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RESPONSES TO SINGLE PHOTONS IN VISUAL CELLS OF *LIMULUS*

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SUMMARY

1. A system proposed in a previous article as a model of responses of visual cells has been analysed with the purpose of predicting the features of responses to single absorbed photons.

2. As a result of this analysis, the stochastic variability of responses has been expressed as a function of the amplification of the system.

3. The theoretical predictions have been compared to the results obtained by recording electrical responses of visual cells of *Limulus* to flashes delivering only few photons.

4. Experimental responses to single photons have been tentatively identified and it was shown that the stochastic variability of these responses is similar to that predicted for a model with a multiplication factor of at least twenty-five.

5. These results lead to the conclusion that the processes responsible for visual responses incorporate some form of amplification. This conclusion may prove useful for identifying the physical mechanisms underlying the transducer action of visual cells.

INTRODUCTION

The process of vision starts with absorption of light in the pigments of visual cells in the eye and ends with the recognition of patterns as evidenced by changes of behaviour or by conscious experience. The initial step in this process is the transformation of light into a signal capable of influencing the activity of nerve cells, and the present paper will be concerned exclusively with these initial events.

The earliest change produced by light must be some alteration of the visual pigment molecules, such as the transformation of rhodopsin from its 11-cis configuration to its all-trans structure (see Wald, 1961). Following this, processes are initiated which lead to a potential change of the membrane of visual cells. In the cells of the compound eyes of invertebrates, membrane potential is decreased by illumination (Hartline, Wagner & MacNichol, 1952; Fuortes, 1959*a*; Naka & Eguchi, 1962), and the following considerations will be limited to the visual responses recorded from cells in the eyes of *Limulus*. One would hope, however, that the conclusions may have some general validity.

THEORY

Hodgkin's model. Examining responses of visual cells of *Limulus*, Hodgkin observed that the main features of the potential changes evoked by light can be reproduced by a system such as that represented by the electrical network of Fig. 1*B* (Fuortes & Hodgkin, 1964). In this model, the shape of responses to a given input is a function of the number of stages n. For a given number of stages, the amplitude of the responses is controlled by both parameters $\tau_1 = RC$ and $\tau_2 = C/\mu$, whereas the time course of the response is controlled by τ_1 only. If the input is an instantaneous impulse $\bar{v}_0 \Delta t$ at $t = 0$, the output $v_n(t)$ will be

$$v_n(t) = \bar{v}_0 \Delta t \, \frac{t^{n-1} e^{-t/\tau_1}}{(n-1)!\,\tau_2{}^n}. \tag{1}$$

Plots of equation (1) for different values of n are shown in Fig. 1*A*.

The change of membrane potential $V(t)$ in visual responses evoked by flashes of intensity I can be related to the output of the model by introducing two constants of proportionality $A = \frac{V}{v_n}$ and $B = \frac{I}{\bar{v}_0}$ One obtains then

$$V(t) = \frac{A}{B} I \Delta t \, \frac{t^{n-1} e^{-t/\tau_1}}{(n-1)!\,\tau_2{}^n}. \tag{2}$$

as the expression describing the electrical response of a visual cell to a brief flash of light.

The network of Fig. 1 is a linear system and, therefore, it can reproduce visual responses only over their limited linear range. Non-linearities similar to those observed in visual cells can be simulated by this model if τ_1 is placed under the control of the output v_n, for instance using the relation

$$\frac{1}{\tau_1} = \frac{1}{\bar{\tau}_1}\left(1 + \frac{v_n}{w}\right). \tag{3}$$

It then becomes possible to reproduce responses over a wider range of stimuli. The best fit between theoretical and experimental responses has been obtained by Marimont (1965) using a slightly different form of gain control and is illustrated in Fig. 2.

Gain. A typical feature of the model of Fig. 1 is the possibility to provide gain. Considering responses to an instantaneous impulse, it is convenient to define gain as the ratio

$$G = \frac{\int_0^\infty v_n(t)\,\mathrm{d}t}{\bar{v}_0 \Delta t} = \left(\frac{\tau_1}{\tau_2}\right)^n. \tag{4}$$

It is easily seen that if each stage has a gain of g, over-all gain is g^n where g can be greater than unity.

Particle models. Other models have been proposed which lead to equation (1) but cannot supply gain. Borsellino, Fuortes & Smith (1965)

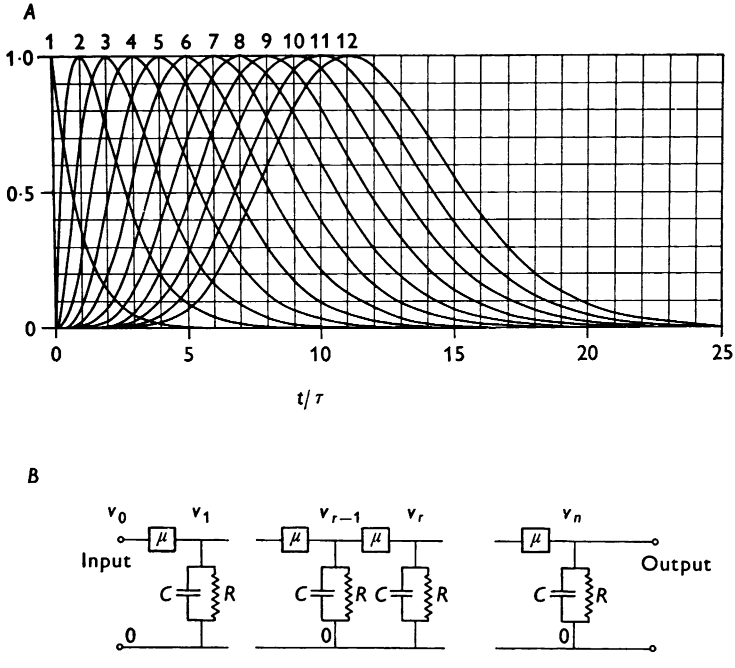

Fig. 1. *A*. Response of the model of Fig. 1*B* to an instantaneous impulse. The figure shows normalized plots of equation (1) for $n = 1, 2, \ldots, 12$. Voltages are measured in ordinate in units of peak output voltage, V_{max}.

B. Electrical network reproducing kinetics of visual responses. The network consists of n identical stages including the two time constants each: $\tau_1 = RC$ and $\tau_2 = \mu/C$. The components separating the successive stages are amplifiers of infinite input impedance and of mutual conductance μ. In order to incorporate suitable non-linearities in this model the conductances $1/R$ are made linearly dependent upon the output voltage v_n (see equation 3). (Modified from Fuortes & Hodgkin, 1964.)

have pointed out that equation (1) is satisfied by a system in which a response is produced when a particle capable of moving forward and backward along a chain of n sites reaches the end of the chain. However, some important features of visual responses involving non-linearities can be reproduced by this system only if its multiplication factor (defined as the number of particles reaching the output per number of particles introduced at the input) is much less than unity. More recently, Levinson (1966) has proposed a model based on the assumption that absorbed photons activate 'sites' in the visual cell and that a response is obtained if n

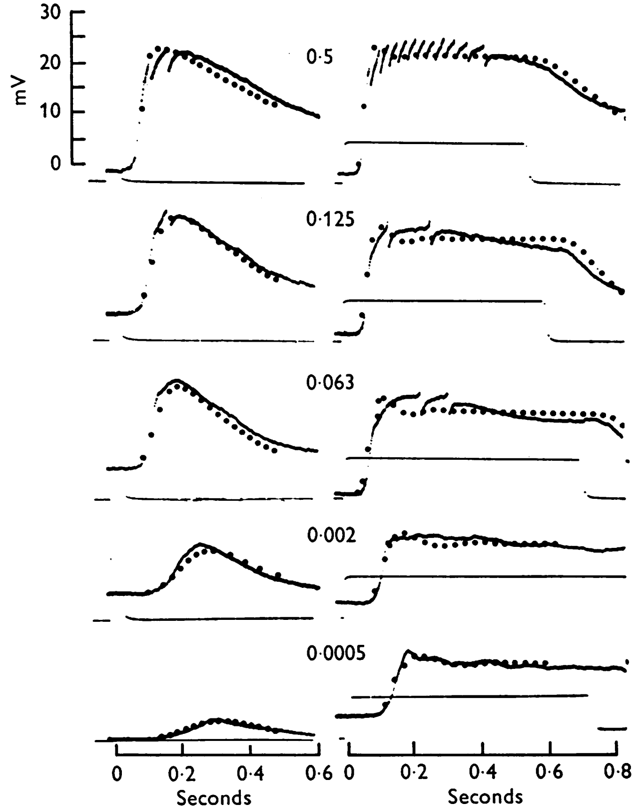

Fig. 2. Comparison of experimental and theoretical responses. Solid lines are photographs of responses recorded from a visual cell in the eye of *Limulus* by means of an intracellular electrode, following stimulation with flashes or steps of light. Light intensity in arbitrary units is indicated near each pair of records. The dots show points calculated from the model of Fig. 1*B*; gain control was obtained using the relation $1/\tau_1 = 1/\bar{\tau}_1\{1+(v_{n-1}/w)\}$ rather than equation (3). This corresponds to moving the origin of the gain control loop from the last stage to the stage before last. In this way, a faster feed-back is obtained and excessive oscillations of the output of the model can be avoided. Temperature 13° C. (Modified from Marimont, 1965.)

particles of a pre-existing substance S_0 reach a site, provided that the site is not inactivated by arrival of particles of another substance S_i. The multiplication factor of this system (defined as the number of responses per number of activated sites) is necessarily less than unity.

Diffusion of a substance satisfies input–output relations similar to those of Hodgkin's model but again it implies a gain less than unity.

Compartmental model. It seems useful to determine whether visual responses have the features predicted by models providing amplification or by systems without gain since different physical processes are suggested by these two classes of models.

In order to study this question, we found it convenient to represent the model of Fig. 1*B* as a system of compartments because compartmental models are especially suitable for analysis of stochastic properties, and stochastic properties are predictably related to gain. A compartment is defined as the state or location of particles (Solomon, 1961; Berman, Shahn & Weiss, 1962). The number of particles in compartment i is called q_i and the fractional rate of transition of particles from one compartment to another is called λ_2. The network of Fig. 1 is analogous to a sequence of $n+1$ compartments ($n = 0, 1, 2,\ldots, n$) arranged as shown in Fig. 3 in which compartment 0 may be thought to represent absorption of light and the rapid transformations induced by light on the pigment molecules (the so-called 'light reactions'). This system is characterized by the equation

$$\frac{\mathrm{d}q_i}{\mathrm{d}t} = -\lambda_1 q_i + \lambda_2 q_{i-1} \quad (i = 1, 2,\ldots, n). \tag{5}$$

It is assumed that a flash of light of intensity I and duration Δt activates a number αM of particles in compartment 0, proportional to the number of photons in the flash, $M = I\Delta t$. The average life τ_0 of these particles is supposed to be very short so that the average number of particles present during the flash will be $\bar{q}_0 = \alpha M\tau_0/\Delta t = \alpha I\tau_0$. The number of particles introduced into compartment 1 during the flash will be $\lambda_2\bar{q}_0\Delta t$, and if Δt is brief compared with the time course of the response (which is controlled by the time constant $1/\lambda_1$), this number may be regarded as the initial number of particles in compartment 1: $q_1(0) = \lambda_2\bar{q}_0\Delta t$. With this initial condition, the solution of equation (5) is

$$q_n(t) = \bar{q}_0\Delta t\lambda_2^n \frac{t^{n-1}\mathrm{e}^{-\lambda_1 t}}{(n-1)!}. \tag{6}$$

It might be objected that the correct initial conditions for compartment 1 are

$$q_1(0) = 0 \text{ and } \frac{\mathrm{d}}{\mathrm{d}t}\, q_1(0) = \alpha\, M\lambda_2 = \frac{1}{\tau_0}\, \lambda_2\bar{q}_0\Delta t.$$

However, the initial condition mentioned previously is equivalent to these for all practical purposes if τ_0 and Δt are much shorter than the time constant $1/\lambda_1$; more precisely, if $\tau_0 \ll \Delta t \ll 1/\lambda_1$. The recorded electrical response $V(t)$ is assumed to be proportional to the number of particles in the last compartment

$$V(t) = \bar{v} q_n(t). \tag{7}$$

Equation (6) can be simplified introducing a parameter with dimensions of rate

$$\Lambda = \frac{\lambda_2}{(n-1)!}\left(\frac{\lambda_2}{\lambda_1}\right)^{n-1}$$

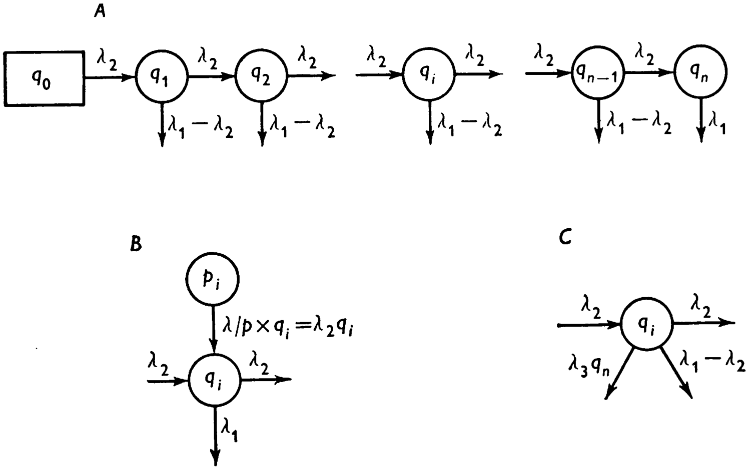

Fig. 3. Compartmental representation of the model of Fig. 1*B*. The parameters of this model correspond to the parameters of the electrical network as follows:

Compartmental model	q_i	λ_1	λ_2	n
Electrical model	v_i	$1/RC = 1/\tau_1$	$\mu/C = 1/\tau_2$	n

In the electrical model, the elements μ supply current to the stages $i+1$ without draining current from the preceding stages i. In order to reproduce this feature in the compartmental model, $i+1$ must receive particles from i, but i must not lose particles in the process. Thus, the outflow from i into $i+1$ ($\lambda_2 q_i$) must be compensated by an equal influx ($-\lambda_2 q_i$). This can be accomplished by subtracting λ_2 from the rate of decay λ_1. One obtains then

$$\frac{dq_i}{dt} = -(\lambda_1-\lambda_2)q_i - \lambda_2 q_i + \lambda_2 q_{i-1} = -\lambda_1 q_i + \lambda_2 q_{i-1}.$$

A chemical system satisfying this relation has been described in a previous article and is reproduced in *B*: a precursor reacts with an enzyme in compartment i to give a new enzyme in compartment $i+1$ (Borsellino *et al.* 1965).

The gain control of equation (3) can be incorporated in the compartmental system using the relation

$$\lambda_1 = \bar{\lambda}_1 + \frac{\bar{\lambda}_1}{w} q_n = \bar{\lambda}_1 + \lambda_3 q_n$$

and a typical compartment of the non-linear system is illustrated in *C*.

and a dimensionless variable $\theta = \lambda_1 t$. One obtains then

$$q_n(t) = \bar{q}_0 \Delta t \Lambda \theta^{n-1} e^{-\theta}. \tag{6B}$$

For the case of inputs in the form of an impulse, gain G is defined as

$$G = \frac{\int_0^\infty q_n(t)\,dt}{\bar{q}_0 \Delta t} = \left(\frac{\lambda_2}{\lambda_1}\right)^n. \tag{8}$$

Since the average life of particles is τ_0 in compartment 0 and $1/\lambda_1$ in compartment n, the average number of particles introduced by the flash in compartment 0 will be $\bar{q}_0 \Delta t/\tau_0$ and the total number of particles in compartment n will be

$$\lambda_1 \int_0^\infty q_n(t)\,dt.$$

The ratio between these two numbers will be called the 'multiplication factor' F of the chain of compartments:

$$F = \frac{\lambda_1 \int_0^\infty q_n(t)\,dt}{\bar{q}_0 \Delta t/\tau_0} = \lambda_1 \tau_0 G. \tag{9}$$

Finally, the ratio between the number of particles generated by the flash in compartment 0

$$\alpha M = \frac{\bar{q}_0 \Delta t}{\tau_0}$$

and the number of absorbed photons $Q = KM$ will be called the 'initial quantum yield' Y_0

$$Y_0 = \frac{\alpha M}{Q} = \frac{\alpha}{K}. \tag{10}$$

In the following treatment we shall assume that $Y_0 \leqslant 1$. In physiological experiments, the directly measurable quantities are the voltage change $V(t)$, and the number of absorbable photons in the flash $M = Q/K$. In order to evaluate F from equation (9), it is necessary to know in addition the value of $\bar{v}$ in equation (7), of Y_0 in equation (10), and of K. Since these parameters are unknown, F cannot in general be deduced directly from the experimental results. However, if the input of the model of Fig. 3 is a single particle ($\bar{q}_0 = \tau_0/\Delta t$), its output should show random variations and their statistical properties should be recognizably different for different values of F. The features of the responses to be expected for different amplifications can be deduced from the considerations outlined below and given in greater detail in the Appendix.

Stochastic treatment of compartmental model. Each particle in a compartment can either decay or it can generate particles in the following compartment. The number of particles in each compartment will then be considered as a stochastic variable $X_1(t)$, $X_2(t),\ldots, X_n(t)$ and each of these variables can assume the integer values $x = 0, 1, 2,\ldots$.

We shall define

$$P_{i,x}(t) = \text{Prob}\{X_i(t) = x\} \tag{11}$$

as the probability that the number of particles in the ith compartment at time t is x.

The average number of particles in the ith compartment is

$$\sum_{x=0}^{\infty} P_{i,x}(t)x = q_i(t) \tag{12}$$

where $q_i(t)$ is the solution of equation (6).

As explained in the Appendix, the probability that the number of particles at time t is x, is given by the Poisson equation

$$P_{i,x}(t) = \frac{1}{x!}\{q_i(t)\}^x e^{-q_i(t)}. \tag{13}$$

If one assumes that the measured latency of a response is the time at which the first particle appears in the nth compartment, latency distribution of responses can be predicted as follows:

The average rate of arrival of particles at the last compartment at the time t is

$$\nu(t) = \lambda_2 q_{n-1}(t) \tag{14}$$

The average number of particles arriving within t will then be

$$\int_0^t \nu(t)\,dt = \lambda_2\int_0^t q_{n-1}(t)\,dt \tag{15}$$

and the probability that no particle arrives within t is (see Appendix)

$$p_{n,0}(t) = \exp\left\{-\lambda_2\int_0^t q_{n-1}(t)dt\right\}. \tag{16}$$

This can be considered also as the probability that all particles arrive after t; therefore the probability that the first particle arrives between t and $t+dt$ is

$$p_{n,0}(t) - p_{n.0}(t+dt) = -\frac{d}{dt}p_{n,0}(t)\,dt \tag{17}$$

and the probability density of arrival of the first particle is

$$P^*(t) = -\frac{d}{dt}\,p_{n,0}(t) = \lambda_2 q_{n-1}(t)\exp\left\{-\lambda_2\int_0^t q_{n-1}(t)\,dt\right\} \tag{18}$$

The probability $P^*(t)$ can be expressed as a function of the total number of particles arriving at compartment n. We shall call this number β and we shall define it as

$$\beta = \lambda_2 \int_0^\infty q_{n-1}(t)\,\mathrm{d}t = \lambda_1 \int_0^\infty q_n(t)\,\mathrm{d}t = \bar{q}_0 \Delta t \frac{\lambda_2^n}{\lambda_1^{n-1}}. \tag{19}$$

With this definition, equation (18) can be written

$$P^*(t) = \lambda_1 \beta \frac{(\lambda_1 t)^{n-2} \mathrm{e}^{-\lambda_1 t}}{(n-2)!} \exp\left\{-\lambda_1 \beta \int_0^t \frac{(\lambda_1 t)^{n-2} \mathrm{e}^{-\lambda_1 t}}{(n-2)!}\,\mathrm{d}t\right\}. \tag{18B}$$

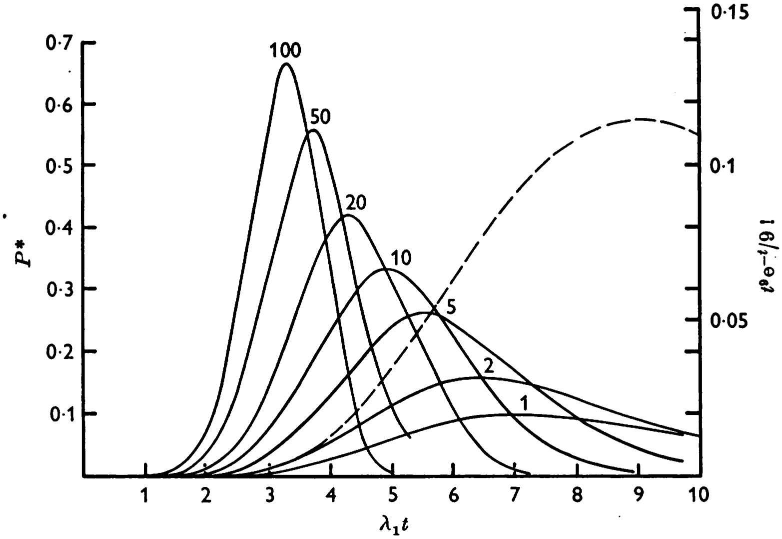

Fig. 4. Probability of arrival of first particle in compartment n when responses are produced by different numbers of particles. Curves calculated from equation (18 C) taking $n = 10$ and $\lambda_1 = 1$. The value of β (the total number of particles arriving at n) is indicated near each curve. The dashed curve is the solution of equation (6) with $n = 10$, $\bar{q}_0 \Delta t / \tau_0 = 1$ and $\lambda_1 = \lambda_2 = 1$. This solution is: $q_{10}(t) = t^9 \mathrm{e}^{-t}/9!$ and describes the average response to one particle in compartment 0 when the multiplication factor F is unity.

Knowing β, the numerical evaluation of this expression can be easily performed noting that, when $\lambda_1 \doteq 1$

$$P^*(t) = \beta \left[\frac{t^{n-2} \mathrm{e}^{-t}}{(n-2)!}\right] \exp\left\{-\beta \left[\sum_{r=n-1}^{\infty} \frac{t^r \mathrm{e}^{-t}}{r!}\right]\right\} \tag{18C}$$

where the expressions in square brackets are found in the tables of the Poisson distribution.

Values of P^* from (18C) for $n = 10$ and for different values of β are plotted in Fig. 4. The curves in this Figure illustrate therefore the latency distributions expected when the total number of particles arriving at the last compartment has the values indicated by the figures near each curve. This number, β, is related to the amplification of the system by the expression:

$$\beta = \frac{\bar{q}_0 \Delta t}{\tau_0} F = \lambda_1 \tau_0 \frac{\bar{q}_0 \Delta t}{\tau_0} G, \quad \text{where} \quad \frac{\bar{q}_0 \Delta t}{\tau_0}$$

is the number of particles introduced in compartment 0, F is the multiplication factor and G is the gain. Therefore, if the input $\bar{q}_0 \Delta t/\tau_0$ is known, it should be possible to estimate the amplification of the system from the latency distribution of responses.

Features of quantal responses in systems with different gain. The relation between the number of particles $q_n(t)$ and the electrical response $V(t)$ is given by equation (7) as $V(t) = \bar{v} q_n(t)$, where $\bar{v}$ defines the voltage change produced by one particle in compartment n. When we consider the number of particles as a random variable $X_n(t)$ the electrical response $\underline{V}(t)$ will also be a random variable defined by $\underline{V}(t) = \bar{v} X_n(t)$. In order to reconstruct these stochastic responses we suppose that the fluctuations of $X_n(t)$ occur only at discrete, equally spaced times of interval $1/\lambda_1$; in this way each particle arriving at n evokes an 'elementary response' of height $\bar{v}$ and of duration $1/\lambda_1$ (the average life of particles in compartment n).

Figure 5 illustrates possible individual responses and their expected averages for inputs consisting of a single particle ($\bar{q}_0 \Delta t/\tau_0 = 1$) and for different amplifications. It has been assumed that $1/\lambda_1 = 1$. Latency distributions have been retraced from Fig. 4 noting that when the input is a single particle, $\beta = F$. It has been mentioned above that the response $\bar{v}/\lambda_1$, evoked by one particle in compartment n, will be called 'elementary response'; the response to injection of Y_0 particles in compartment 0 (corresponding to absorption of one photon) will be called 'quantal response'. A quantal response may be composed of several elementary responses if $F > 1$.

We have then in the plots of Fig. 5 the average form of the response, the latency distribution and the possible organization of individual responses to be expected from systems with different amplification when the input $\bar{q}_0 \Delta t/\tau_0 = 1$. Comparison of the experimental results with the curves of Fig. 5 should give an estimate of the number of particles

$$\beta = \lambda_1 \int_0^\infty q_n(t)\,\mathrm{d}t$$

contributing to the experimental response. If it could be established in addition that the responses considered are evoked by a single particle at the input, the number β would give an estimate of the multiplication factor. In general we would expect that if $F \leqslant 1$, the response to one particle in compartment 0 should be a brief transient appearing with widely variable latency, whereas if $F \gg 1$ the response should resemble the usual generator potentials and latency scatter should be restricted.

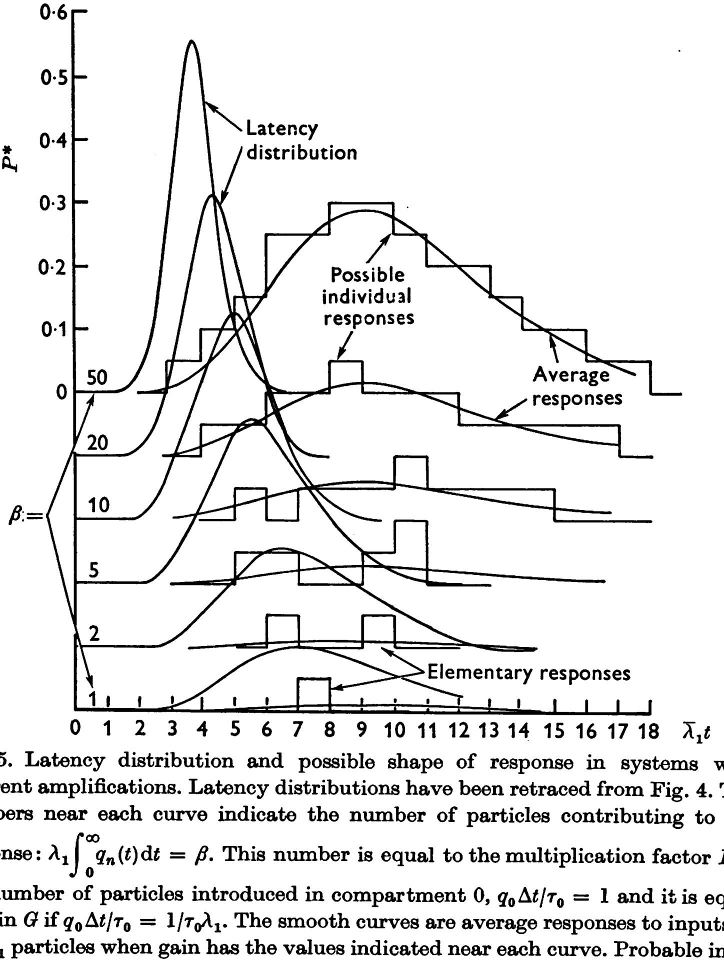

Fig. 5. Latency distribution and possible shape of response in systems with different amplifications. Latency distributions have been retraced from Fig. 4. The numbers near each curve indicate the number of particles contributing to the response: $\lambda_1 \int_0^\infty q_n(t)\,\mathrm{d}t = \beta$. This number is equal to the multiplication factor F if the number of particles introduced in compartment 0, $q_0 \Delta t/\tau_0 = 1$ and it is equal to gain G if $q_0 \Delta t/\tau_0 = 1/\tau_0\lambda_1$. The smooth curves are average responses to inputs of $1/\tau_0\lambda_1$ particles when gain has the values indicated near each curve. Probable individual responses are the function $\underline{V} = \bar{v}X_{10}(t)$ and have been constructed assuming that fluctuations of $X_{10}(t)$ occur only at equally spaced intervals, as explained in the text.

METHODS

The potential changes occurring in visual cells following illumination were led off by means of intracellular micro-electrodes. The general methods used for stimulating and recording have been described in previous articles (Fuortes, 1959*a*; Fuortes & Hodgkin, 1964); in addition a Mnemotron computer (C.A.T.) was used for detecting responses to dim flashes occurring in the presence of the large 'noise' which is usually characteristic of deeply dark-adapted preparations. The output of this computer, giving the average response to the flashes, was recorded on an *x*–*y* plotter. Controls were routinely performed to make sure that the conditions of the preparation were stable while these records were taken.

RESULTS

At first sight, the experimental results seem to favour the view that absorption of a photon elicits the type of response expected from a system without gain. Several years ago, Yeandle (1957) found that dim lights applied to dark-adapted preparations evoke sharp discrete waves (Fig. 6*A* and *B*) which could be produced (according to statistical criteria) each by absorption of a single photon. It was later observed that, for dim illuminations, frequency of occurrence of these discrete waves is linearly related to light intensity and that their latency distribution following flashes is similar to the time course of composite generator potentials (Fuortes & Yeandle, 1964). It seemed reasonable to suggest on the basis of these findings that these discrete waves are unitary responses to single absorbed photons and that the generator potentials evoked by brighter lights result from summation of many discrete waves. However, two observations are difficult to reconcile with the view that Yeandle's discrete waves are the elementary components of generator potentials; firstly, discrete waves disappear during light adaptation (as shown in the experiment of Fig. 6*C*), although it is well known that generator potentials can

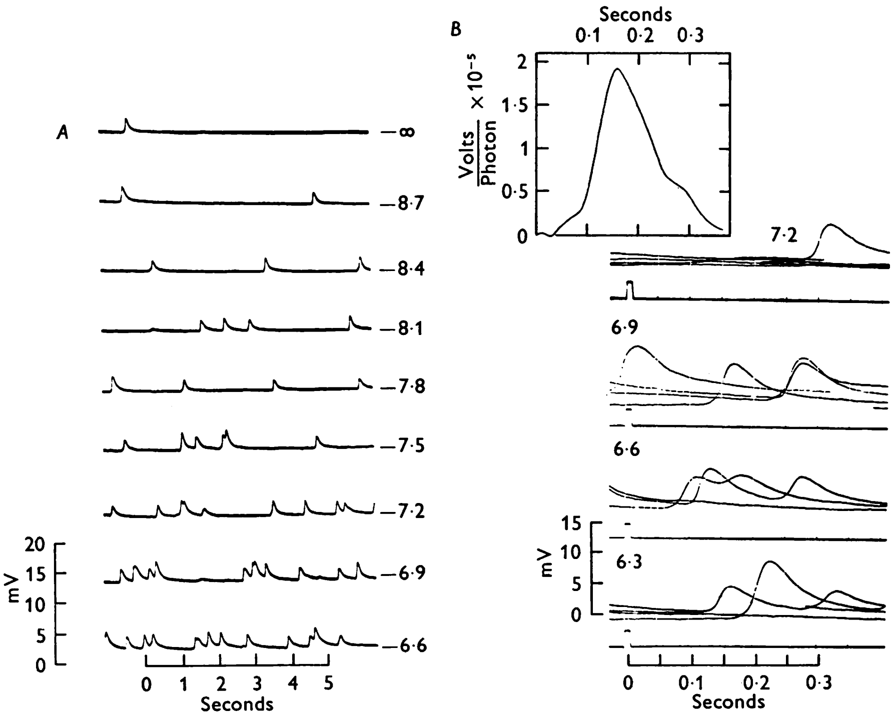

Fig. 6, *A* and *B*. For legend see opposite page.

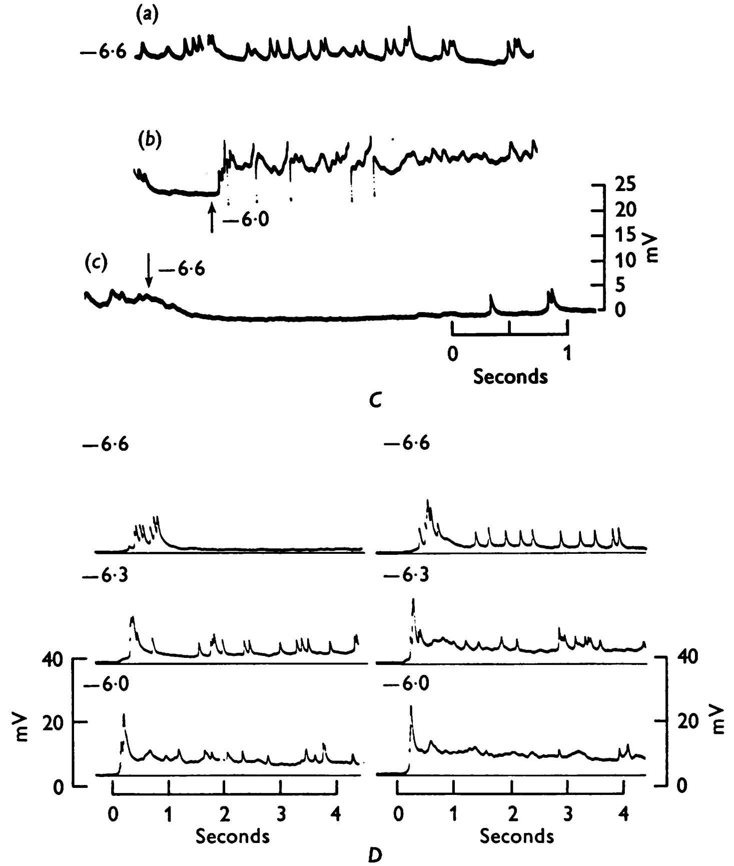

Fig. 6*A*. Discontinuous activity recorded during constant illumination. Occasional discrete waves were recorded from this cell in the absence of light as shown in the top record (−∞). Frequency of discrete waves increased with illumination, as shown in the other records. Figures at right measure intensity of constant light in logarithmic units. Temperature 7° C.

B. Discrete waves recorded following flashes. Numerous discrete waves were present in this unit in darkness. Probability of occurrence of these waves increased transiently following dim flashes. This increase is not apparent in the few samples shown in the illustration but could be demonstrated by summing responses to many flashes, as shown in the inset. Inset: Mnemotron record obtained by summing 512 responses to flashes of intensity −7·2; 256 responses to flashes of intensity −6·9; and 128 responses to flashes of intensity −6·6. Temperature 24° C.

C. Changes elicited by light-adaptation. (*a*) is a record of the activity present during dim, constant illumination; light intensity was increased by a factor of four in (*b*) and returned to the original intensity in (*c*). The discrete waves which appeared after a few seconds of silence in (*c*) had approximately the same size as those recorded in *a*.

D. Activity recorded following steps of light. Steps of light of 5 sec duration and of intensity indicated (in arbitrary logarithmic units) near each record were applied at time 0. Illumination evokes a sustained depolarization with superimposed discrete waves. Frequency of discrete waves is greater at the beginning of the illumination. All records in this figure are from the same unit. The preparation was more deeply dark-adapted when the records on the right-hand side were taken. Temperature 20 °C.

still be evoked in these conditions. In order to interpret this finding it may be suggested that discrete waves do not disappear during light adaptation but rather become too small for detection. However, there is no evidence in the records of Fig. 6*C* to support this view, since the first discrete waves which appear as the preparation recovers (record *c*) have approximately the same size as those present in control conditions (record *a*). These findings are certainly not crucial but they appear to suggest that the principal effect of light adaptation (as produced in this experiment) is to decrease the frequency rather than the size of the discrete waves.

Secondly, it can be shown on occasions that discrete waves evoked by dim illumination are superimposed on a smooth generator potential. This type of result is illustrated in Fig. 6*D* (see also Fuortes, 1959*b*). It is evident in these records that the small sustained depolarization of the two tracings labelled $-6{\cdot}6$ cannot be made up of the relatively large discrete waves recorded from this cell. It seems unlikely, therefore, that generator potentials result necessarily from combination of discrete waves.

In view of these difficulties, it is important to re-examine the features of response to dim flashes.

Linearity of responses to dim light. It has been assumed so far that one absorbed photon is sufficient for eliciting an electrical response. However, since this assumption could not be proved unequivocally in previous research (Fuortes & Yeandle, 1964) it is important to reconsider the question because arguments and conclusions might change if this assumption were not valid. Responses to large number of flashes of different intensities were summed and averaged on a Mnemotron Computer. Figure 7 shows average responses obtained from a cell using three different light intensities. The flashes used to obtain the curve labelled $-7{\cdot}2$ delivered 37·5 absorbable photons in the average. Of these, not more than four passed through the rhabdome where the pigment is located. The optical density of the rhabdome is difficult to estimate, and our attempts to measure the absorption of the visual pigment have not given very precise results. However, it appears from our measurements that in the experimental conditions used in this study (the beam of light was perpendicular to the optical axis of the ommatidium) the rhabdome absorbs less than 10% of the light impinging upon it. Therefore, if four photons are delivered to the rhabdome, less than 0·4 can be absorbed ($K < 0{\cdot}1$). For the other curves ($-6{\cdot}9$ and $-6{\cdot}6$) light intensity was doubled, and the number of stimuli was halved so that a constant total number of photons was delivered in each set. It is seen that the averaged response curves are approximately equal in size and shape. This finding is consistent with the view that single absorbed photons elicit an electrical response, but is inconsistent with the hypothesis that co-operation of two or more photons is required, since in this case the area of the curves should increase with increasing light intensity. The reasons for this can be clarified with reference to Fig. 8 (see also Fuortes & Yeandle, 1964); if each usefully absorbed photon produces a response, then the number of quantal responses should be proportional to number of photons absorbed. Thus, equal total numbers of photons

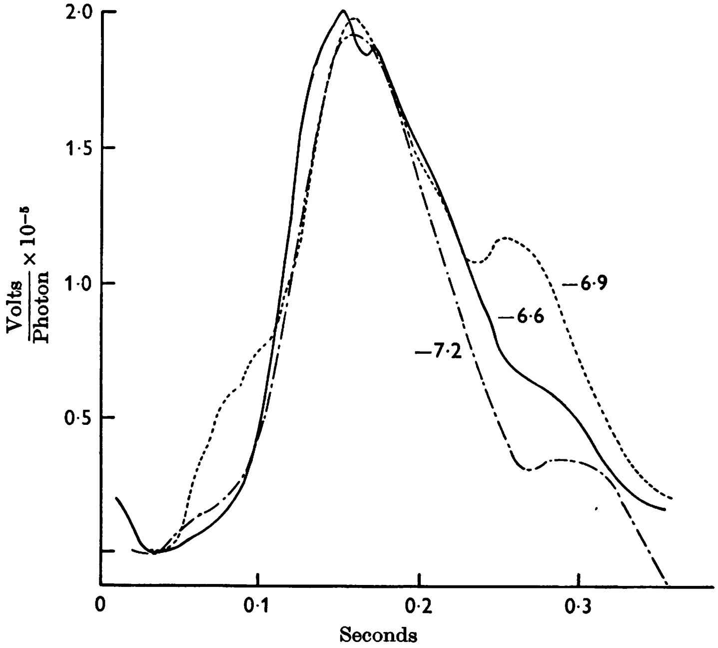

Fig. 7. Average responses to flashes. The Figure shows curves obtained by summing responses to a large number of flashes of three different intensities. The characteristics of the stimulation were as follows:

Light intensity	−7·2	−6·9	−6·6
Number of photons between 4000 Å and 8000 Å in each flash	37·5	75·0	150·0
Number of photons through rhabdome/flash, *M*	3·75	7·5	15·0
Number of photons absorbed in rhabdome/flash, *Q*	$< 0·375$	$< 0·75$	$< 1·5$
Number of flashes delivered	1024	512	256
Total number of photons through rhabdome	3840	3840	3840
Total number of photons absorbed	< 384	< 384	< 384

The ordinate scale was obtained dividing the voltage of the summated response by the total number of photons through the rhabdome. Temperature 24° C.

delivered using flashes of different intensities should produce equal response curves, as is the case in Fig. 7. If instead, a response can occur only if two photons are absorbed during the flash, then one response will be obtained if two or three photons are usefully absorbed; two responses will occur if four of five photons are absorbed, etc. Thus if Q is the average number of photons absorbed per flash, the number of quantal responses would be

$$N_2 = \sum_{r=0}^{\infty} r\left\{\frac{(Y_0Q)^{2r}}{(2r)!} + \frac{(Y_0Q)^{2r+1}}{(2r+1)!}\right\} e^{-Y_0Q} = \tfrac{1}{4}(e^{-2Y_0Q} + 2Y_0Q - 1) \qquad (20)$$

(see Appendix). As shown in Fig. 8, the number of quantal responses would increase more than proportionally to light intensity as long as Q is less than about 1·5. With more photons absorbed, linearity between number of responses and light intensity is predicted even if coincidence of two or more photons is required for one response. It was estimated in the experiment of Fig. 7 that less than 0·4, 0·8, and 1·6 photons per flash were absorbed at the three light intensities used. If this estimate is correct then the area of curve $-6·6$ should be more than twice the area of curve $-7·2$. If this estimate were in error by a factor of four, curve $-6·6$ should still be about 25 % larger than curve $-7·2$. Since this does not seem to be the case, it may be concluded that absorption of a single photon can produce an electrical response, and the curves of Fig. 7 can be regarded as average quantal responses although correction of the voltage scale may be required due to the uncertainty of the value

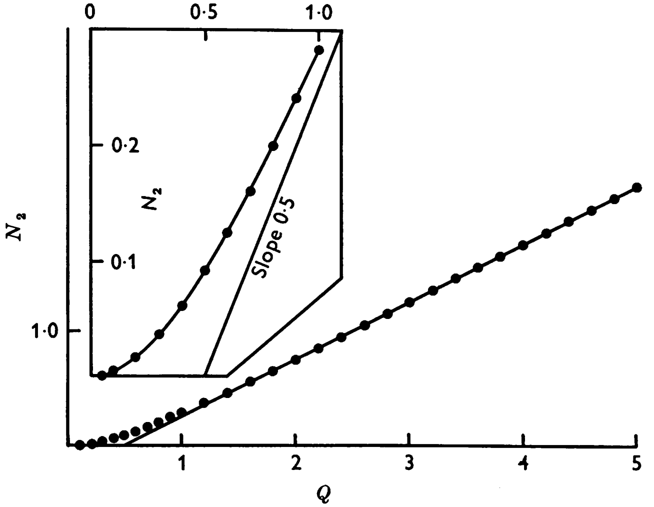

Fig. 8. Relation between number of responses N_2 and number of photons absorbed Q. This figure is constructed assuming that pairs of photons absorbed anywhere in the rhabdome following one flash produce a response, but single photons have no effect. Under these assumptions the average number of responses elicited by one flash (N_2) is defined by equation (20). The relation is appreciably non-linear only if $Q < 1·5$, as seen in greater detail in the inset of the figure.

$$\alpha = KY_0 = \frac{\bar{q}_0\Delta t/\tau_0}{M}$$

(the ratio between number of particles in compartment 0 and incident photons).

Linearity of the summation curves can be demonstrated only over a narrow range of light intensities; with flashes delivering more than 40 photons (of which less than four can be absorbed according to the estimates given above) the size of summated waves evoked by a constant number of photons decreases and their shape changes as illustrated in Fig. 9*A*.

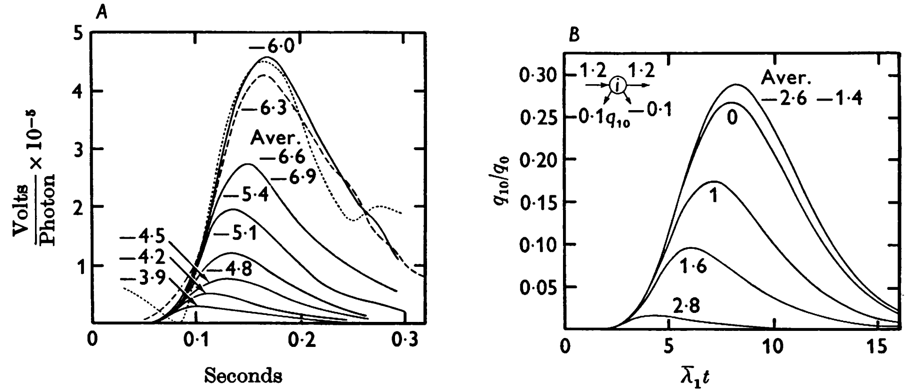

Fig. 9*A*. Summed responses to constant total number of photons and different light intensity. Mnemotron records as in preceding Figures (6*B* and 7). The curves were obtained by summing 2048 responses to flashes of intensity −6·9; 1024 responses to flashes of intensity −6·6, etc. The curves have a common rising phase, but time-to-peak and peak voltage decrease as light intensity is increased. Temperature 25° C.

B. Responses of a chain of ten compartments to impulse inputs of different intensities. The Figure illustrates that the qualitative features of the nonlinearities are similar to those observed experimentally.

These changes are qualitatively similar to those occurring at the output of Hodgkin's model when input intensity is increased, as shown in Fig. 9*B*. It is apparent that significant similarities exist in the properties of the nonlinearities of the summated responses to dim flashes and of the output of the model described by equations (1) and (3).

The relation between area of the average response to one flash and flash intensity is plotted in Fig. 10 for two different units.

The study of average responses described in the preceding section indicates that electrical responses can be elicited by absorption of single photons, but does not reveal whether these responses have the features expected from systems providing gain (such as Hodgkin's model) or from the more extensive class of systems without gain (such as Levinson's model). Analysis of individual responses is required in order to investigate this question.

Classification of discrete waves. Examining individual responses to dim flashes, we have observed that discrete waves often appear to divide into two groups (Adolph, 1964); some are large and fast, others are smaller and slower. In different preparations either the large or the small waves may predominate so that different amplitude histograms may be obtained (Fig. 11). When amplitude distribution is not clearly bimodal the two types of waves may be separated by plotting peak height as a function of time of rise, as shown in Fig. 12. Moreover, it can be observed in the records of Figs. 13 and 14 that fluctuations of latency are greater for the large than for the small waves. One may then conclude that discrete waves can often be classified in two groups defined by the following properties: group I—small, slow and synchronous (S-waves) and group II—large, fast and asynchronous (L-waves). The two types of discrete waves can be demonstrated more easily using flashes of moderate intensity; with brighter flashes the dispersion of the larger waves decreases and with still

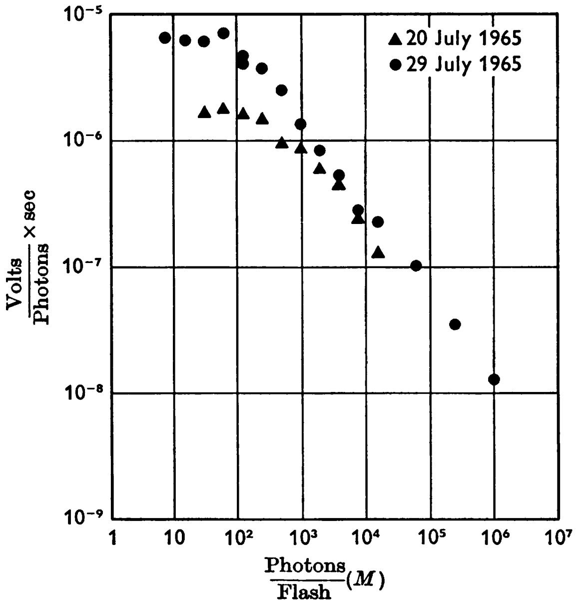

Fig. 10. Relation between area of average responses to flashes and light intensity. Summed responses as illustrated in Figs. 6*B* (inset) and 7 were elicited by flashes of different intensities and their normalized area was plotted as a function of the number of absorbable photons in each flash (M). Units in ordinate are: Area of response/Total number of absorbable photons ($M \times$ number of flashes). With this type of plot area of response is constant as long as the responses to individual photons do not interact. Constancy of response can be demonstrated in practice only over a narrow limit of light intensity but presumably it extends indefinitely towards the left-hand side of the plot.

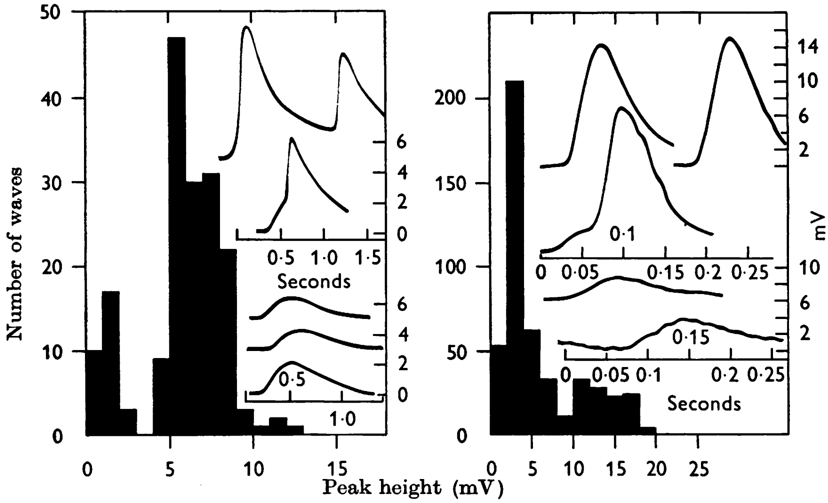

Fig. 11. Amplitude histogram of discrete waves. The tracings show examples of waves recorded in darkness or following illumination from two cells. The larger and faster waves often start from a slower depolarization, similar to the rising phase of the slower waves (see Adolph, 1964, fig. 9). The histograms indicate that the waves can sometimes be divided into two groups with respect to peak amplitude. Temperature: Left, 7° C; Right 25 °C.

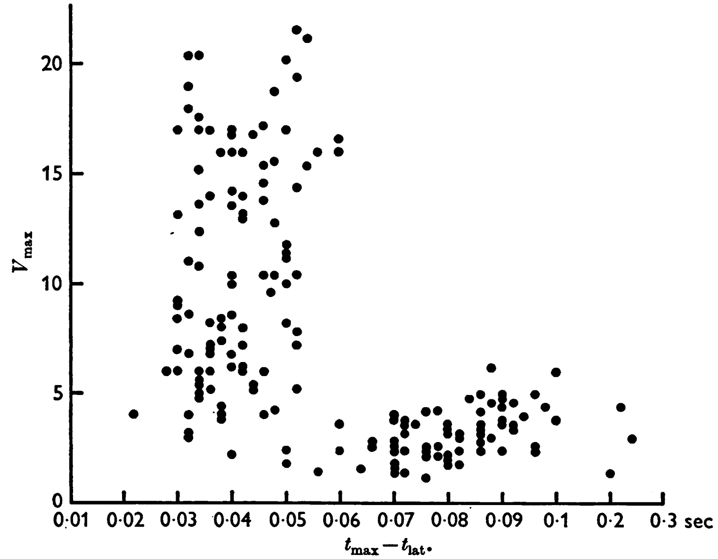

Fig. 12. Relation between amplitude and time of rise of discrete waves. Discrete waves were evoked by flashes of different intensities and peak amplitude of each wave was plotted as a function of its rise time ($t_{max}-t_{lat}$). It is apparent that two populations of waves are included in this plot. In this experiment, waves with rise time longer than 0·06 sec and peak height of less than 6 mV were classified as S-waves, while waves with rise time shorter than 0·06 sec and peak height greater than 6 mV were classified as L-waves. The remaining waves were discarded because their classification was considered uncertain. Temperature 25° C.

higher intensities S- and L-waves fuse and the responses cannot be decomposed into discrete components. Strong non-linear effects become apparent at this stage. These features of responses to flashes of increasing intensities are illustrated in Fig. 13.

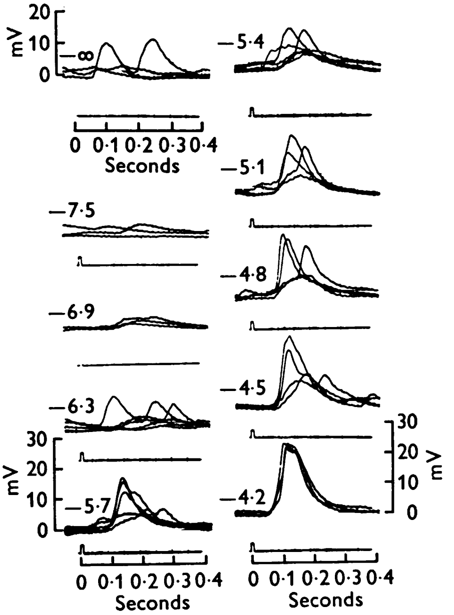

Fig. 13. Waves recorded following flashes. Superimposed sweeps of responses to flashes of intensity indicated near each record. Both slow and fast waves were present in darkness. Following flashes, the slow waves tend to superimpose better than the faster transients. Temperature 25 °C.

In some cells these two groups of discrete waves cannot be recognized, apparently because S-waves are too small to be detected when they appear singly. In these cases, however, it is usually possible to demonstrate them by superposing several responses to flashes. Small superimposed waves can be seen in Fig. 14 (attenuation 6·0); with this intensity every flash evokes a small wave at a fixed time after the stimulus. With dimmer flashes small waves appear occasionally at the appropriate time after the flash, but they are too small to be identified with confidence.

Latency distribution of waves evoked by flashes. Latency distribution of S- and L-waves is plotted in Fig. 15 together with theoretical curves computed from equation (18C). It is seen that the experimental measurements fit the theoretical curves only very roughly. We see, however, that the distribution of S-waves is similar to that predicted in a chain of ten compartments following arrival of about twenty-five particles in the last

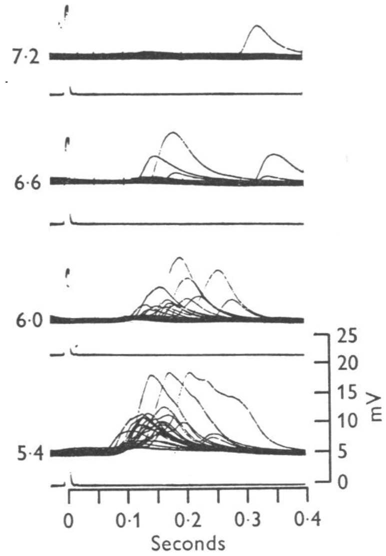

Fig. 14. Discrete waves evoked by flashes. In this unit, only few waves were present in the absence of illumination. Flashes evoked small superimposed waves starting with a latency of about 80 msec, and larger waves of variable latency. The superimposed waves are interpreted as small S-waves. Temperature 24° C.

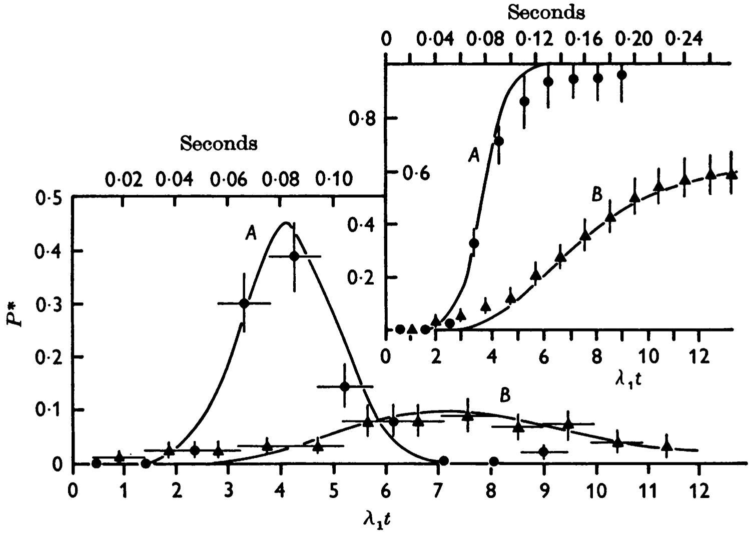

Fig. 15. Latency distribution of discrete waves evoked by flashes. Discrete waves recorded following flashes were classified in the two groups, defined in text. Measurements on S-waves are given by circles and on L-waves by triangles. Probability of occurrence of waves of each group at given times after the flash was plotted after subtracting the probability that the same type of wave occurred in darkness. Solid lines are the function P^* for $\lambda_1 = 1$ and $\beta = 25$ (curve A) or $\beta = 1$ (curve B). *Inset:* Same data plotted as the cumulative distribution of latency of the two groups of waves. The theoretical curves are the integrals of the corresponding curves in the main figure. Temperature 25 °C.

compartment while the distribution of L-waves resembles that expected if only one particle arrived at the last compartment following one flash (see Fig. 4).

A different plot of the same data is shown in the inset of Fig. 15 where the percentage of responses occurring within the time t is compared to the expected integral distribution given by:

$$\int_0^t P^*(t)\,\mathrm{d}t = 1 - p_{n,0}(t) \tag{21}$$

In this plot, the points relating to S-waves fit the theoretical curve reasonably well, but the measurements of latency of L-waves show a vertical displacement. This discrepancy is due to the contribution of the first four points which remained above zero after subtraction of the average resting frequency. It is difficult to say whether this deviation should be ascribed to chance fluctuations of the noise or whether it should be taken to reveal a real inconsistency of the model. However, it seems reasonable to conclude that the restricted latency scatter of S-waves in this experiment indicates that they are produced not by a single event but by combination of several events and we shall assume that each S-wave was due to arrival of about twenty-five particles at the nth compartment.

Relation between failures and light intensity. It becomes important at this point to determine the value of the input $\bar{q}_0 \Delta t/\tau_0$ in this same experiment, and we shall attempt to estimate it from measurement of the number of photons in the flash and from the relation between this number and the probability of obtaining or not obtaining a response. No response will be obtained in the model if no particle arrives at the last compartment. This will occur (1) if the flash fails to produce particles in compartment 0; this probability is $\exp\{-Y_0 Q\}$where $Y_0 Q$ is the average number of particles introduced by one flash; (2) if particles are produced but they all decay before reaching the last compartment. As explained in Appendix, the total probability that no particle arrives at the last compartment when the average number of photons absorbed per flash is Q, is

$$P_0 = \exp\left\{-Y_0 Q\{1 - \mathrm{e}^{-\gamma(\lambda_2/\lambda_1)^{n-1}}\}\right\}, \text{ where } \gamma = \lambda_2 \tau_0. \tag{22}$$

In the experiment of Fig. 15, the number of photons delivered by each flash, M, was approximately 24; since according to our optical measurements the ratio between absorbed and delivered photons is less than 0·1, we can take as a maximum value: $Q = 2{\cdot}4$. The number of particles introduced in compartment 0 by the flash will then be at the most: $Y_0 Q = 2{\cdot}4 Y_0$. It was concluded on the basis of latency distribution that S-waves evoked by this input were due to about twenty-five particles in the last compartment. Thus we can write

$$\lambda_2 \int_0^\infty q_{n-1}(t)\,\mathrm{d}t = 2{\cdot}4\gamma Y_0 \left(\frac{\lambda_2}{\lambda_1}\right)^{n-1} = 25 \tag{23}$$

(see Appendix, formula (32*A*)).

Taking $n = 10$, we have

$$\gamma \left(\frac{\lambda_2}{\lambda_1}\right)^9 = \frac{25}{2{\cdot}4 Y_0}$$

and substituting this value in formula (22) we obtain

$$P_0 = \exp\left\{-2{\cdot}4\,Y_0\{1 - \mathrm{e}^{-25/2\cdot 4Y_0}\}\right\} \tag{22B}$$

If $Y_0 \leqslant 1$ as we have assumed, $\mathrm{e}^{-25/2\cdot 4Y_0} \ll 1$ and we have with good approximation:

$$P_0 = \mathrm{e}^{-2\cdot 4Y_0} \tag{24}$$

Since we consider only linear responses, this conclusion can be generalized for any value of Q and we obtain

$$P_0(Q) = \mathrm{e}^{-Y_0 Q} = \mathrm{e}^{-u} \tag{25}$$

This means that if the multiplication factor of the chain

$$F = \lambda_1 \tau_0 \left(\frac{\lambda_2}{\lambda_1}\right)^n = \gamma \left(\frac{\lambda_2}{\lambda_1}\right)^{n-1}$$

is large, then the probability of failure is controlled by the probability that no particle enters compartment 0 following a flash.

Figure 16 is a plot of the experimental probabilities of failure for different values of M, while the curve through the experimental points is the theoretical function $P_0(Q)$. It is seen that an acceptable fit is obtained for $u = KY_0M = 0{\cdot}01\,M$, giving $KY_0 = 0{\cdot}01$. This fit suggests that in this experiment about one in a hundred photons through the rhabdome produced one particle in compartment 0; thus, since 24 photons were delivered with each flash, 0·24 particles/flash were produced in the average. We can conclude from this that the large majority of responses observed were elicited by a single particle at the input and thus that the multiplication factor F was about 25. It should be noted that this value is obtained without taking into account possible fluctuations of the parameters λ_1 and λ_2. If such fluctuations occur the observed latency distribution would be consistent with a larger value of F.

This evidence suggests that S-waves arise as a consequence of absorption of single photons but are brought about by the combined action of many events, interpreted as the arrival of particles in the last compartment. According to this point of view, S-waves are essentially identical to the generator potentials evoked by flashes delivering many photons.

Origin of L-waves. L-waves have instead the features which one would expect from a system with $F = 1$. Figure 15 shows that their latency distribution is similar to the probability of arrival of the first particle in the last compartment when their average number β is unity. In order to explain production of two types of waves with different properties one may assume that one photon can activate one channel giving multiplication of particles and producing the S-waves and another non-multiplying channel giving origin to the L-waves. However, it may be simpler to assume that both S- and L-waves originate from a single channel. For instance, one could assume that a photon activates only one multiplying channel; one particle in compartment 0 will then produce several particles in n. Each of these particles has a small probability H of producing an L-wave and a much larger probability $(1-H)$ of producing a small voltage change lasting a brief time $1/\lambda_1$.

In these conditions, the average number of particles arriving at the nth compartment following a flash Q will be

$$\lambda_2\int_0^\infty q_{n-1}(t)\,\mathrm{d}t = Y_0QF$$

and the average number of L-waves will be $u' = HY_0QF$. The probability P'_0 that no L-wave is generated will then be

$$P'_0(Q) = \mathrm{e}^{-HY_0QF} = \mathrm{e}^{-u'} \tag{26}$$

Figure 16 shows how this curve fits the experimental measurements of probability of failure of L-waves. This fit indicates that $u' = 1$ when $M = Q/K = 500$. We have then: $1 = 500KY_0FH$. Using the values determined in the previous section: $KY_0 = 0{\cdot}01$; $F = 25$. We obtain

$$H = \frac{1}{500\times 0{\cdot}01\times 25} = 0{\cdot}008.$$

Based on these conclusions, we can propose the following scheme: each usefully absorbed photon gives one particle in compartment 0; particles multiply along the chain and one particle at the input produces on average twenty-five particles at the output. Each particle at the output produces a small (elementary) voltage change $\bar{v}/\lambda_1$ and the summation of the twenty-five elementary responses produced by absorption of a single photon makes up an S-wave. In about one case in one hundred, a particle in the last compartment does not produce a small response $\bar{v}/\lambda_1$ but evokes instead a large effect resulting in an L-wave. L-waves will then appear with a probability proportional to the number of particles in the last compartment and, therefore, proportional to the instantaneous height of the S-wave.

Convolution of S- and L-waves. If the hypothesis proposed above is essentially correct, it should be possible to reconstruct the average responses to flashes by summation of *S*- and *L*-waves taking into account shape, frequency of occurrence and latency distribution of these waves. This reconstruction was performed as follows: several records of *S*-waves were averaged and this average $\bar{v}_S(t)$ was normalized to unit area; similarly, several records of *L*-waves were superimposed to obtain an average *L*-wave, $\bar{v}_L(t)$. According to the model advanced in the previous section, $\bar{v}_S(t)$ describes the latency distribution of *L*-waves. The contribution of both types of waves to the average response to a flash should then be given by the convolution of their average shape with their latency distribution. This convolution is described by:

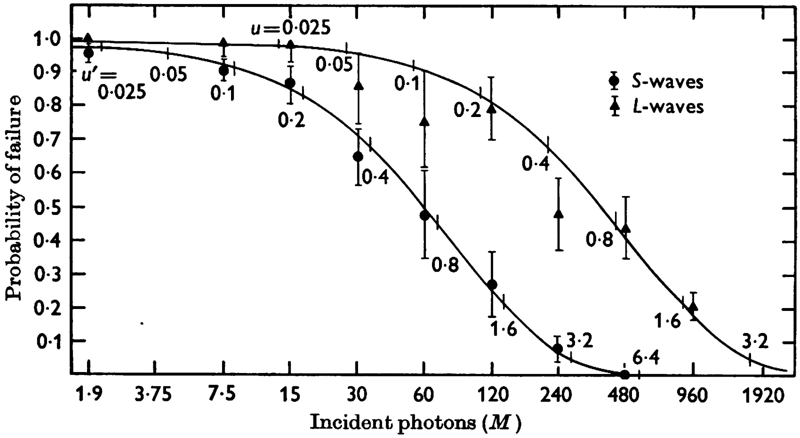

Fig. 16. Probability that *S*-waves or *L*-waves do not occur following flashes of different intensities. Experimental measurements of probability of failure of *S*- and *L*-waves were fitted to the curves $P_0(Q)$ and $P'_0(Q)$ given by equations (25) and (26) respectively. As explained in text, the ratios u/M and u'/M should give an estimate of both quantities KY_0 (the probability that an incident photon produces a particle in compartment 0) and H (the probability that a particle in the *n*th compartment produces an *L*-wave). Data were taken from the cell which gave the latency distribution curves of Fig. 15. Temperature 25° C.

$$W_L(t) = \int_0^\infty \bar{v}_s(\tau)\bar{v}_L(t-\tau)\,d\tau. \tag{27}$$

In the experiment considered, N stimuli evoked N_S *S*-waves and N_L *L*-waves, and the area of the *S*-wave was a in the average. The average response to one stimulus could then be predicted to be

$$V(t) = \frac{N_S}{N} a\bar{v}_S(t) + \frac{N_L}{N} W_L(t). \tag{28}$$

The computation of this expression is shown in the dotted curve of Fig. 17, superposed to experimental responses averaged by a Mnemotron computer. This comparison reveals good agreement of theoretical and experimental curves, and therefore it supports the notions proposed above.

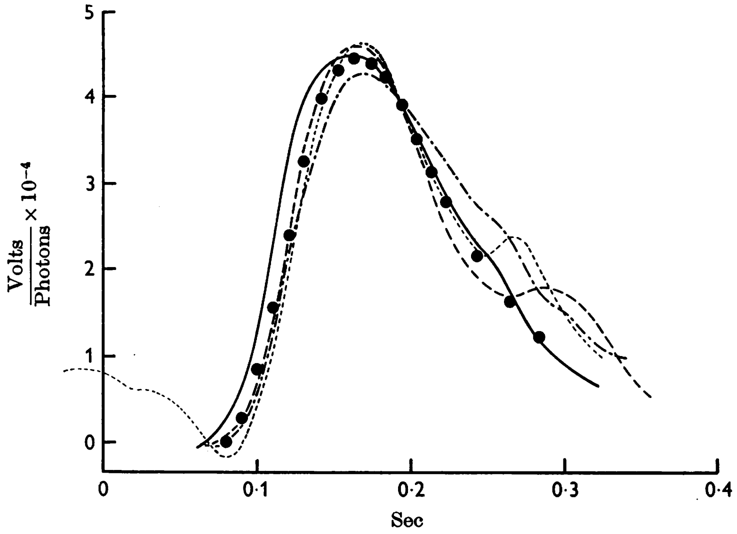

Fig. 17. Comparison of theoretical and experimental average responses to flashes. Average responses to dim flashes were recorded on a Mnemotron computer and the curves obtained following flashes of four different intensities are shown. The heavy dots are computed from expression (28), assuming that latency scatter of *S*-waves is negligible and probability of occurrence of *L*-waves is proportional to the time course of *S*-waves. The reconstruction is based on the experimental values of shape and frequency of occurrence of the discrete waves of the two groups.

DISCUSSION

We conclude from these results that responses to flashes delivering only few photons have the properties expected if they are brought about by processes supplying some form of amplification. Although we do not know what processes correspond to the components and parameters of this model, a chemical hypothesis may be proposed as an example. It may be thought (as already suggested by Wald, 1956) that each photon absorbed in the rhabdome activates a molecule of visual pigment with the probability Y_0; each activated pigment molecule (E_0) reacts with a substrate to produce on average q_1 particles of a new substance E_1. E_1, in turn, reacts with another substrate forming a product E_2 and so on for n steps until $q_n (q_n \gg 1)$ particles of substance E_n are generated. Each particle of E_n combining with the membrane of the visual cell produces an elementary response consisting of a brief and minute depolarization. The sum of q_n elementary responses is the average response to one photon or 'quantal response'. These quantal responses are the slow S-waves described in this paper. These events occur both with dim and bright illumination; however, the substance E_n increases the rate of decay of all particles in accordance with the relation

$$\lambda_1 = \bar{\lambda}_1\left(1+\frac{q_n}{w}\right) \tag{29}$$

(see equation (3)). This relation is responsible for the non-linearities observed following brighter illumination and described in a previous article (Fuortes & Hodgkin, 1964) as well as in the present study. If the ratio $\bar{q}_0\Delta t/M\tau_0$ is indeed about 0·01 (see p. 16), then the non-linearities shown in Fig. 10 become appreciable as soon as the average number of photons absorbed in each flash exceeds one.

S-waves are regarded therefore as small generator potentials since both unitary S-waves and composite generator potentials are supposed to originate from combination of many undetectably small responses.

L-waves are considered in this interpretation to be accessory events. Their general features can be explained assuming that they originate due to a large amplification of a minority of elementary responses; the majority of E_n particles produce small elementary responses but an occasional particle generates instead a large response or L-wave. Since both S- and L-waves can be present in darkness one must assume that both E_0 and E_n particles may exist in the absence of illumination.

When L-waves occur, they will contribute to the shape and features of visual responses, but if they are abolished (as it may be the case during light adaptation) generator potentials can still be produced by the more usual small elementary responses.

It should be stated, however, that different processes such as transport of particles or states would be equally consistent with the experimental results, provided that these processes include some mechanism supplying amplification, as required for consistency with the findings described in this paper.

APPENDIX

Amplification chain as a stochastic process

The differential equations for the probability functions $P_{i,x}(t)$ at time t can be obtained by computing the probability $P_{i,x}(t+\mathrm{d}t)$ for the number x of particles in the compartment i at the later time $t+\mathrm{d}t$.

This probability is given by the sum of: (1) the probability $P_{i,x}(t)$ that the number of particles $X_i(t)$ is already x at time t, multiplied by the probability that it does not change during the interval $\mathrm{d}t$; and (2) the probability that the number $X_i(t)$ being $y \neq x$ at time t changes during $\mathrm{d}t$ by the right amount to become x.

The leakage mechanism gives a probability $\lambda_1 \mathrm{d}t$ that one of the x particles disappears during the interval $\mathrm{d}t$; the probability that two particles disappear during $\mathrm{d}t$ is proportional to $(\mathrm{d}t)^2$ and can be ignored.

The second mechanism for changing the number of particles in compartment i is given by the probability $\lambda_2 \mathrm{d}t$ that any one particle in compartment $i-1$ generates a new particle in compartment i. Thus, the probability that one particle is added to compartment i during the interval $\mathrm{d}t$, when the number of particles in compartment $i-1$ is y, is $\lambda_2 y \mathrm{d}t$.

Since y can assume all the values $y = 0,1,2,\ldots$, the probability that $X_i(t)$ increases by one particle in compartment i during $\mathrm{d}t$ is given by:

$$\sum_{y=0}^{\infty} P_{i-1,y}(t)\lambda_2 y\,\mathrm{d}t.$$

The probability that more than one particle is added to compartment i during the interval $\mathrm{d}t$ can be ignored for the reasons mentioned above.

We have therefore

$$P_{i,x}(t+\mathrm{d}t) = P_{i,x}(t)[1-\lambda_1 x\,\mathrm{d}t - \sum_{y=0}^{\infty} P_{i-1,y}(t)\lambda_2 y\,\mathrm{d}t] + P_{i,x+1}(t)\lambda_1(x+1)\,\mathrm{d}t + P_{i,x-1}(t)\sum_{y=0}^{\infty} P_{i-1,y}(t)\lambda_2 y\,\mathrm{d}t$$

and we can write

$$\frac{\mathrm{d}}{\mathrm{d}t}P_{i,x} = -\lambda_1 x P_{i,x} - \lambda_2 P_{i,x}\sum_{y=0}^{\infty} P_{i-1,y}\,y + \lambda_1(x+1)P_{i,x+1} + \lambda_2 P_{i,x-1}\sum_{y=0}^{\infty} P_{i-1,y}\,y. \quad (1A)$$

The sum in the right-hand side of equation $(1A)$ represents the average number of particles in each compartment

$$m_i(t) = \sum_{x=0}^{\infty} P_{i,x}(t)\,x \quad (2A)$$

so we can write

$$\frac{\mathrm{d}}{\mathrm{d}t}P_{i,x} = -(\lambda_1 x + \lambda_2 m_{i-1})P_{i,x} + \lambda_1(x+1)P_{i,x+1} + \lambda_2 m_{i-1} P_{i,x-1}. \quad (3A)$$

The system of infinite equations $(3A)$ gives the probability functions $P_{i,x}(t)$ describing the stochastic properties of our amplification chain and we look for the solution with the initial conditions:

$$\left.\begin{aligned} P_{1,x}(0) &= \begin{cases} 1 & \text{if } x = m, \\ 0 & \text{if } x \neq m; \end{cases} \\ P_{i,x}(0) &= 0 \quad \text{for} \begin{cases} i = 2, 3, \ldots, n, \\ x = 0, 1, 2, \ldots; \end{cases} \end{aligned}\right\} \quad (4A)$$

corresponding to the injection of exactly m particles at time $t = 0$ in the first compartment. We show first that the average number $m_i(t)$ is the same as the quantities $q_i(t)$ of equations (5) of the text. In order to demonstrate this we multiply equation (3A) by x and sum over x, obtaining:

$$\begin{aligned}\frac{dm_i}{dt} &= -\lambda_1\mu_i - \lambda_2 m_{i-1}m_i + \lambda_1(\mu_i - m_i) + \lambda_2 m_{i-1}(m_{i+1}) \\ &= \lambda_1 m_i + \lambda_2 m_{i-1}, \end{aligned} \tag{5A}$$

where

$$\mu_i(t) = \sum_{x=0}^{\infty} P_{i,x}x^2 \tag{6A}$$

are the second moments of the distributions and we made use of:

$$\left.\begin{aligned}\sum_{x=0}^{\infty} P_{i,x+1}x(x+1) &= \sum_{x=0}^{\infty} P_{i,x}(x-1)x = \mu_i - m_i \\ \sum_{x=0}^{\infty} P_{i,x-1}x &= \sum_{x=0}^{\infty} P_{i,x}(x+1) = m_i + 1\end{aligned}\right\} \tag{7A}$$

taking into account that $P_{i,x} = 0$ for $x < 0$ and

$$\sum_{x=0}^{\infty} P_{i,x}(t) = 1. \tag{8A}$$

The initial values (4A) imply

$$m_1(0) = m; \quad m_2(0) = m_3(0) = \ldots = m_n(0) = 0. \tag{9A}$$

Comparing equations (5A), (9A) with equations (5, text), we conclude that the solutions $q_i(t)$ of the deterministic model are identical with the averages $m_i(t)$ computed from the stochastic treatment of the same process, if we put $m = \lambda_2\bar{q}_0\Delta t$; therefore, we have

$$m_i(t) = q_i(t) = m(\lambda_2 t)^{i-1}\frac{e^{-\lambda_1 t}}{(i-1)!}. \tag{10A}$$

To solve equations (3A) we introduce the probability generating functions

$$F_i(s,t) = \sum_{x=0}^{\infty} P_{i,x}(t)s^x \tag{11A}$$

defined for $-1 \leqslant s \leqslant 1$ and for $t \geqslant 0$.

Multiplying equation (3A) by s^x and summing over x, we obtain the partial differential equations for F_i

$$\frac{\partial F_i}{\partial t} = \lambda_1(1-s)\frac{\partial F_i}{\partial s} - \lambda_2 q_{i-1}(t)(1-s)F_i \tag{12A}$$

that must be solved with the initial and boundary values:

$$\text{for}\quad t = 0: F_i(s, 0) = 1 \quad (i > 1),$$

$$F_i(s, 0) = s^m \tag{13A}$$

$$\text{for}\quad s = 1: F_i(1, t) = \sum_{x=0}^{\infty} P_{i,x}(t) = 1 \tag{14A}$$

which are deduced from (14A) and (8A). For $i = 1$, we must put in (13) $q_0(t) = 0$ (or $\lambda_2 = 0$).

To solve equation (12A) we define

$$\theta = \lambda_1 t; \quad \xi = \ln(1-s); \quad \psi_i = \ln F_i. \tag{15A}$$

Using expression (10A) for $q_i(t)$, we obtain

$$\frac{\partial \psi_i}{\partial \theta} + \frac{\partial \psi_i}{\partial \xi} = A_i \theta^{i-2} e^{\xi-\theta}, \tag{16A}$$

where

$$A_1 = 0; \quad A_i = \left(\frac{\lambda_2}{\lambda_1}\right)^{i-1} \frac{m}{(i-2)!} \quad \text{for} \quad i > 1. \tag{17A}$$

Equation (16A) has the general solution, for $i > 1$

$$\psi_i = A_i e^{\xi-\theta}\left(\frac{\theta^{i-1}}{i-1} + C_0\right) = (s-1)q_i(t) + C_1 e^{-\lambda_1 t}, \tag{18A}$$

where C_0, C_1' are arbitrary constants. The boundary condition (14A) is satisfied; to satisfy the initial conditions (13A) we must take $C_1' = 0$ and we obtain

$$F_i(s, t) = \exp\{(s-1)q_i(t)\}. \tag{19A}$$

We obtain in this way the probability generating functions for a Poisson process with intensity $q_i(t)$ *dependent* on time. We can write, therefore, the probability functions, for $i > 1$

$$P_{i,x}(t) = \frac{\{q_i(t)\}^x e^{-q_i(t)}}{x!}. \tag{20A}$$

For the first compartment ($i = 1$) equation (16A), considering (17A) can be written

$$\frac{\partial F_i}{\partial \theta} + \frac{\partial F_1}{\partial \xi} = 0 \tag{21A}$$

with the general solution

$$F_1(\theta, \xi) = \phi(\theta - \xi) \tag{22A}$$

where $\phi(x)$ is an arbitrary function. For $t = 0$ we have using equations (13A), (14A), (15A)

$$F_1(0, \xi) = \phi(-\xi) = s^m = (1 - e^{\xi})^m \tag{23A}$$

therefore

$$\phi(x) = (1-e^{-x})^m. \tag{24A}$$

We obtain in conclusion

$$F_1(s,t) = (1-e^{\xi-\theta})^m = [1-(1-s)e^{-\lambda_1 t}]^m \tag{25A}$$

that is the probability generating function of a binomial distribution with probability of success $e^{-\lambda_1 t}$. The probability functions are therefore given by

$$P_{1,x}(t) = \frac{1}{x!}\left(\frac{\partial^x F_1}{\partial s^x}\right) = \binom{m}{x}[1-e^{-\lambda_1 t}]^{m-x}e^{-x\lambda_1 t} \tag{26A}$$

for $x \leqslant m$; for $x > m$, $P_{1,x}(t) = 0$.

We consider now the (fundamental) solution for the case $m = 1$, that is one particle injected in the first compartment. We have in the first compartment

$$\left.\begin{aligned} P_{1,0}(t) &= 1-e^{-\lambda_1 t},\\ P_{1,1}(t) &= e^{-\lambda_1 t},\\ P_{1,2}(t) &= P_{1,3}(t) = \ldots = 0.\end{aligned}\right\} \tag{27A}$$

For the last compartment we have

$$P_{n,x}(t) = \frac{\{q_n(t)\}^x e^{-q_n(t)}}{x!}. \tag{28A}$$

Number of responses occurring if two photons are required for each response

If QY_0 is the average number of photons usefully absorbed per flash, the number of photons in each flash will have a Poisson distribution around the average QY_0. Therefore the probability to have m photons will be

$$(QY_0)^m(e^{-QY_0})/m$$

To obtain r responses, the number of photons must be $2r$ or $2r+1$; therefore the average number of responses per flash is given by

$$N_2 = \sum_{r=0}^{\infty} r\left\{\frac{(QY_0)^{2r}}{(2r)!}+\frac{(QY_0)^{2r+1}}{(2r+1)!}\right\}e^{-QY_0}$$

as already mentioned in the text. This equation can be written

$$\begin{aligned} N_2 &= e^{-QY_0}\left\{\frac{QY_0}{2}\frac{d}{d(QY_0)}\left(\sum_{r=0}^{\infty}\frac{(QY_0)^{2r}}{(2r)!}\right)+\frac{(QY_0)^2}{2}\frac{d}{d(QY_0)}\left\{\frac{1}{QY_0}\sum_{r=0}^{\infty}\frac{(QY_0)^{2r+1}}{(2r+1)!}\right\}\right\}\\ &= \tfrac{1}{2}QY_0e^{-QY_0}\left\{\frac{d}{d(QY_0)}\cosh(QY_0)+QY_0\frac{d}{d(QY_0)}\frac{\sinh(QY_0)}{(QY_0)}\right\}. \end{aligned} \tag{29A}$$

From this we obtain

$$N_2 = \frac{QY_0}{2}-\frac{1}{4}+\frac{1}{4}e^{-2QY_0}. \tag{30A}$$

Probability of failure in the stochastic chain

The average number of particles arriving at compartment n from time 0 to ∞ is given by

$$\lambda_2 \int_0^\infty q_{n-1}^{(m)}(t)\,dt,$$

where $q_{n-1}^{(m)}$ is the average number of particles in compartment $n-1$ when m particles are injected at time 0 in compartment zero. The actual number of particles $X_{n-1}(t)$ at a given time t fluctuates around the average $q_{n-1}^{(m)}(t)$ with a Poisson distribution. It can be shown that also the integral

$$\int_0^\infty X_{n-1}(t)\,dt$$

is a Poissonian random variable with average value $\int_0^\infty q_{n-1}^{(m)}(t)\,dt$. Therefore the probability to have no particles in compartment n from zero time to infinity when m particles are initially at compartment zero is

$$\exp\left\{-\lambda_2 \int_0^\infty q_{n-1}^{(m)}(t)\,dt\right\}.$$

Compounding this probability with the probability to have m particles in compartment zero when their average number is $Y_0 Q$, we obtain for the total probability P_0 to have no particles in compartment n

$$P_0 = \sum_{m=0}^{\infty} \frac{(Y_0 Q)^m}{m!} \exp(-Y_0 Q) \exp\{-\lambda_2 \int_0^\infty q_{n-1}^{(m)}(t)\,dt\} \quad (m = 0, 1, 2, \ldots). \tag{31A}$$

Using the relation

$$\lambda_2 \int_0^\infty q_{n-1}^{(m)}(t)\,dt = m\lambda_2 \tau_0 \left(\frac{\lambda_2}{\lambda_1}\right)^{n-1} \tag{32A}$$

and defining $\lambda_2 \tau_0 = \gamma$ we have

$$\begin{aligned} P_0 &= \exp(-Y_0 Q) \sum_{m=0}^{\infty} \frac{(Y_0 Q)^m}{m!} \exp\{-\gamma m (\lambda_2/\lambda_1)^{n-1}\} \\ &= \exp(-Y_0 Q) \sum_{m=0}^{\infty} \frac{\{Y_0 Q \exp[-\gamma(\lambda_2/\lambda_1)^{n-1}]\}^m}{m!} \\ &= \exp(-Y_0 Q) \exp\{Y_0 Q \exp[-\gamma(\lambda_2/\lambda_1)^{n-1}]\} \\ &= \exp\left\{(-Y_0 Q)\{1 - \exp[-\gamma(\lambda_2/\lambda_1)^{n-1}]\}\right\}. \end{aligned} \tag{33A}$$

The second term in the exponent describes the fluctuations along the chain. If $\gamma(\lambda_2/\lambda_1)^{n-1} \gg 1$ then probability of failure is controlled only by the fluctuations at the input and we have

$$P_0 = \exp(-Y_0 Q). \tag{34A}$$

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