then McDonald's Protect-A-Coat lacquer on the surface, which made the insert serviceable for about 6 weeks.
10. Three separate lights were projected through band-pass interference filters (450 nm, 545 nm, and 690 nm) and, to eliminate colored shadows, superimposed by two-way mirrors to form a common projection beam (Fig. 1). The beam passed normal to the face of a water prism onto the entire Mondrian at a 45° incident angle. White plastic inserts on the aquarium floor and opposite wall prevented undue specular reflections. Before each test session, the relative intensities of the long-, middle-, and short-wave light reflected from the colored target paper in the Mondrian and other Mondrian papers relatively near in hue were measured with a telephotometer (Spectra Pritchard, 1980A).
11. J. Levine, *Sens. Processes* **3**, 95 (1979).
12. N. W. Daw, *J. Physiol. (London)* **197**, 567 (1968).
13. I thank E. H. Land for detailed discussion of retinex theory, constructive criticism of the experimental methods, and design of the tricolor illuminator; A. R. Chase for careful training and testing of the animals; B. Young and C. Easson for technical consultation; and S. H. Perry for assistance with the manuscript.

6 August 1984; accepted 6 December 1984

Heat Generated by the Dark-Adapted Squid Retina in Response to Light Pulses

Abstract. *A rapid increase in the temperature of the dark-adapted squid retina evoked by a brief light pulse was detected with a pyroelectric detector. The amount of heat generated by the retina in response to a pulse of blue light of moderate intensity was far greater than that produced by direct conversion of the stimulating light by the retinal pigments into thermal energy.* D-*Glucose in the medium was required to maintain the ability of the retina to produce light-evoked thermal responses.*

In a variety of invertebrate eyes, readily detectable electrical responses can be evoked by a very small number of photons absorbed by visual pigments (*1*). It is generally believed that the photochemical reaction initiated by absorption of light by visual pigments is followed by a succession of biochemical reactions that amplify the effect of light stimulation and eventually lead to the generation of electrical responses (*2*). How this amplification takes place is not precisely known.

We report here that exposure to a brief light pulse evokes a rapid increase in the temperature of the dark-adapted squid retina and that the amount of heat generated is far greater than that associated with the stimulating pulse itself. We also present evidence in support of the view (*3*) that the process of energy transduction in the retina is dependent on the oxidation of sugar.

To detect heat production we used a pyroelectric detector constructed with poly(vinylidene fluoride) (PVDF) film (Kureha Chemical). PVDF is a synthetic polymer that becomes highly pyroelectric when heated and stretched in the presence of a high electrical field (*4*). The film we used was about 9 μm thick and had a 10-nm layer of aluminum deposited on each surface; a 1°C change in the temperature of this film produces a change of about 5×10^{-9} C/cm^2 in the electrical charge on the aluminum layer. Radiant heat of about 1 μsec in duration has been detected by PVDF film (*5*). We have measured the heat generated by the crab nerve during excitation with a time resolution of about 1 msec (*6*).

A piece of PVDF film about 0.4 by 1 cm^2 in area was glued to a thin (25-μm) platinum plate and was attached to a 4-μm-thick sheet of Mylar; it was connected to an operational amplifier (Analog Devices model 515) with a high (2×10^9 ohm) load resistance and a small (about 3×10^{-12} farad) capacitor (Fig. 1). The output of the operational amplifier was led to a signal averager (Nicolet Instrument model 1072) through an a-c–coupled amplifier with a gain of 200.

The magnitude of the signal recorded under these conditions is proportional to the rate of change of the temperature of the film (*7*). When the heat capacity of the sample under study is far larger than that of the temperature-sensitive portion of the detector, the rate of increase in the temperature of the sample is given by V/apR), where V is voltage at the output of the operational amplifier, a is the area of the PVDF film (in square centimeters), p is the pyroelectric coefficient of the PVDF film (in coulombs per square centimeter per degree Celsius), and R is the load resistance (in ohms). The validity of the relation was substantiated by measuring the heat generated by pulses of green light of known intensities absorbed by a slice of 2 percent gelatin gel that was stained with a mixture of chlorphenol red and phenol red and that absorbed 95 percent of the transmitted light. The time resolution of the detector, determined primarily by the time constant of the operational amplifier, was approximately 6 msec.

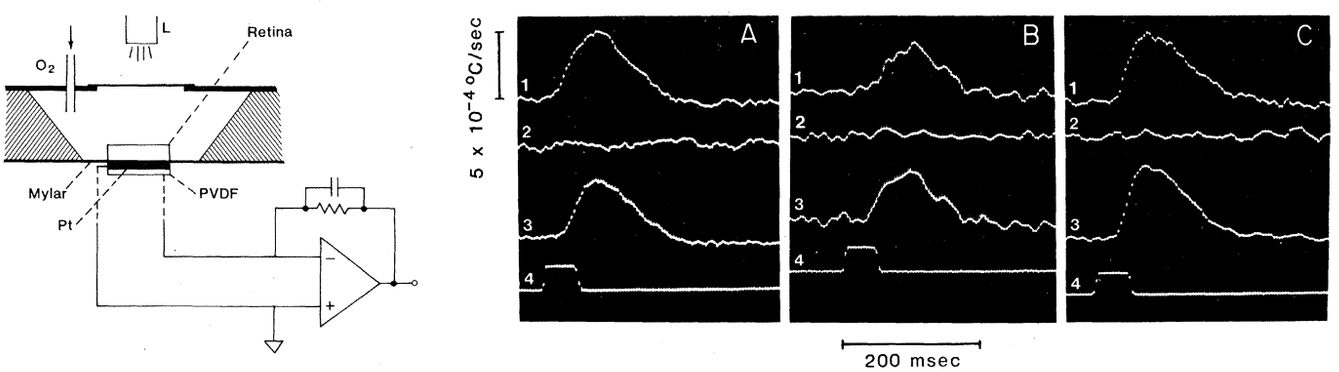

Fig. 1 (left). Schematic diagram of the experimental setup used for measuring the rate of increase in the temperature of the retina caused by stimulating light pulses. L represents a bundle of optical fibers for guiding light from an incandescent lamp; Pt, a thin platinum plate; and PVDF, a sheet of poly(vinylidene fluoride). The potential difference across PVDF film was recorded with an operational amplifier. Platinum was inserted to ensure a spatially uniform increase in the temperature of the PVDF film and to prevent possible mechanical changes in the retina from affecting the PVDF film. Fig. 2 (right). Records showing the temperature increase (expressed in degrees centigrade per second) of the dark-adapted squid retina evoked by light pulses (indicated by traces 4). The light used was 500 nm in wavelength, 25 μW/cm^2 in intensity, and 50 msec in duration. Trace 2 in (A) was taken 10 minutes after switching oxygen to nitrogen; trace 3, 7 minutes after the resumption of oxygen flow. Trace 2 in (B) was taken 40 minutes after immersion of the retina in artificial seawater free of D-glucose; trace 3, 10 minutes after addition of D-glucose (2 m*M*) to the medium. Trace 2 in (C) was obtained 18 minutes after application of 1 m*M* sodium azide; trace 3, 12 minutes after removal of azide. All the records were taken after averaging the signals over 16 trials repeated at 9-second intervals at 20°C.

Eyes of the squid *Loligo pealeii* Lesueur were excised under dim red light. The sclera was removed in artificial seawater containing tris(hydroxymethyl)aminomethane (0.2 m*M* at *p*H 8) and D-glucose (2 m*M*) and the retina was cut into slices approximately 400 μm wide. The heat-sensitive area of the detector was covered with about six slices (approximately 20 mg), with their cut surfaces making direct contact with the Mylar sheet. A plastic cover with a glass window at the top was placed above the detector and a flow of oxygen, moistened by passing through seawater, was maintained above the slices. A light-emitting diode or a 100-W quartz-iodine lamp (in conjunction with an interference filter, neutral density filters, and an electromechanical shutter) was used for stimulation. Precautions were taken to suppress mechanical disturbances reaching the detector (*6*).

Figure 2 shows typical examples of the thermal responses recorded from slices of the dark-adapted squid retina. With brief (about 50-msec) pulses of light 5 to 50 $\mu W/cm^2$ in intensity and about 500 nm in wavelength, a fairly rapid increase in temperature was found to start approximately 20 msec after the onset of the light pulse at 20°C. The rate of the increase in temperature reached a maximum usually 70 to 110 msec after the onset. Duration of the response was generally between 150 and 250 msec. When the light intensity was about 25 $\mu W/cm^2$ or greater, the maximum rate of temperature increase observed was 3×10^{-5} to 5×10^{-5} degree Celsius per second. The maximum rate decreased when the light intensity was reduced.

The rate of the increase in temperature can be converted into the heat generated by multiplying the specific heat of the slices (roughly 1 cal/deg) by the rate. When the loss of heat from the slices (by conduction, convection, and radiation) is neglected, the total heat generated by the slices can be estimated from the area under the observed curve representing the rate of temperature increase (*8*). By estimating the area, the total amount of heat associated with the response shown in Fig. 2A (trace 1) is roughly 4×10^{-5} cal per gram of retina. This value is much greater than that of the heat generated by the vertebrate peripheral nerve, about 3×10^{-6} cal/g per impulse at 20°C (*9*), but is far smaller than that associated with a twitch of the frog muscle, roughly 10^{-3} cal/g (*10*).

The thermal responses described above are distinct from the heat generated by the stimulating light absorbed by rhodopsin molecules and pigment granules in the photoreceptor cells. We have seen repeatedly that the thermal responses are reversibly suppressed by mild light adaption. Anoxia resulting from circulation of nitrogen above the retina also suppressed the thermal responses within 5 to 10 minutes (Fig. 2A). These findings indicate that the heat generated by the stimulating light itself is negligibly small compared to the heat associated with the response triggered by the stimulus.

By increasing the light intensity after suppression of the thermal responses or by choosing red light for stimulation, it was possible to detect the heat generated by the stimulating light itself. By extrapolating the results obtained, we found the heat produced by the stimulus to be slightly smaller than one-tenth of the maximum rate of heat production associated with the thermal responses shown in Fig. 2. Thus, we find that the total amount of heat associated with the production of a single thermal response is far (at least 25 times) larger than the total amount of heat generated by the retinal pigments during the 50-msec period of stimulation. In all probability the pigment granules located in the vicinity of the rod base (*11*) play a dominant role in the process of direct conversion of light into heat. It is evident, therefore, that there is a large amplification of energy in the production of a thermal response.

Figure 2B demonstrates the necessity of D-glucose in the artificial seawater for the maintenance of the thermal responses. After taking records of thermal responses from the slices in the detector, we introduced sugar-free artificial seawater into the detector. The seawater was oxygenated and gently circulated over the surface of the slices (without detaching them from the Mylar sheet). From time to time the seawater was removed and thermal responses of the slices were recorded. By this procedure we found that slices immersed in a sugar-free medium lost their ability to develop thermal responses within 20 to 100 min. Addition of D-glucose, D-fructose, or D-mannose to the medium restored the responses within 3 to 8 minutes. No restoration was observed when 2-deoxyglucose, D-galactose, D-xylose, D-fucose, L-glucose, L-fructose, or L-mannose was added. These findings are consistent with the biochemically well-grounded view (*3*) that the energy derived from oxidation of D-glucose, D-fructose, or D-mannose is essential for maintenance of the retinal responses (*12*).

Finally, we examined the effects of several well-known inhibitors of oxygen utilization. Figure 2C shows records obtained with 1 m*M* azide in seawater (containing D-glucose) applied to the slices in the detector. Azide reversibly suppressed the production of thermal responses. A similar reversible suppression was observed with cyanide (0.3 to 1 m*M*). Dinitrophenol, an uncoupler of oxidative phosphorylation, had a similar effect of 0.2 m*M*. Iodoacetate, a classical inhibitor of glycolysis, suppressed the responses at about 1 m*M*; the effect was, however, irreversible. The effects of these inhibitors on cells and tissues are more complicated than their effects on isolated enzymes (*13*). Nevertheless, the results strongly suggest that oxidation of organic fuels underlies the process of amplified energy transduction in the retina. It should be noted that all the reagents that suppressed thermal responses also suppressed electrical responses of the retina at about the same concentration.

Further studies are required to clarify the interrelations among the mechanical (*14*), thermal, and electrical responses.

ICHIJI TASAKI
TOSHIO NAKAYE
Laboratory of Neurophysiology, National Institute of Mental Health, Bethesda, Maryland 20205, and *Marine Biological Laboratory, Woods Hole, Massachusetts 02543*

References and Notes

1. W. A. Hagins, R. D. Penn, S. Yoshikami, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 403 (1965); M. G. F. Fuortes and P. M. O'Brian, in *Handbook of Sensory Physiology*, vol. 7, part 2, *Physiology of Photoreceptor Organs*, M. G. F. Fuortes, Ed. (Springer, Berlin, 1972), pp. 326–338; S. Laughlin, *ibid.*, vol. 7, part 6B, *Vision in Invertebrates*, H. Autrum, Ed. (Springer, Berlin, 1981), pp. 209–211.
2. G. Wald, *Science* **150**, 1028 (1965).
3. H. J. Autrum and H. Tschartke, *Z. Vgl. Physiol.* **45**, 695 (1962); W. Sickel, in *Handbook of Sensory Physiology*, vol. 7, part 2, *Physiology of Photoreceptor Organs*, M. G. F. Fuortes, Ed. (Springer, Berlin, 1972), pp. 667–727.
4. K. Nakamura and Y. Wada, *J. Polym. Sci.* **9**, 161 (1971); A. L. Robinson, *Science* **200**, 1371 (1978).
5. A. M. Glass, J. H. McFee, J. G. Bergman, Jr., *J. Appl. Phys.* **42**, 5219 (1971).
6. I. Tasaki and K. Iwasa, *Biochem. Biophys. Res. Commun.* **101**, 172 (1981).
7. C. B. Roundy, *Proc. Soc. Photo-Opt. Instrum. Eng.* **62**, 191 (1975).
8. The loss of heat from the detector was estimated by measuring the temperature increase resulting from absorption of light of known intensity; with light pulses of 100 msec the loss was roughly 16 percent of the total heat absorbed.
9. J. V. Howarth, R. D. Keynes, J. M. Richie, *J. Physiol. (London)* **194**, 745 (1968).
10. A. V. Hill, *Proc. R. Soc. London Ser. B* **148**, 397 (1958).
11. H. V. Zonana, *Bull. Johns Hopkins Hosp.* **109**, 185 (1961).
12. Weak alkali isomerizes a portion of fructose and mannose to form glucose [W. Pigman, *The Carbohydrates* (Academic Press, New York, 1957), p. 60]; therefore, it seems difficult to conclude that fructose or mannose is utilized directly as the source of energy.
13. J. L. Webb, *Enzyme and Metabolic Inhibitors* (Academic Press, New York, 1963), vol. 1, pp. 427–486.
14. I. Tasaki and T. Nakaye, *Science* **223**, 411 (1984).
15. We thank T. Araki and H. Obara of Kureha Chemical Co. for supplying the PVDF film and H. Gainer for his helpful advice during this research.

20 September 1984; accepted 1 November 1984