SENSITIVITY TO LIGHT IN THE SEA ANEMONE
METRIDIUM SENILE (L.)

II. STUDIES OF REACTION TIME VARIABILITY AND THE EFFECTS
OF CHANGES IN LIGHT INTENSITY AND TEMPERATURE*

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In the first paper of this series it was shown by North and Pantin (1) that the sea anemone Metridium senile is not uniformly sensitive to radiation throughout the visible spectrum; maximum sensitivity generally occurred in the blue-green at about 500 mμ, although considerable variability was found from animal to animal. Light and dark adaptation phenomena were also demonstrated.

The entire body surface of Metridium is apparently photosensitive, and the animal usually responds to light by local contraction of the parietal musculature; unilateral stimulation, therefore, results in bending, Bohn (2), Parker (3), Batham and Pantin (4). North and Pantin were unable to find either macroscopic or microscopic units suggestive of photoreceptors in the animals, hence the actual site of light perception is still unknown. Both Batham and Pantin and North and Pantin used high light intensities for times up to several minutes. Responses were often movements of a centimeter or more and there was a delay of 20 to 40 seconds between commencement of illumination and the onset of bending. Neither the magnitude of bending, however, nor the time delay were free from variability.

The present investigation deals with much dimmer stimuli and a response of slightly different character. The magnitude of this response is rarely a bending traverse of more than a millimeter; here only bending is involved whereas the high intensity stimuli occasionally cause complete closure, suggesting a more general effect, perhaps even an avoidance reaction. The low intensity response is also involved at high intensities, but may escape notice since it is completed in less than a second with a movement of perhaps a millimeter or less. The action spectra of the two responses are similar, however, and each under the proper conditions displays a reaction time (delay between initiation of stimulus

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715

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and response). Because of its greater uniformity, the present study will utilize only the low intensity response.

Materials and Methods

The illuminator used to supply test stimuli was a model 370 Spencer microscope lamp supplied with a General Electric, vertical ribbon filament, 110 watt projection bulb, type 18AT10/1-6v. The lamp was fitted into a holder containing an additional lens, a 5 cm. plexiglas cell filled with 0.1 M acidic copper sulfate solution, and a slotted holder for 8¼ cm. square filters. The illuminator produced a 1½ x 8 cm. rectangular image of the filament, which was sharply defined at distances from 22 to 35 cm. in front of the forward lens of the system. The input voltage was stabilized by two Sola constant voltage transformers, types CV and CVH, rated at 500 watts each and connected in series. Both transformers were rated to give 1 per cent or better regulation through ±20 per cent fluctuations in line voltage; through the series connection, the regulation was refined to 0.01 per cent. Daily and momentary line voltage fluctuations at the laboratory rarely exceed ±0.10 per cent.

The regulated voltage was transformed to 6 volts through a triad No. F22-A transformer connected to a variac which controlled the voltage to the illuminator. The latter voltage was kept constant at 6.00 volts by means of a Weston AC voltmeter.

Within the illuminator infrared radiation was removed by a Corning aklo No. 3966 heat filter, 2.5 mm. thick, and by copper sulfate kept in the aforementioned plexiglas cell of 5 cm. pathlength. An Ilford No. 603 spectrum filter, 8½ x 8½ cm., made of gelatin, was used to provide blue-green light. This filter had a maximum transmission of 12 per cent at 493 mμ (near the maximum of the anemone's action spectrum) and a transmission of 1 per cent at 473 and 520 mμ; transmissions substantially lower than 460 mμ or beyond 550 mμ were 0.001 per cent or less. Intensities were regulated with Ilford neutral density gelatin filters. All filters were calibrated in a Beckman DU spectrophotometer.

The illuminator was calibrated with the aklo, copper sulfate, and 603 filters in place, by means of an Eppley No. 2470 vacuum thermopile, placed 25 cm. in front of the forward lens of the system, and positioned in the center of the image. The thermopile was calibrated by means of a National Bureau of Standards secondary radiation standard No. C-613, and the output was measured with a Leeds and Northrup type 2284B reflecting galvanometer. Corrections for losses due to absorption, reflection, and scattering by glass surfaces and sea water in the aquarium and aquarium housing totalled 10 per cent and were determined with a General Electric type DW-68 exposure meter.

Throughout the experiments to be described, the animal was illuminated from above by a 2 watt GE 46 radio bulb, 40 cm. distant, shining through an Ilford No. 608 red filter. Voltage across this bulb was regulated as described above. This arrangement cast a general illumination of about 10⁴ quanta/cm.² of light with wave length values greater than 620 mμ upon the surface of the anemone. Light of this dimness and spectral character was assumed to impose no adaptation to the blue-green stimulus. Variation of the intensity of the red light by about threefold did not change the sensitivity of animals to the blue-green stimulus.
The containing aquarium had a capacity of about 10 liters and was kept in a heat-insulated housing in a dark room. The housing was sufficiently large to permit moisture, condensed on the exterior of the aquarium, to be wiped away before each determination. Sea water in the aquarium was circulated continuously by a Jabsco stainless steel pump, through a stainless steel cooling coil and a charcoal column 45 cm. high. Plexiglas tubing was used for interconnecting the components of the circulating system, and water was cycled completely through the aquarium at a continuing rate of about once every 5 minutes. Temperature was controlled by a Fenwall No. 17851 thermoswitch which maintained constancy of ±0.5°C. The entire system was drained and flushed with 40 liters of fresh sea water once or twice weekly. At this time the walls of the aquarium were thoroughly cleaned.

An upright grid of inert black bakelite bars 2 mm. square in cross-section, and arranged to provide 8 mm. square holes, was positioned in the aquarium between the anemone and the eye of the observer. During stimulation the head of the observer was fixed by a rest, the direction of view being perpendicular to the stimulating light beam. Any motion by the animal was readily apparent through the bars of the grid. The anemone was kept on a rotatable, tiltable dais which enabled the observer cautiously to line up the leading edge of the animal with one of the vertical bars of the grid; movements of ½ mm. or less were thus readily detectable. Before each stimulation the anemone was closely observed for at least 30 seconds to ensure that no movement might be in process. If movement was detected, observations were continued until none occurred during a 30 second period. During an experiment the stimulus was extinguished a few seconds after response and at least 5 minutes of darkness intervened between successive stimuli. Measurements of height and diameter were made at intervals during the course of an experiment to ensure that an animal maintained approximately the same shape and volume. Any radical changes in these quantities might alter the total number of photoreceptive units within the illuminated area. Short times (120 seconds or less) were measured with a stop-watch.

Moderate sized specimens of Metridium (about 3 cm. diameter and 10 cm. high while expanded) were collected intertidally along the California coast between Pt. Conception and Pt. Reyes. They were allowed to adhere to plexiglas discs and when not under observation were maintained in running sea water cooled to 13°C. Healthy specimens have been thus maintained at La Jolla for 18 months, being fed occasionally on living Artemia salina, a small pelagic brine shrimp which Metridium readily captures on contact. All data presented were obtained using the white color phase of this anemone, although most of the other color phases described by Fox and Pantin (5) are available. Any variations in sensitivity due to different concentrations of insensitive body pigments were thus minimized.

RESULTS

Variability of the Reaction Time.—The reaction time in Metridium (the period elapsing from the instant of illumination until a response is observed) is a feature of great importance, since at any given intensity it is a measure of the number of quanta delivered to the animal. Illumination is apparently necessary for the entire duration of the reaction time; in 70 trials no response was ob-
served if the light was extinguished before the onset of bending. The magnitude of the response (the distance moved during bending) does not appear to correlate with the length of the reaction time.

Unfortunately even under the most uniform environmental conditions obtainable, all animals studied displayed considerable variations in reaction time from one stimulus to the next. Fig. 1a shows a typical record obtained on an anemone from 38 observations during a 7 hour period. Since a light source of constant intensity was used to stimulate, the number of quanta necessary to cause a response varied fivefold in this graph. A sevenfold variation was, in fact, common.

Irregular and large as the fluctuation was, the reaction times of any given anemone always appeared to oscillate around a given average value which did not change over periods of at least a week. It was found, in fact, that when large numbers of observations were studied, statistical similarities among data from different anemones became apparent, and a scheme could be constructed to predict the expected error when only small numbers of observations were available.

If, say, 100 observations were made on an animal, and the reaction times were grouped into classes (for example, out of the 100 observations, 7 might be in the 1.6 to 2.5 second class; 11 in the 2.6 to 3.5 second class; 18 in the 3.6 to 4.5 second class, etc.), a characteristic skewed distribution was always obtained with a degree of kurtosis (peakedness) somewhat greater than ordinarily found in a normal or Gaussian distribution. The anemone data could be adequately fitted by curves calculated from the Poisson distribution

\[ F_t = \frac{(Kt)^m e^{-Kt}}{m!}, \]

in which \( F_t \) is the frequency of any class of reaction time \( t \), and \( K \) and \( m \) are constants characteristic of each distribution. The reasons for choosing the Poisson distribution will become apparent below but it should be pointed out that the experimental data could probably be adequately fitted by other distributions as well, such as the Gram-Charlier with a correction factor for kurtosis.

Fig. 1b shows the results of 4 large groups of observations made upon 4

1 *Metridium* is different in this respect from certain other animals such as *Ciona* and *Mya* (Hecht (6, 7)), *Lampetra* (Steven (8)), or *Cerianthus* (Moore (9)) possessing dermal photosensitivity. The reaction times in the latter group are divisible into a "sensitization period" during which the stimulating light must continue if a response is to be obtained, and a "latent period" during which the animal may remain in darkness and still respond. Both sensitization periods and latent periods might run into several seconds' duration. Any latent period in *Metridium* is probably less than a second, as the animal responds in less than a second to intense flashes of about \( \frac{1}{400} \) second duration.
different animals under uniform conditions of environment and stimulus. The data were calculated as cumulative frequency, plotted upon probability paper, and the Poisson distribution of closest fit was drawn. The most obvious difference among the curves is their vertical separation, evidenced by the different values of $K$ needed to fit the curves to the data. This difference suggests that the average reaction time for each animal is different, or the average number of quanta necessary to cause a response varies from animal to animal.

North and Pantin (1) have shown that there are factors, such as body pigmenta-

![Graph](image)

**Fig. 1 a.** Fluctuations in the length of reaction time observed over a 7 hour period; $T = 12^\circ\text{C}$. The points were arbitrarily connected by straight lines. Since any latent period is a negligible fraction of the reaction time, the length of the latter can be used as a measure of the radiant energy necessary to elicit a response. Energy of the stimulus was $8.6 \times 10^8$ quanta/cm.$^2$ sec. of blue-green light.

...that can cause permanent variations in base line sensitivity to blue-green light among individuals of *Metridium*. Undoubtedly there are unknown causes as well, and the existence of such differences is hardly surprising, although well worth future study. The main point to be made here is that the vertical spread of the curves does not necessarily indicate fundamental differences in the patterns of variability found in the animals studied.

Variation of $K$ in Equation 1 involves a slight change of slope in the curves of Fig. 1 b, but the top curve is of such different slope that it requires an increase in the value of $m$ to best fit the data. In order to determine whether this might indicate a fundamental difference between this animal and the others, a simple statistical device was constructed to run analyses.

The device consisted of a number of marbles of the same size and weight, placed in an opaque urn. Several colors of marbles were available, and a given color was assigned...
to each reaction time class. $F_t$ values for $m = 5 K = 1.00$ (see Equation 1) were then calculated, and the number of marbles corresponding to each $F_t$ were placed in the urn. Marbles were withdrawn by touch one at a time, the class noted, the marble replaced, and the urn shaken thoroughly before the next withdrawal.

Ten distributions of 100 withdrawals each were thus obtained, and the two distributions with the most extreme slopes are plotted in Fig. 1 c. These extremes are closely fitted by curves for which $m = 4$ and $m = 8$, hence it is concluded from the available data that the differences of slope in the curves of Fig. 1 b probably have no fundamental significance. A total of eight distributions of 60 or more observations each on eight different anemones have not shown significant deviations from each other when analyzed in the above manner.

It would seem, then, that the patterns of variability in different anemones appear essentially alike when sufficiently large numbers of observations are analyzed. This suggests that the cause of the variability may be a mechanism common to all anemones of this species.
One further question needs to be answered; is there a correlation between the values of consecutive reaction times when series of observations are made, or do the values distribute themselves at random? A rapid and easy way of determining autocorrelation is to count the number of oscillations in a given series of observations. If the values occur at random, the number of maxima and the number of minima found should each be about 1/3 of the total number of observations. Large deviations from the 1/3 value indicate that autocorrelation exists.

To take an example, the record shown in Fig. 1a consists of 38 observations and contains 11 maxima and 11 minima (a point at the start or the end of a series being arbitrarily judged a maximum or a minimum if it deviates from the average by more than 1 second). The series of observations is therefore almost certainly composed of values distributed at random. This conclusion is supported by data obtained from the distributions of Fig. 1b, collected in Table I. In addition, the range of variation is shown in the 10 distributions obtained from the random marble withdrawals which illustrate the variation that can be expected. Our conclusion is that there may be a factor or mechanism in the photosensory processes of Metridium which causes as much as a sevenfold random variation in reaction time.

*Expected Error for Small Numbers of Observations.*—Determination of the large distributions shown in Fig. 1b requires not only considerable time but also a degree of good fortune, since animals may not maintain constant position, shape, or activity for the necessary long periods. Many easier yet significant experiments could be performed, however, based upon smaller numbers of...
observations, if the experimenter had a fairly accurate estimate of the errors that might be expected in the procedure he employs. In experiments to be discussed below, for example, it was found necessary to determine the means, the per cent deviation from the mean, and the degree of randomness of small groups of reaction time determinations. A description of the method of determining the expected error in these quantities follows.

It was decided to adopt ten observations as a convenient total for a given series. This number can usually be made in an hour or less, and, as will be seen presently, does not give rise to large expected errors.

Since it has been shown that large numbers of observations of reaction times occur randomly in a distribution resembling the Poisson, it is possible to calculate theoretically the expected error of, say, the mean of a group of 10 obser-

<table>
<thead>
<tr>
<th>Experiment (Fig. 1 b)</th>
<th>No. of observations × 1/3</th>
<th>No. of maxima</th>
<th>No. of minima</th>
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<td>28</td>
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<tr>
<td>A</td>
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<td>31</td>
</tr>
<tr>
<td>B</td>
<td>32.7</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>D</td>
<td>33.3</td>
<td>29</td>
<td>29</td>
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</tbody>
</table>

TABLE I
Statistical Data on Four Large Distributions of Reaction Time from Four Different Specimens of Metridium senile and from Ten Distributions Obtained from a Statistical Analyzer Described in the Text

vations. It is much less laborious, however, to perform a thousand marble withdrawals in the statistical analyzer, divide them into groups of ten, determine the means of each group, and plot a frequency curve. The validity of this curve can then be checked against results obtained from anemones. Fig. 2 shows the results of such determination of the means, per cent deviations from the mean, and degree of randomness, employing the series of 1000 marble withdrawals used in the previous section. Likewise included in the figures are values obtained from the four anemone distributions shown in Figure 1 b. The correspondence is fairly close, and the curves of Fig. 2 will be used in the following section as a basis for estimating the expected error or the expected range of variation when groups of about ten observations each are studied.

Relationship between Reaction Time and Light Intensity.—

Over the range of intensities studied, it has been found that the photosensitive response of Metridium obeys, on the average, the Bunsen-Roscoe law of reciprocity

\[ I \times t = K, \] (2)
in which \( I \) is the intensity of the stimulating light, \( t \) is the length of illumination time necessary to elicit response (for the case of Metridium, the reaction time), and \( K \) is a constant which for Metridium appears to be in the neighborhood of \( 5 \times 10^9 \) blue-green quanta/cm\(^2\), but varies among individuals by a factor of perhaps three.

Fig. 3a shows the results of 62 observations over a 55 hour period, made on a white animal at different intensities. Each point represents the mean of ten
or eleven consecutive observations. Assuming that the animal obeyed the Bunsen-Roscoe reciprocity relationship, the 62 observations were recalculated as if they had been determined at a constant intensity and the mean of the entire group taken. On the basis of this mean, still assuming reciprocity, the theoretical mean at the different intensities of Fig. 3 a was then determined, as well as the limits within which it could be expected that nine out of ten points, such as are shown in the figure, would lie. The limits are indicated by the dotted lines, and it is seen from the points that a satisfactory hyperbolic relationship was obtained between the reaction time and the intensity of the stimulating light. 306 observations on eight different anemones, covering the range shown in Fig. 3 a, have produced no evidence to suggest that the reciprocity relationship is incorrect.

We are now in a position to eliminate an uncertainty of Fig. 1 a. It will be recalled that in constructing this figure the points were arbitrarily connected by straight lines. Suppose, however, that the fluctuations in reaction time occur much more rapidly and instead of connecting the points with straight lines, two or three or more oscillations between each observation should be...
indicated. If such were the case, it would be expected that records made at
dim intensities in which reaction times may average a minute or so, would show
not only a decrease in the per cent deviation from the mean, but also in random-
ness, since minor peaks would be levelled as uniform readings.

Fig. 3 b shows the effect of change of average reaction time on the per cent
deviation and on the randomness. Many of the readings were taken at higher
temperatures in the hope that this would increase the number of fluctuations

per unit time and enhance the effect we are seeking, but the next section will
show that there is apparently no temperature effect.

There is no marked change in either the randomness or the per cent deviation
at longer average reaction times. There is perhaps an unexpectedly large number
of values of 8 for the maxima plus minima sum, but in three of the four in-
stances at the longer times they are caused by minor fluctuations such as 52,
54, 51 seconds, which at ten times the intensity would have been recorded as
5, 5, 5, seconds (readings are taken to the nearest second).

The values of per cent deviation at the longer reaction times are slightly
low, lying mostly in the second quartile of the frequency curve of Fig. 2. The
significance of this will be left for a future investigation, but it is felt that the
SENSITIVITY TO LIGHT IN SEA ANEMONE. II

slight change does not constitute evidence for a short period fluctuation. We conclude that if irregular fluctuations in the state of the animal are responsible for the observed variability in reaction time, they are probably fairly accurately portrayed by observations made every 5 minutes.

Anemones often require about a minute to respond to light stimuli of $10^8$ quanta/cm. sec. This is roughly the illumination cast by an ordinary street light upon a normal surface about a mile away. About half an hour of dark adaptation is required before an investigator can accurately judge responses by the animals.

**TABLE II**

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>No. of observations</th>
<th>Average reaction time</th>
<th>Temp. °C</th>
<th>No. of observations</th>
<th>Average reaction time</th>
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<td>10</td>
<td>6.8</td>
<td>17.5</td>
<td>10</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Limits within which 9 out of 10 average reaction times would be expected to fall: 5.0-7.4

**Effects of Temperature upon the Reaction Time.**—Table II shows the average reaction times found for two anemones over a range of about 12°C. There seems to be no significant trend or change over this range, which approximately covers the environmental temperatures for the regions where *Metridium* is found along the Pacific coast. A total of 300 observations were made on eight different anemones over the temperature range 4.3—21.0°C, and no significant changes in average reaction time with temperature were found.

As noted above, if a latent period exists in the response of *Metridium* to light it is very short, probably less than a second in duration. A latent period might include thermally affected processes such as nerve conduction time, muscular contraction time, etc., which could conceivably require less than a second to execute. Although muscular contraction is often a slow process

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For an oscillation of average wave length 2 minutes or less the per cent deviations should lie between 0 and 18; for an oscillation of average wave length 2 to 4 minutes the per cent deviation should be distributed around 18 as an average.
Metridium can execute a considerable contraction of the longitudinal musculature in a fraction of a second, and conduction in the nerve net of Metridium has been shown by Parker (11) to proceed at a rate of about 13 cm./sec. at 21°C. Pantin (12) found similar rates in Calliactis for the general nerve net. Photochemical reactions, on the other hand, are well known to be independent of temperature. Since average reaction time does not vary

![Figure 4](image)

**Fig. 4.** Data calculated from 290 observations of reaction time on eight different anemones, showing the distribution of per cent deviation from the mean and of degree of randomness at different temperatures. Each point represents 9 to 11 observations. Energy of the stimulus in all cases was $8.6 \times 10^9$ quanta/cm.$^2$ sec. of blue-green light.

with temperature, it seems safe to assume that most of the reaction time involves photochemical processes.

Lack of a temperature effect upon the length of the reaction time would thus have a plausible explanation. If, however, the variability should arise from changes in physiological state within the animal, one might expect the rate of such changes to display a temperature effect. By way of illustration, suppose that the variability is caused by variance in the tone of the musculature. The body column of a normal Metridium is in a state of constant activity, bending about (Batham and Pantin (13)); so a short reaction time might represent an instance when the animal was stimulated just as it was about to execute a forward (i.e. "lightward") bend, while a long reaction time might represent an
instance when the stimulus arrived just prior to a backward (or "darkward") bend. The rate of such complex activity should, however, be affected by temperature, and it is indeed found that animals at low temperature are much freer of perceptible random movement and therefore much easier to study. Hall and Pantin (14) found a $Q_{10}$ of 2 for the rate of contraction and relaxation in *Metridium*. The rate of spontaneous bending should, therefore, be considerably retarded at low temperatures. If our hypothesis about muscular tone is correct, the effect on a record such as depicted in Fig. 1a would be to lengthen the time scale. Thus, at 2°C. the abscissa of Fig. 1a might be expected to cover a period of 14 hours or more instead of 7 hours, without any other change in the diagram. This suggests that the average number of oscillations per unit of time would be expected to decrease, which could be detected in our measure of degree of randomness. If the temperature effect were considerable, then, for a small number of observations, the per cent deviation from the mean might also decrease. These predictions are not, however, borne out by the experimental evidence, presented in Fig. 4. Over the range of temperatures shown, at least a three- to fourfold change in the rate of ordinary physiological reactions might be expected. Such a change would decrease the sum of maxima and minima to around 2 or 3, for ten observations taken 5 to 7 minutes apart. It can be concluded that the response of *Metridium* to photic stimulation exhibited no temperature effect in experiments so far devised to test for such an effect.

**DISCUSSION**

*Ecological Aspects of the Photosensitive Response.*—The experiments with different intensities have shown that *Metridium* is able to integrate absorbed photic energy over periods of a minute or more, and to utilize the effect to produce a directional response. Several eyeless animals have been shown to possess this ability. Probably the most intensive studies were done on *Ciona* and *Mya* by Hecht (6, 15).

The mechanism of integration of light might conceivably be of advantage to an anemone in a dark environment, causing orientation of the body and tentacles towards steady but weak sources of bioluminescence, thus perhaps increasing the chances of catching microplanktonic luminescent animals for food. Flashes of bioluminescence, presumably from single cells, are often observed in the experimental aquarium, and appear to the human eye many times brighter than illuminations eliciting anemone responses in say, 5 seconds. The ability to integrate radiant energy over the entire body surface might have great potential survival value to other animals as well in the unlighted depths of the oceans where translucency or transparency among organisms is common. Clearly the phenomenon needs to be studied further, not only with regard to

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1 *Metridium* is dredged from depths of 300 to 400 meters in Puget Sound (D. L. Ray, personal communication).
its occurrence, already demonstrated in several phyla, but also with regard to actual uses of it in nature.

_Cause of Reaction Time Variability._—Pirenne (16) notes that many investigators have observed variability of response to light in their experimental material when the region of the threshold is approached. In the case of the human eye the source of the variability arises from the uncertainty of quantum capture at dim threshold intensities. For _Limulus_, however, Hartline _et al._ (17) were unable to distinguish between quantal uncertainty and the possibility of variation in nerve thresholds known to occur.

With _Metridium_, for lack of further data, we are at the present time faced with something of the same dilemma, although the current experimental evidence favors the hypothesis of quantal uncertainty. As mentioned above, any variable physiological state such as the tone of a muscle or the threshold of a nerve, depending upon a vast array of physiological and biochemical reactions within the organism, can be expected to display a temperature effect of some sort. In order to explain the observed variability by assuming a fluctuating change in the condition of the animal, we should probably look for a single simple temperature-independent chemical process or, at most, two balancing processes, one with a positive and the other with a negative temperature coefficient. Such processes would need to cause sevenfold changes in photosensitivity of the animal over short periods of time; they have yet to be discovered.

If one assumes that _Metridium_ has such a low concentration of photosensitive pigment that only a few quanta may be captured in the brief exposure time at the intensities used, explanation of variability in the reaction times follows. This hypothesis of uncertainty of quantum capture has so far proven entirely adequate as an explanation of the data. Since any latent period is a negligible part of the anemone’s reaction time, the latter could be expected to be entirely temperature-independent under this hypothesis. Reaction time distributions would need to conform to the Poisson distribution. And indeed they do, although this is a necessary but not a sufficient requirement. If we assume quantal uncertainty, cumulative curves derived from experimental data have all shown values of _m_ (as defined in Equation 1 above) less than 10, suggesting that the animals responded to less than ten absorbed quanta.

At first thought it seems difficult to conceive that when more than one billion quanta are administered to an anemone, the animal would effectively absorb less than 10 of these. Indeed this is probably not so; measurements upon the body wall of white _Metridium_ by North and Pantin (1) have shown transmissions of roughly 50 per cent at 500 mμ. Of the remaining 50 per cent a large fraction is undoubtedly reflected, scattered, or otherwise dispersed, but nonetheless considerably more than 10 quanta are probably absorbed by the
tissues. It must be remembered, however, that there is apparently a specific pathway by which absorption of quanta must occur and still elicit a response by the animal; action spectra for *Metridium*, with maxima lying between 500 and 600 mμ, have been found by North and Pantin (1), suggesting that quanta must be absorbed by a certain pigment or perhaps a few pigments. The concentration of such a photosensitive pigment, therefore, is the critical factor that must be considered.

Unfortunately hardly a beginning has been made towards isolating possible photosensitive pigments from *Metridium*. Indeed if we assume that the hypothesis of quantal uncertainty is correct, even a rough calculation quickly indicates the futility of attempting to isolate such a pigment in any reasonable quantity, much less determine the concentration accurately. We note

Let us assume, as a rough conservative estimate from the data presented above, that on the average 1 quantum in 10⁸ is absorbed by the photosensitive pigment in the illuminated area of the animal. Assume, further, a quantum efficiency of unity and let the concentration of pigment be \( c \), its molar extinction coefficient at the wave length of maximum absorption be \( E \), and assume that the thickness of the body wall of the anemone is \( 10^{-1} \) cm. Then

\[
\epsilon = \frac{\ln(I_o/I)}{E \times l} = \frac{\ln\left(\frac{10^9}{10^9 - 1}\right)}{E \times 10^{-1}} = \frac{10^{-8}}{E} \text{, expressed as moles/liter.}
\]

In order to obtain a fairly good absorption spectrum in a Beckman DU spectrophotometer, with a 1 cm. path-length cell, the concentration of pigment should be such as to render the maximum per cent absorption not much less than 10 per cent. Such a concentration of pigment would be

\[
\epsilon = \frac{\ln(100/90)}{E \times 1} = \frac{10^{-1}}{E},
\]

again expressed as moles/liter. A concentration factor of \( 10^3 \) is therefore indicated.

An average sized *Metridium* contains perhaps 10 cc. of tissue, therefore, one million such animals would be expected, according to our calculation, to yield 1 cc. of dilute extract for spectrophotometric analysis, assuming no losses during the extraction procedure. To conduct such an experiment in California would require several man-years of effort just to collect the necessary anemones; the chemical extraction might well be complicated by similar but non-photosensitive pigments which would almost certainly be present in the 100 tons or so of material. To attempt the collection and analysis hardly seems practical.

The hypothesis of quantal uncertainty can be shown to lead to sensible results if we assume a value of \( E \) in the equation above, of \( 10^3 \), a value common among carotenoids at the wave length of maximum absorption. It can then be calculated that our average sized *Metridium*, fully expanded, would have \( 10^4-10^5 \) photosensitive pigment molecules per cm.² of body surface. The animal would need perhaps \( 10^{14} \) of such molecules per cm.² before it would appear faintly colored.
in passing that *Metridium* may represent a biological extreme in photosensitive machinery. On the one hand we have receptors such as the human eye, where evolution has conveniently concentrated the photosensitivity pigment. Threshold studies here must deal with the uncertainty of the exact number of quanta contained in any stimulating flash. In similar studies with *Metridium*, on the other hand, the number of quanta per stimulus is readily determinable with sufficient accuracy, but the uncertainty element may still be present, since the pigment concentration may be so dilute as to render any adequate determination of it all but impossible.

In retrospect, the critical data that support the hypothesis of quantal uncertainty are the variabilities in reaction time and especially the demonstration of a complete lack of a temperature effect. At the same time the length of the reaction time is intimately related to illumination since it can easily be varied by changing the intensity of the stimulus. While these considerations constitute strong arguments for the hypothesis, they are perhaps not the conclusive proof that is needed for complete acceptance. In dealing with the properties of such a dynamic fluid as protoplasm, of which we possess only a fragmentary knowledge, it would seem wise to view with reservation conclusions dependent upon indirect evidence.

**SUMMARY**

1. The reaction time of the photosensitive response of *Metridium* was found to be composed almost entirely of a sensitization period. If a latent period exists, it is too short to be detected by the methods used and is probably less than a second in duration. The length of the reaction time, therefore, was used as a measure of the radiant energy necessary at any intensity to elicit a response.

2. The length of the reaction time was found to vary randomly by a factor of seven under constant environmental and stimulating conditions. Determination of large numbers of reaction times on several anemones, grouped according to length, gave closely similar distributions resembling Poisson distributions. It was suggested that the variability may be caused by the same factor or mechanism in each individual. An experimental scheme was presented for determining the expected error and variation in statistical quantities when groups of ten observations are used.

3. The means of groups of reaction times determined at different intensities formed a hyperbolic relationship when plotted against intensity, suggesting that the animal obeys the Bunsen-Roscoe law of reciprocity. No marked changes were noted of per cent deviation from the mean or of randomness at different intensities. Stimulation of *Metridium* requires roughly $5 \times 10^8$ incident quanta/cm$^2$ of blue-green light.

4. No temperature effect could be found on either the means, the per cent
deviation from the mean, or the degree of randomness of series of about ten observations, studied over a 17°C. range. It was concluded that the variability in reaction time was not due to changes in any complex physiological state such as muscular tone.

5. A possible use of the photosensitive response is suggested and the potentialities of "integrative" photosensitivity are discussed.

6. Possible mechanisms to explain the variability in reaction time are discussed. In the light of evidence presented, the most likely hypothesis appears to be the uncertainty of quantum capture caused by low concentrations of photosensitive pigment. Assuming the validity of this hypothesis, evidence suggests that the anemones responded to less than 10 quanta of absorbed light. Caution, however, is recommended in accepting the hypothesis because of the indirect nature of the supporting evidence.

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