by the kidney (*15*), we expected the subjects to reach equilibrium rapidly and to excrete in urine a steady-state concentration of nitrate that would represent some fraction of that consumed. Instead, we found wild fluctuations in urinary nitrate on a day-to-day basis on both the 0.8-g and protein-

Table 1. Urinary nitrate in subjects consuming a protein-free diet.

Subject	Total daily nitrate in urine (μmole $NaNO_3$)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
1	985	462	978	1610	1350	2270	279	325	289	275
2	4050	1410	1330	791	2400	1720	1000	1390	815	
3	1140	5150	1260	1140	3040	2210	1220	918	840	1070
4			1550	2640	1960	355	1140	981		883
5	406	378	219	1550	833	233	861	579	530	971
6	1130	1720	635	1550	1430	1320	1380	448	2210	462

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