Probability of Occurrence of Discrete Potential Waves in the Eye of *Limulus*

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ABSTRACT Discrete potential waves can be recorded from cells in the eye of *Limulus* both in darkness and in dim illumination. With constant illumination the frequency of these waves is linearly related to light intensity and the distribution of intervals between waves follows an exponential function. The latency of waves evoked by short flashes of light is usually long and variable and the number of waves evoked by a flash varies randomly, obeying approximately a Poisson distribution. The results of experiments with flashes of light have been compared with the predictions derived from the hypotheses that one, two, or three quanta of light are required for production of one wave. The agreement of the data with the theory can be considered acceptable for the “one quantum” hypothesis, is less satisfactory for the “two quanta” hypothesis, and is very poor for the “three quanta” hypothesis.

Activity of visual cells of *Limulus* has been studied for a long time by recording impulse discharges from the axons of eccentric cells. Using such techniques, the effects of lights too dim to evoke impulse firing cannot be detected directly, but evidence of subliminal changes can be obtained by means of studies of excitability changes. Studies of this sort have been performed by Hartline, Milne, and Wagman (1947), Wagman, Hartline, and Milne (1949), and Hartline, Wagner, and MacNichol (1952), who investigated the excitability changes resulting from a weak flash of light and the fluctuations of threshold occurring in conditions of dark and light adaptation. The results obtained in this work have not yet been reported in full, but important data from unpublished work by Hartline and Wagner have been reported and discussed by MacNichol (1958).

Subliminal activity can be recorded as a change of voltage when intracellular microelectrodes are used to measure membrane potential of visual cells (MacNichol, 1958). Using this method, Yeandle (1957 and 1958) has found that potential changes consisting of an irregular succession of discrete depolarizing waves sometime occur in dark-adapted preparations. Yeandle
has found that probability of occurrence of these waves is increased by illumination and he also obtained some evidence consistent with the view that a wave originates from absorption of a single quantum of light. Later experiments gave reasons for thinking that light evokes depolarization of visual cells through the action of a "transmitter substance" liberated by the photoreceptor and acting upon the neighboring visual nerve cells (Fuortes, 1959a; Rushton, 1959). It was thought then that the waves of visual cells might be produced by the action of discrete "droplets" of the postulated transmitter, and not necessarily by absorption of individual photons (Fuortes, 1959b). The present article reports in greater detail than before the properties of these discrete potential changes of visual cells. The experiments described here were performed between 1958 and 1962, partly in Bethesda and partly in Woods Hole.

METHODS

The general methods employed in this research for impaling cells in the Limulus eye, recording potentials, and passing currents through the cell's membrane, have already been described in previous papers (Frank and Fuortes, 1956; Fuortes, 1959a).

The light used for stimulation originated from a 36 watt car headlight lamp and was attenuated as desired by means of calibrated neutral filters. A mechanical shutter consisting of a flag operated by a fast electromagnetic device was used in order to apply flashes or steps of light.

When no attenuation was introduced the flux of quanta of wavelengths included between 400 and 600 μm was of the order of $10^{14}$ quanta sec$^{-1}$ cm$^{-2}$ at the cornea, as measured by means of a calibrated thermopile. It can be inferred from the spectral sensitivity curves published by Graham and Hartline (1935) that the quanta which can be absorbed by the photopigment of Limulus are about one-third of those entering the ommatidium in the range between 400 and 600 μm. Thus, the flux of absorbable quanta was about $3 \times 10^{14}$ sec$^{-1}$ cm$^{-2}$ at the cornea. With a spot of light of 50 μ in diameter (as was used in most experiments), the flux was about $6 \times 10^{13}$ quanta sec$^{-1}$. Therefore, the number of absorbable quanta in a flash of 50 msec. duration attenuated by 6 logarithmic units (as in Experiment 81960, intensity 1) was about $3 \times 10^{9}$ at the cornea. Unfortunately, it is very difficult to estimate what fraction of the quanta impinging on the cornea is focused on the rhabdome and what further fraction may be caught by the photopigment. Thus, considerable uncertainty still remains concerning the number of quanta available for absorption by the photopigment, with the stimuli employed in this research.

For the type of study described in this paper, it is desirable to reduce to a minimum the amount of activity present in the absence of applied stimulation. To accomplish this, the preparation should be protected from stray light and should be kept at a low temperature. In addition, it is sometimes useful to maintain the membrane potential at a high value by means of a constant hyperpolarizing current. The effects of currents and of temperature on the discrete waves to be discussed in this article have not yet
Figure 1. Effects of temperature and of currents on the discrete waves. A, retinula cell. Steady hyperpolarizing current of $-3 \, \text{nA}$ ($1 \, \text{nA} = 10^{-9} \, \text{A}$) throughout the experiment. Ink writer record. Upper record shows the activity recorded in darkness at $22^\circ\text{C}$. Lower record shows the activity recorded immediately after lowering temperature to $10^\circ\text{C}$. At this lower temperature, flashes of light (indicated by downward deflections in the bottom tracing) still evoke waves similar to those originating spontaneously at $22^\circ\text{C}$, but of slower time course. Upward pips in lower tracings are at 5 sec. intervals. B, eccentric cell. About $20^\circ\text{C}$. Ink writer record as above. Activity was recorded in darkness, in upper record without currents and in lower record with a constant current of $-10 \, \text{nA}$ between outside fluids and intracellular electrode. With hyperpolarizing currents, average size of the waves increases and their frequency of occurrence decreases.

been studied in detail but their qualitative actions are illustrated in Fig. 1 (see also Fuortes, 1959 b).

Since the experiments performed in this research often required several hours to be satisfactorily completed, it sometimes happened that some change occurred in the impaled cell or in other parts of the system so that measurements taken at different times did not give consistent results (see for example Fig. 4). For this reason it was
necessary to reject many results and it was important to formulate criteria to avoid a selection of data based upon their agreement with preconceived hypotheses. Membrane potential was continuously monitored on an ink-writer (frequency response essentially flat between 0 and 50 c/s) and results were discarded if baseline changes exceeding a few millivolts occurred during an experimental run. In some experiments, steps of depolarizing current were applied at regular intervals and the number of spikes in the response was observed, since any change of membrane potential would affect the response and could, therefore, be detected and distinguished from drift in the amplifiers. Also, results were rejected if the responses to a given illumination were not constant, within confidence limits, throughout the experiments. To check this, the preparation was kept in darkness for suitably long periods at different times during an experiment and changes in the average frequency of "spontaneous" waves were noted. In addition, identical stimulations were repeated several times during an experiment and results were accepted only if the measurements taken at different times were consistent within statistical errors. On some occasions, membrane conductance was repeatedly measured in darkness by the bridge balance method already mentioned in a previous article (Fuortes, 1959 a).

With a selection based upon these criteria, the number of data accepted for analysis was greatly reduced but it was hoped that greater confidence could be attached to the interpretation of the results.

RESULTS

Potential Changes in Darkness or during Constant Illumination

RELATION BETWEEN FREQUENCY OF OCCURRENCE OF THE WAVES AND INTENSITY OF CONSTANT LIGHTS

Fig. 2 illustrates the general appearance of the waves under discussion. The records in this figure were taken from a cell producing large spikes (presumably an eccentric cell, according to Fuortes, 1958) but appearance and properties of the waves are similar when they are recorded from cells producing small spikes and presumed to be retinular cells (see Yeandle, 1958).

It is seen in this figure that the individual waves rise rapidly to a peak and decay more slowly, following an approximately exponential course. They may have different sizes, are separated by irregular intervals, and may sum with one another, giving rise to composite waves. With normal membrane potential, their peak height is often several millivolts and in some cells single waves may reach a peak height of over 10 mv.

It has already been mentioned that the average frequency (number of waves/time) of these waves is increased by dim illumination (Yeandle, 1957; Fuortes, 1959 b). As long as weak, constant lights are used average frequency of the waves is linearly related to light intensity (Fig. 3).

Assuming that activity in darkness is due to a process equivalent to illumination, $i_0$, the origin of the abscissa can be shifted to the point of intersection...
between the abscissa itself and the straight line through the experimental points. This straight line expresses then the relation:

$$n = b(i + i_o)$$  \hfill (1)$$

where $b$ is a constant, $n$ is the frequency of the waves, $i$ is light intensity, and $i_o$ is the "equivalent light" responsible for spontaneous activity. The linear relation between frequency of waves and light intensity is valid only for dim illuminations. With stronger lights proportionality breaks down and in all cases examined the frequency failed to increase in proportion to the light intensity.

![Figure 2. Activity in darkness and during constant illumination. Eccentric cell. Steady hyperpolarizing current of 0.8 nA. Temperature 7°C. Top record was taken in darkness and following records during constant illumination at the relative intensities indicated by the figures at the left.](image)

It has been mentioned under Methods that in some cases the responses to a given light were significantly different at different times during one experiment. Although these results had to be discarded for most purposes, it was still possible to deduce from them that frequency of waves is approximately proportional to light intensity within short periods of time. In the experiment illustrated in Fig. 4, the eye was illuminated for periods of about 2 minutes at various times as indicated on the abscissa. The average values of intensity used were in the ratio 1/2/4. No activity was detected at any time during this experiment while the eye was left in darkness. Average frequency of the waves which could be clearly distinguished above noise during each period of illumination was measured and plotted as a function of time. The scale of the frequency meas-
measurements was different for each light intensity used, being in the ratio 1/2/4 as for the intensities themselves. When the data are plotted in this fashion, it is seen that all points follow approximately the same smooth line, showing that despite the large "drift" in the preparation, frequency is roughly proportional to light intensity at any given time. Two sharp increases of activity (arrows) coincided with manipulations of the cooling system and might have been due to increases of temperature.

![Graph](image-url)

**Figure 3.** Relation between number of waves and intensity of a constant light, Retinula cell. No current. Temperature 20°C. Experiment as in the previous figure. The number of waves occurring within 1 minute was counted with the preparation in darkness, or during illumination at various intensities. The five points fall approximately on a straight line of slope b. It is assumed that activity in darkness is due to a process equivalent to illumination at intensity $i_0$ defined by the relation $bi_0 = n_o$, where $n_o$ is the frequency of waves in darkness. In the experiment illustrated, $i_0 = 0.8$.

**Distribution of intervals between waves** Both in darkness and during dim, constant illumination, successive waves are separated by irregular, apparently random, intervals as shown in Fig. 2. If intervals are counted during constant illumination and grouped according to their duration, it is seen that the number in each group is a decaying exponential function of the duration of the intervals. This relation is characteristic of random processes occurring in accordance with the Poisson distribution.
Let \( \tau \) be the interval between one wave and the next, and \( N \), the number of intervals in which \( \tau \) is greater than some value \( t \). Then, if the Poisson distribution applies, the following relation should be valid:

\[
N_t = N_0 e^{-nt}
\]

where \( N_0 \) is the number of intervals greater than 0 (thus the total number of intervals considered) and \( n \) is the average frequency of the waves. In the experiment of Fig. 5, intervals between responses have been measured in darkness and during constant illumination at relative intensities 1, 2, and 4. The lines in this figure are the theoretical curves derived from expression (2). They satisfy the requirements that both \( N_0 \) and \( n \) be proportional to light intensity if it is assumed that the responses in darkness are evoked by a process equivalent to illumination at relative intensity 0.8.

![Figure 4](image-url)
Similarly, the number $N_{\Delta t}$ of intervals having a value between $t$ and $t + \Delta t$ should be determined by the expression:

$$N_{\Delta t} = N_0 e^{-\mu \Delta t}$$  \hspace{1cm} (3)

where $N_0$ and $\mu$ are defined as in (2). Since a large total number of waves must be recorded in order to count a significant number of intervals included within the short range of variation $\Delta t$, it was impractical to repeat the measurements with different values of illumination, and Fig. 6 shows the results obtained counting intervals during a long period of darkness. The same analysis per-

![Figure 5. Distribution of intervals between waves. Same cell as in Fig. 3. Activity in darkness is supposed to be due to a process equivalent to illumination at relative intensity 0.8 and this value has been added to the intensities of the applied lights, as explained in the preceding figure. Solid lines are a plot of relation (2) where the experimental values of $N_0$ and $\mu$ have been slightly modified, to make them rigorously proportional to the corrected values of light intensity. (Note that intercepts of solid lines with ordinate do not coincide with experimental points.) The points are counts of the number of intervals longer than the times shown in abscissa, as measured during illumination at the different intensities indicated. A total $\chi^2$ test calculated by summing the $\chi^2$ values of the data relating to the individual light intensities gave a level of significance between 0.02 and 0.05.](image-url)
formed on another experiment (81960) during constant dim illumination gave comparable results.

The agreement between theoretical curves and experimental points found with both methods gives support to the view that intervals between successive waves follow approximately the Poisson distribution within the limits of light intensity considered.

**Figure 6.** Relation between number and duration of intervals between waves in darkness. Retinula cell. Hyperpolarizing current $-8 \, \text{nA}$. Temperature 20°C. The number of intervals of duration between $t$ and $t + \Delta t$ are plotted as a function of $t$. The solid line is a plot of relation (3) in which the constants have values measured experimentally, as follows:

$$N(t) = N_0 e^{n t}$$

Length of vertical bars is twice the square root of the number of intervals. Significance level estimated by $\chi^2$ test is between 0.7 and 0.5.

**Responses to Flashes of Light**

**Distribution of Latencies** When activity is evoked by flashes, the discrete waves usually appear with long and variable latencies. For flashes of fixed intensity, mean latency increases with hyperpolarizing currents (Fuortes, 1959 b) and with decreasing temperature. In the experiment of Fig. 7, cur-
rent and temperature were kept constant and responses were evoked by flashes of 50 msec. duration and of relative intensities 1, 2, and 4. The three types of shading in the histogram represent the three light intensities used. The histogram was constructed by plotting first the latencies for intensity 1, then adding to these the latencies for intensity 2, and finally by adding those obtained with intensity 4. Statistical comparison shows that the three distributions (although similar) are not identical. In no case was the distribution of a simple Poisson type, and certainly it does not reflect the distribution in time of absorbed quanta since these are all absorbed within the first 50 msec. Rather, the latency distribution must reflect some features of the unknown intermediary processes between light absorption in the photopigment and production of responses in the nerve cell.

**Relation Between Total Number of Responses and Flash Intensity**

Additional information on the properties of the waves of visual cells can be obtained by analyzing responses to flashes, as has already been done by Yeandle (1958). In the present experiments, flashes of constant intensity and duration were applied at regular intervals (usually one every 2.5 to 10 sec.) until a suitable series of stimuli was completed. With these rates of stimulation, it was highly improbable that a response evoked by a certain flash would occur.

![Graph showing latency distribution](image-url)
after the following flash (see above, Distribution of latencies). Light intensity was then changed and other similar series of flashes were applied. In order to check the stability of the preparation during the several hours required to complete an experiment, series of flashes of a given intensity were repeated at different times and responses were counted during periods of darkness interposed between the different series of stimuli. Light intensity was such that responses were evoked only by a fraction of the stimuli. In the experiments selected for analysis, successes and failures occurred at random.

In order to test whether responses to flashes occurred at random, a response (consisting of one or more waves) was scored as a + and a failure was scored as a −. Sequences of the same sign were called “groups;” for instance the succession ++−−++−+− contains six groups.

In the first method used to test randomness, the number of groups occurring in a series of trials was counted and the level of significance was determined from the tables prepared by Swed and Eisenhart (1943), which show the probability that less than a certain number of groups will occur. A sequence was considered random only if the level of significance exceeded 5 per cent, as defined by Swed and Eisenhart (1943).

In the second method, frequency of occurrence of groups of a certain length is determined. If \( P \) is the probability of obtaining a response, \( 1 - P \) will be the probability of a failure and, if the distribution of responses is random,

\[
P(1 - P)^a
\]

will be the probability of occurrence of a group of \( a \) minus signs. The agreement between theory and experimental results was determined by means of a chi square (\( \chi^2 \)) test.

The third method is based on the argument that if the responses occur at random, the probability \( P \) of obtaining a response should not be influenced by the occurrence of a previous response. The fraction of flashes evoking a response was computed separately for the first, second, third, and fourth trials following a flash which produced a response. These four measurements were compared to the average probability of occurrence of a response and a statistical method devised by Kullback, Kupperman, and Ku (1962) was applied as a test of homogeneity.

As was observed for stimulation with continuous lights, the total number of waves evoked by a given number of flashes was found to be approximately in linear relation with light intensity. This result is illustrated in Fig. 9 and will be discussed in a later section.

**DISTRIBUTION OF NUMBER OF WAVES IN RESPONSES TO FLASHES** The results described in the previous section merely confirm what had already been shown with continuous lights, namely that the number of waves evoked by light is an approximate linear function of the total quantity of light. But stimulation with flashes offers a convenient method for testing whether the Pois-
### Table I

**Comparison of Experimental and Theoretical Results for One Experiment (81960)**

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<th>Relative light intensity</th>
<th>1</th>
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<td>$m_1$</td>
<td>0.440</td>
<td>0.800</td>
<td>1.520</td>
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<tr>
<td>$m_2$</td>
<td>1.26</td>
<td>2.040</td>
<td>3.600</td>
</tr>
<tr>
<td>$m_3$</td>
<td>2.19</td>
<td>3.540</td>
<td>5.640</td>
</tr>
</tbody>
</table>

| No. of stimuli | 291 | 316 | 200 |

| Total No. of waves evoked by stimuli | 146 | 265 | 282 |

| Total waves | 0.505 | 0.640 | 1.410 |

| No. of stimuli | 5 |

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<th>Experimental fraction of stimuli evoking $r$ waves</th>
<th>$r = 0$</th>
<th>$r = 1$</th>
<th>$r = 2$</th>
<th>$r = 3$</th>
<th>$r = 4$</th>
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<tr>
<td>$m_1$</td>
<td>0.595</td>
<td>0.323</td>
<td>0.069</td>
<td>0.014</td>
<td>0.000</td>
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<tr>
<td>$m_2$</td>
<td>0.283</td>
<td>0.144</td>
<td>0.035</td>
<td>0.003</td>
<td>0.000</td>
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<tr>
<td>$m_3$</td>
<td>0.009</td>
<td>0.038</td>
<td>0.128</td>
<td>0.068</td>
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<td>$r = 0$</td>
<td>0.644</td>
<td>0.283</td>
<td>0.062</td>
<td>0.009</td>
<td>0.001</td>
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<tr>
<td>$r = 1$</td>
<td>0.449</td>
<td>0.360</td>
<td>0.144</td>
<td>0.038</td>
<td>0.009</td>
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<tr>
<td>$r = 2$</td>
<td>0.219</td>
<td>0.332</td>
<td>0.253</td>
<td>0.128</td>
<td>0.068</td>
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<td>$r = 3$</td>
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<td>$r = 4$</td>
<td></td>
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| Chi square | 3.14 | 6.86 | 7.06 | 17.06 |

| Degrees of freedom | 2 | 3 | 4 | 7 |

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<th>Level of significance $\alpha$</th>
<th>$0.20 &lt; \alpha &lt; 0.05$</th>
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<td>0.000</td>
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<td>0.455</td>
<td>0.132</td>
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<tr>
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<tr>
<td>$r = 4$</td>
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| Chi square | 15.07 | 2.18 | 16.76 | 34.00 |

| Degrees of freedom | 2 | 2 | 3 | 5 |

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<table>
<thead>
<tr>
<th>Probability of $r$ waves calculated from $P = P_{t(r)}^{(r)}$</th>
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<th>$r = 1$</th>
<th>$r = 2$</th>
<th>$r = 3$</th>
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<td>0.350</td>
<td>0.024</td>
<td>0.000</td>
<td>0.000</td>
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<tr>
<td>$r = 1$</td>
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<td>0.115</td>
<td>0.007</td>
<td>0.000</td>
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<tr>
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<td>$r = 4$</td>
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</table>

| Chi square | 41.38 | 18.77 | 58.10 | 118.25 |

| Degrees of freedom | 2 | 2 | 3 | 5 |

<table>
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<tr>
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<th>$\alpha &lt; 0.001$</th>
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</thead>
</table>

$m_1$, $m_2$, and $m_3$ are obtained from curves of Fig. 9. $P_{t(r)}^{(1)}$, $P_{t(r)}^{(2)}$, and $P_{t(r)}^{(3)}$ are given respectively by equations (5), (6), and (7). The $\chi^2$ and the degrees of freedom associated with them were calculated as follows. For comparing experimental fraction of stimuli evoking $r$ waves with $P_{t(r)}^{(1)}$, $P_{t(r)}^{(2)}$, and $P_{t(r)}^{(3)}$ for each individual light intensity the standard goodness of fit $\chi^2$ was used. The degrees of freedom for each intensity and each hypothesis are approximately the number of categories minus 1, since not only data from the particular intensity but data from the entire experiment were used in estimating $m_1$, $m_2$, and $m_3$. The total $\chi^2$ for each hypothesis was obtained by summing the $\chi^2$ of each intensity. The degrees of freedom for the total $\chi^2$ are the sum of the degrees of freedom for the $\chi^2$ of each separate intensity minus 2, since two parameters, the number of quanta equivalent to the spontaneous activity and the number of quanta in the arbitrary unit of light, were estimated from Fig. 9.
son distribution applies to the number of waves included in various responses.

If flashes of given intensity and duration evoke on the average a certain number \( n \) of waves, some flashes will evoke more and some flashes will evoke less than \( n \) waves. The Poisson series predicts that the probability of obtaining 0, 1, 2, 3 \( \ldots \) etc. waves is given by the successive terms of the Poisson series, 
\[
\frac{e^{-n} n^0}{0!}, \frac{n^1}{1!} e^{-n}, \frac{n^2}{2!} e^{-n}, \frac{n^3}{3!} e^{-n}, \ldots \]
Both Table I and Fig. 8 show that there is moderately good agreement between the theoretical distribution (represented in the figure by the histograms) and the experimental points. Thus, the results presented in this section encourage the conclusion that the distribution of the number of waves in responses to flashes is related to the average number of responses \( n \) approximately in accordance with the Poisson distribution.

**RELATIONS BETWEEN ABSORPTION OF LIGHT QUANTA AND OCCURRENCE OF WAVES** Since absorption of light quanta by the photopigment also can be presumed to obey the Poisson distribution (at least for the low intensities considered in this work), it seems desirable to determine the relations between the theoretical distribution of quanta absorbed following flashes of different

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**Figure 8.** Number of waves in responses elicited by flashes. Eccentric cell. Hyperpolarizing current \(-5\) nA. Temperature 7°C. The histograms show the theoretical probability of obtaining zero to five waves in response to a flash when average frequency of responses is the frequency \( n \) determined experimentally. These experimental values of \( n \) are shown in line 6 of Table I. The three sets of data were obtained with flashes of relative intensity 1, 2, and 4. The points are uncorrected experimental measurements (block 7, in Table I). The theoretical probability of occurrence of zero, one, or more waves as a function of average frequency is shown in block 8 of the same table. A total \( \chi^2 \) of 15.8 giving (for 6 degrees of freedom) a level of significance between 0.02 and 0.01 was determined for this experiment.
intensities and the observed distribution of waves. The data of Fig. 8 are not sufficient for this purpose since there the parameter \( n \) was taken separately for each light intensity. In order to correlate more rigorously number and distribution of waves to number and distribution of absorbed quanta one must first postulate a proportionality factor relating light intensity \( i \) to the number \( m \) of absorbed quanta; second, one must formulate some hypothesis on the number of quanta required to produce one wave. The following alternative hypotheses have been made: (a) Whenever one quantum is absorbed by the pigment, one wave is recorded in the impaled cell. (b) Whenever two or three quanta are absorbed, one wave is obtained; four or five quanta elicit two waves, and so forth. When an odd number of quanta is absorbed the unused quantum does not leave any residual effect that may influence the response to the following flash. (c) One wave is obtained, when three, four, or five quanta are absorbed, two waves occur following absorption of six, seven, or eight quanta, etc. Again, unused quanta have no residual effect.

It is recognized that these hypotheses are greatly oversimplified but perhaps it would be premature to attempt any greater refinement at this stage.

For the one quantum hypothesis, the probability \( P_{1}(r) \) that \( r \) waves follow a pulse of light obeys the Poisson distribution:

\[
P_{1}(r) = e^{-m_{1}} \frac{m_{1}^{r}}{r!}
\]

while for the two quanta and three quanta hypotheses, the probabilities of occurrence of \( r \) waves following a flash are respectively:

\[
P_{2}(r) = e^{-m_{2}} \left[ \frac{m_{2}^{2r}}{2r!} + \frac{m_{2}^{2r+1}}{(2r+1)!} \right]
\]

\[
P_{3}(r) = e^{-m_{3}} \left[ \frac{m_{3}^{3r}}{3r!} + \frac{m_{3}^{3r+1}}{(3r+1)!} + \frac{m_{3}^{3r+2}}{(3r+2)!} \right]
\]

where \( m_{1} \), \( m_{2} \), and \( m_{3} \) are the average numbers of absorbed quanta.

For the one quantum hypothesis, the relation between the average number \( n \) of waves produced and the average number of quanta absorbed is:

\[
n = \sum_{r=0}^{\infty} r \frac{m_{1}^{r}}{r!} e^{-m_{1}} = m_{1}
\]

For the two quanta hypothesis, the equation is:

\[
n = \sum_{r=0}^{\infty} r \left[ \frac{m_{2}^{2r}}{2r!} + \frac{m_{2}^{2r+1}}{(2r+1)!} \right] e^{-m_{2}}
\]

and finally for the three quanta hypothesis the equation is:
\[ n = \sum_{r=0}^{\infty} r \left[ \frac{m_2^{3r}}{3r!} + \frac{m_3^{(3r+1)!}}{(3r+1)!} + \frac{m_2^{3(r+2)}}{(3r+2)!} \right] e^{-m_1} \]  

where \( r \) has the same meaning as in equations (5) to (7).

Since there is an unknown coefficient of proportionality between average number of quanta in a flash (light intensity) and average number of quanta utilized by the photopigment one can attempt to fit the data to the three curves.

**FIGURE 9.** Relation between number of absorbed quanta and number of waves. 12359, retinula cell. No current. Temperature 7°C. 81960, same cell as in Fig. 8. Curves 1, 2, and 3 obey relations (5), (6), and (7) respectively. Points are experimental measurements of average number of waves occurring in darkness (the two lowest points in each curve) or following flashes of relative intensities 1, 2, and 4. Solid and open symbols indicate results obtained in two different preparations. Complete numerical data for one experiment are shown in lines 4 to 6 of Table I. The same experimental points were fitted to the theoretical curves using the procedures explained in the text.

Length of vertical bars through points is \( 4 \sqrt{\frac{n}{s}} \) where \( n \) is the average number of waves following a flash and \( s \) is the number of flashes delivered.

Curve 1 in Fig. 9 obeys relation (1) where \( b \) includes the coefficient mentioned above. The experimental points were fitted by selecting values of \( b \) and \( i_0 \) to minimize the expression:

\[ \sum_i \frac{(n_i - \bar{n}_i)^2}{n_i} s_i \]  

where \( n_i \) and \( \bar{n}_i \) are respectively theoretical and experimental values of the average number of waves evoked by a flash, \( s_i \) is the number of trials, and the
index $i$ refers to the light intensity used. This is merely a least square fit with the mean equated to the variance since the number of waves following a flash obeys approximately the Poisson distribution.

This method would have been too laborious for curves 2 and 3, and the

![Diagram](image-url)

**Figure 10.** Distribution of number of waves for given average numbers of absorbed quanta. Same cell and same experimental data as in Fig. 8. The histograms show the theoretical probability of obtaining zero to five waves in response to a flash, assuming that the average number of quanta $m$ absorbed following flashes at relative intensities 1, 2, and 4 is in each case the number derived from the theoretical curves of Fig. 9. Histograms in A show the theoretical distribution expected if one absorbed quantum elicits one wave. Histograms in B and C show the distributions expected if absorption of two or three quanta respectively is required for occurrence of one wave. Theoretical probabilities for this experiment are shown in blocks 8, 9, and 10 in Table I and can be compared to the experimental measurements shown in block 7.
points were fitted more crudely by estimating confidence limits. This fit is not satisfactory if it is assumed that the same coefficient (b in Equation 1) controls both average number of waves following a flash and average number of waves in darkness. However, the data can be fitted to either equation (9) or equation (10) by appropriate choice of b and i₀ in the relation

\[ m = bi + i₀ \]  

where b and i₀ have different values for the different hypotheses.

RELATION BETWEEN NUMBER OF QUANTA AND DISTRIBUTION OF NUMBER OF WAVES IN RESPONSES

It is seen in Fig. 9 that in order to fit the same experimental data to the three curves, different values must be assigned both to the abscissa of the first point (corresponding to activity in darkness) and to the proportionality factor relating number of absorbed quanta m to light intensity. Thus, the three hypotheses imply different numbers of absorbed quanta (m₁, m₂, and m₃ respectively) for the same light intensity and predict different distributions in the number of waves evoked by flashes.

To illustrate the above, results of one experiment (81960) will be discussed. According to the one quantum hypothesis, 0.44, 0.80, and 1.52 are the average number mᵢ of quanta absorbed following a flash for relative intensities, 1, 2, and 4, respectively. For the two quanta hypothesis, the average number mᵢ of quanta absorbed for the same flashes of relative intensities, 1, 2, and 4, is 1.26, 2.04, and 3.60, respectively, while for the three quanta hypothesis the corresponding values are, 2.19, 3.34, and 5.64. From these figures, one can calculate \( Pᵢ⁽¹⁾, Pᵢ⁽²⁾, \) and \( Pᵢ⁽³⁾ \) [relations (5), (6), and (7)], and the results of this
calculation are shown by the histograms of Fig. 10, together with the experimental measurements from Fig. 8.

In this experiment the agreement was considerably better for the one quantum than for the two quanta hypothesis. In another experiment the significance of the fit was between 0.01 and 0.001 for both hypotheses. In both experiments the level of significance for the three quanta hypothesis was <0.001.

With the method just described, agreement of the data with theory requires that throughout the experiment no change occur in the effectiveness of absorbed quanta to produce waves. In an attempt to reduce the effect of such changes the results were fitted to distribution histograms constructed for the two quanta and three quanta hypotheses as was done for the one quantum hypothesis in Fig. 8: the values of $m_2$ and $m_3$ were derived directly from the experimental values of $n$ on the basis of equations (9) and (10) respectively. This method gave results comparable to those obtained with the previous procedure: the fit of the data with the histograms was best for the one quantum hypothesis and poorest for the three quanta hypothesis.

**DISCUSSION**

The present article describes some empirical features of the discrete potential waves (originally described by Yeandle, 1958) which can be recorded in certain conditions from cells in the eye of *Limulus* and attempts to determine what general rules govern probability of occurrence and distribution of these waves.

First, an effort has been made to test whether the experimentally observed distribution is in accordance with a simple Poisson distribution. Results such as those illustrated in Figs. 6 and 8 showed that there is fair agreement between theoretical predictions and experimental observations. Second, the alternative hypotheses have been advanced, (a) that each absorbed light quantum evokes one wave; (b) that absorption of two light quanta elicits one wave; or (c) that three quanta are required for production of one wave, and the agreement of the experimental measurements with the predictions derived from these hypotheses has been tested.

All hypotheses imply a number of arbitrary assumptions: among those already stated is the assumption that activity in darkness is due to a process equivalent to dim illumination. In addition, it is assumed that number of quanta absorbed is at all times proportional to light intensity. This requires that no changes occur in time in the concentration of the photopigment. The predictions derived from the three hypotheses are not changed if one considers that a wave is not the necessary consequence of absorption of one, two, or three quanta, but is evoked with a certain probability $P_w$ following absorption of quanta.

There are reasons to believe that $P_w$ changes due to changes of current through the membrane of the impaled cell, due to changes of temperature, and possibly due to other factors. These changes are not taken into account.
by the statistical treatment and could be an important cause of disagreement between theory and experiment. For this reason, hypotheses should perhaps not be discarded, even if the statistical tests yield levels of significance lower than those usually considered acceptable. It may be added that the statistical tests employed in the present work are more stringent than those previously used in the analysis of the same problem. Yeandle (1958), applying to Limulus the technique used by Hecht, Shlaer, and Pirenne (1942) in their studies on human beings, has measured probability of occurrence of a response as a function of average number of absorbed quanta, without differentiating between responses consisting of one or more waves. The probability $P_{(k,m)}$ of obtaining a response (consisting of one or more waves) following a flash is related to the average number $m$ of quanta in the flash by the relation:

$$P_{(k,m)} = \sum_{r=k}^{\infty} e^{-m} \frac{m^r}{r!}$$

where $k$ is the number of photons which must be absorbed in order to produce a response. This method implies no assumptions on the possibility of fluctuations in the number of waves produced by a given number of absorbed quanta, so that, for instance, the experimental measurements could still agree with relation (13) if more than one wave were produced by one absorbed quantum of light. By contrast, the hypotheses proposed in the present paper do not consider the possibility that one quantum may produce more than one wave. With this restraint, the data must necessarily fit the cumulative Poisson distribution if they fit its individual terms. Agreement of the data with relation (13) but not with relations (5) to (7) would not be meaningful in the framework of the hypotheses considered. It seems important to add that the hypotheses proposed above consider specifically absorbed light quanta as the agent controlling distribution of the responses. In fact, however, the statistical tests performed can only give indications on the agreement of the observations with either a simple Poisson distribution (hypothesis 1) or with a distribution resulting from summation of successive terms of the Poisson series (hypotheses 2 and 3) where the parameter $m$ is (under certain assumptions stated before) related to light intensity by an arbitrary coefficient of proportionality. The theoretical predictions could be satisfied not only if $m$ were the average number of absorbed quanta, but also if $m$ were related to number of light quanta by a proportionality coefficient other than unity. For example, the following series of processes could be supposed to occur: (a) Light liberates a hypothetical transmitter substance in the form of droplets and the number of droplets liberated is proportional to the average number of absorbed quanta; (b), each droplet of transmitter has a very small probability $P_t$ of reaching the membrane in its active form so that the probability that $r$ droplets reach the membrane follows the Poisson distribution; (c) one, two, or three droplets
evoke one wave, as specified in hypotheses 1 and 2 respectively, with reference to light quanta. This set of processes is not proposed because it is considered to be a likely model of real events, but simply to illustrate how a Poisson process other than absorption of light quanta could control the observed distribution of responses. It follows from these considerations that even a good agreement between the experimental observations and one or another of the hypotheses proposed above would not be sufficient evidence that the hypothesis is correct. The uncertainty is due to the arbitrariness of the proportionality factor in the relation between light intensity and number of absorbed quanta, and might be reduced if one could obtain reliable information on the optics of the Limulus eye and on the concentration of the photopigment.

It has been seen that the attempt to fit the data to the theoretical curves derived from the alternative hypotheses proposed has shown that the measurements agree with the one quantum (or "one event") hypothesis better than with either of the other two. However, the agreement is not sufficiently close to support this hypothesis unequivocally. The only general conclusion that appears legitimate at the present time is that the results of this analysis are consistent with the view that one quantum of light is sufficient for production of one wave, but other hypotheses are acceptable and a choice among the various possibilities should be based upon better experimental data or upon more specific models and hypotheses than those which could be offered in the present work.

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