RETINAL ACTION POTENTIAL — THEORY AND EXPERIMENTAL RESULTS FOR GRASSHOPPER EYES[1]

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THREE FIGURES

INTRODUCTION

When photoreceptors are illuminated a sequence of measurable changes occur which are instrumental in mediating the sense of vision. These changes are: (1) the absorption of radiant energy by a photolabile pigment or pigments and changes in the state(s) of the pigment (Granit, '47; Wald, '51); (2) a relatively long latent period following the onset of illumination during which no electrical changes are detectable; (3) a potential change measurable across the retina with extracellular electrodes called the retinal action potential (Hartline, Wagner and MacNichol, '52) which begins at the end of the latent period; (4) the initiation of nerve impulses in the axons of the sense cells (Hartline and Graham, '32; Granit, '47).

It has been frequently suggested (Hartline, '35; Wulff, '43; Granit, '47) that the retinal action potential is a generator potential producing local currents and initiating the trains of impulses in optic nerve axons. Recently it has been demonstrated (MacNichol, Wagner and Hartline, '53) with an intracellular electrode, that illumination causes a prolonged depolarization of the sense cells (presumably the eccentric sense cells) in the ommatidia of the Limulus lateral eye and that this depolarization is accompanied by the ap-

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pearance of nerve impulse trains in the same cell. The depolarization measured with the intracellular electrode presumably is the intracellular sign of the retinal action potential recorded with extracellular electrodes. Although the retinal action potential has been shown to be directly associated with the nerve impulse discharge only in the case of the lateral eye of Limulus, we subscribe to the general idea that retinal action potentials are generator potentials.

Between the photochemical event in vision and the production of the retinal action potential processes and/or reactions must occur which produce the potential change. These coupling reactions between the photochemical event and subsequent events in vision have long received the attention of investigators (Hecht, '19; Hartline, '28; Jahn, '47; Wald, '51). Despite these efforts, the nature of the coupling processes remains obscure. It is the purpose of this paper to present a possible kinetic model of coupling processes which quantitatively describes many characteristics of the retinal action potential. It has been used in a predictive capacity to indicate fruitful new directions for experimental work.

Wulff and Pandazi ('51) reported that flash durations from 15 msec. to 1.0 second at constant intensity administered to a dark adapted photoreceptor produced electrical responses whose magnitude increased but whose latent periods were constant. These data thus indicated that two processes may be involved — one controlling the potential, the second controlling the latency. Two models were formulated, the first to describe the relation between intensity, flash duration and magnitude of the retinal action potential; and the second to describe the relation between intensity, flash duration and the latent period of the retinal action potential.

METHOD

The experiments were performed on dark adapted grasshoppers *(Melanoplus differentialis)*. The animals were securely fastened and a chamber was built about each of the

two lateral compound eyes. These chambers were filled with 0.9% NaCl solution, and each chamber made contact with a calomel half cell through a salt bridge. The half cells were connected to the input grids of a D.C. amplifier. The animal was so placed in a plastic container that the cornea of one eye could be exposed to a light flash admitted by the opening of a series of shutters, while the other eye remained in total darkness.

The interior of the chamber was ventilated by a constant stream of air at the temperature of water which circulated constantly through two copper heat exchangers built into the top and bottom of the animal chamber. Temperature was measured in terms of voltage developed by a copper-constant in thermocouple, with reference to a similar junction at 0°C, using a potentiometer circuit. Temperatures were regulated within ± 0.2°C of any given value. The air inside the chamber was approximately saturated with water vapor.

The animal chamber was securely fastened to a movable stage inside a Faraday cage, and the eye of the animal was oriented so that the cornea was at the focus of the light beam. The cage was then shielded from light and the animal was permitted to dark adapt.

Experiments were begun after two to 12 hours of dark adaptation. An interval of one hour between flashes proved to be necessary to permit complete recovery from the effects of preceding flashes. It was desirable to obtain a complete set of data from each animal; consequently the experiments were of several days duration. The grasshoppers, however, were not adversely affected by the experimental treatment and even after two weeks in the experimental chamber, resumed a normal existence upon return to the colony.

After the temperature had reached the desired level the experimental procedure consisted of adjusting the intensity of the light to the desired level, using Wratten neutral filters, and admitting a flash of light of 100 microseconds duration.[4]

[4] The high speed shutter was designed by Frank J. Fry and assembled under his supervision. We express our appreciation to him.

The electrical responses of the photoreceptor and of a photocell, to which a portion of the light beam had been diverted, were fed into D.C. amplifiers the outputs of which were recorded by pens on moving paper and by photographing the display on the face of a two-channel cathode ray tube. The paper receiving the ink tracing moved at 25.0 ± 0.25 cm per second, and the sensitized paper photographing the cathode ray tube display moved at 50.0 ± 0.25 cm per second. After each record the deflection produced by a pulse of known voltage was recorded. A perforated disk driven by a synchronous motor past a light source supplied the time base of the camera. After an adequate interval for dark adaption, another flash was admitted. Flash duration was varied by steps of 0.5 log units from 100 microseconds to 0.5 seconds. Intensity was varied by a factor of 10 from 10^{0} (unit intensity = 11,800 foot candles at the cornea) to 1×10^{-4}.

A total of 16 experiments were performed on grasshoppers. Although all the data obtained exhibited similar relations, single sets of data were selected for the text.

The measurements of magnitude and the latency were made as follows: (1) the base line was extended below the response and the point of greatest deflection was measured and the voltage computed; (2) the point where the tracing could be seen to leave the base line was marked as the end of the latent period and the interval from onset of illumination to this point was measured and converted to seconds. The experimental error has been estimated at ± 3% and the reproducibility of data from the same experimental animal was found to be about ± 10%.

A. Data and theory pertaining to retinal action potential magnitude

When the eye of a grasshopper is illuminated with a short flash of light there ensues a period of time during which no measurable electrical change occurs. This is followed by

a gradually developing negativity at the illuminated cornea which reaches a crest and then declines (fig. 1). If the flash duration is maintained constant and the intensity is decreased, a series of responses are obtained of decreasing magnitude and increasing latency. Similar series of responses have been obtained with a variety of flash durations.

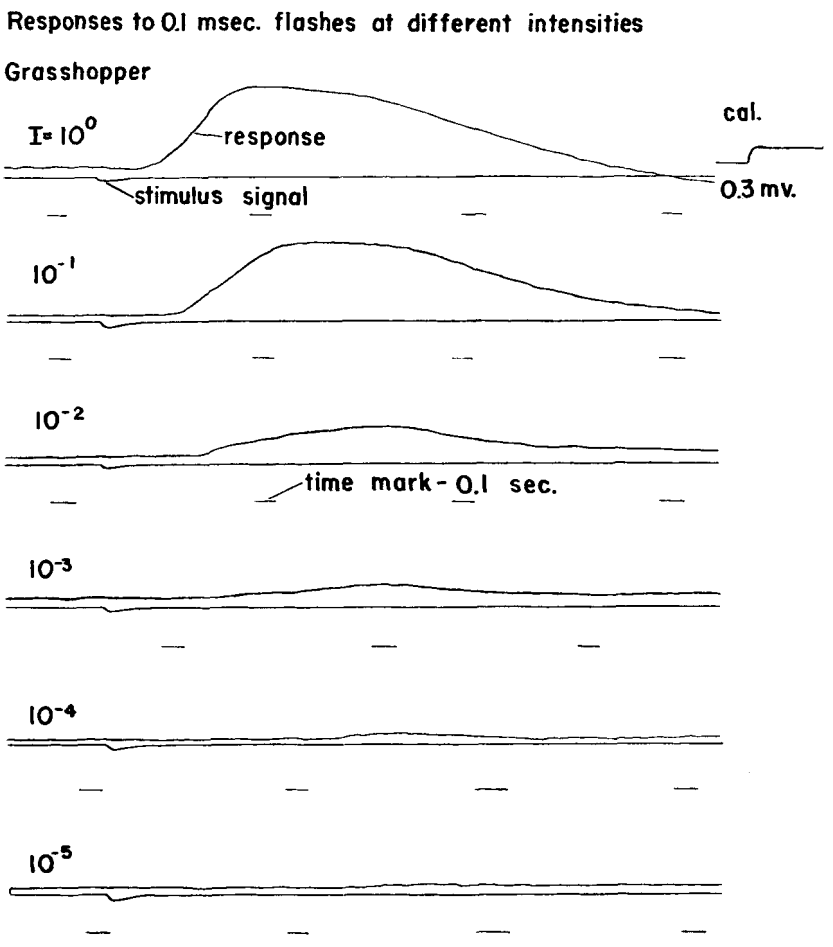

Fig. 1 A series of electrical responses recorded from the eye of a grasshopper *(Melanoplus differentialis)* illuminated with flashes of 0.1 millisecond duration and varying intensity. The recording paper speed for the grasshopper records was 50 cm per second. The records have been retouched.

1. The effect of intensity and duration of illumination on the retinal action potential magnitude. The data obtained from one grasshopper maintained in the dark at 30°C are presented in figure 2. The dots of figure 2 relate peak magnitude of the retinal action potential to the logarithm of the flash duration, measured in seconds, at 5 different intensities.

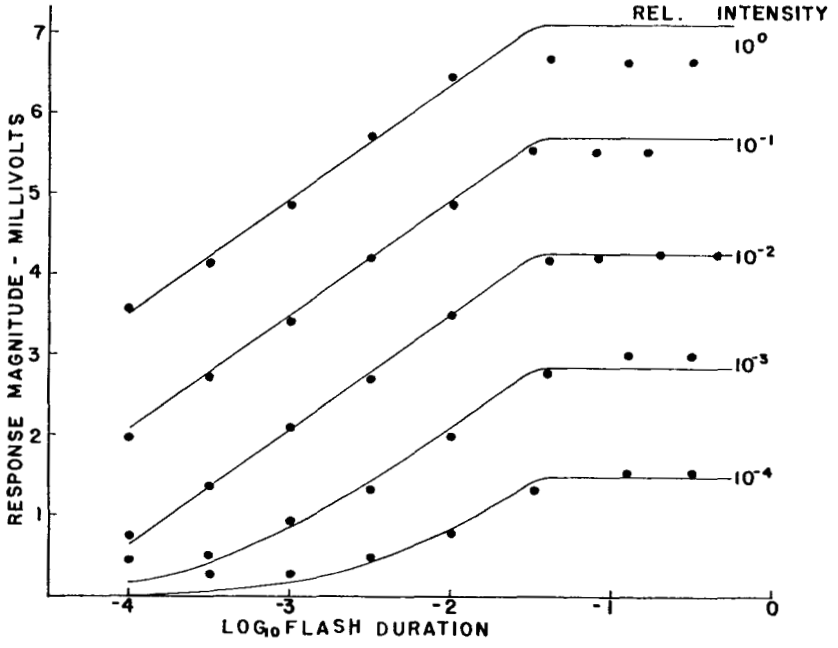

Fig. 2 The closed circles represent the maximum voltage recorded from grasshopper eyes in response to illumination with different intensities and exposures. The curves represent the theoretical relationship between voltage, intensity and flash duration given by expression (4) of the analysis.

The response magnitude at constant intensity increases with increasing flash duration and tends to reach a constant value at flash durations in the range 0.01 seconds to 0.025 seconds. (The specific value is determined by the intensity.) Similar data were obtained in 15 other experiments.

2. The kinetic model of a potential producing reaction. The model formulated to describe the magnitude characteristic

of the retinal action potential may be verbally stated as follows: (1) light acts on a photosensitive substance S in the photoreceptor producing a material C; (2) the substance C accumulates at a rate proportional to the light intensity and is depleted at a rate proportional to the difference between its concentration at any time and its concentration in the dark adapted, unilluminated eye; and (3) the potential magnitude developed after the lapse of the latent period is proportional to the logarithm of the concentration of C at the instant the flash ends.[5]

In the formal description of the essential characteristics of the model, capital letters designate substances and lower case letters designate concentrations and/or magnitudes. We assume that the exposure of a substance S to light produces a substance C (Wald, '51). Let the time rate of conversion of S be given as follows:

$$\frac{ds}{dt} = -aI, \tag{1}$$

which implies that the amount of S is not appreciably depleted during illumination. We assume that N molecules of C follow from the one of S and that the concentration of C changes in accordance with the following expression:

$$\frac{dc}{dt} = bI - k(c - c_1), \tag{2}$$

where b is proportional to $N \cdot a$ and k is the decay constant. If the concentration of C in the dark adapted unilluminated eye is c_1, and if the illumination begins at $t = 0$ and stops at $t = t_f$ then from (2) it follows that the concentration of C at a time equal to or greater than t_f is

$$c = c_1 + \frac{bI}{k}(1 - e^{-kt_f}). \tag{3}$$

We assume that the magnitude of the voltage difference, E, measured across the retina is proportional to the logarithm of the ratio of c to c_1. This is expressed as follows:

$$E = a \log \left[1 + \frac{bI}{c_1 k}(1 - e^{-kt_f})\right], \tag{4}$$

[5] The theory is being extended to include the depletion of the substance C after termination of the flash.

where E is the voltage, t_f is the flash duration and α is the proportionality constant.[6]

The continuous lines in figure 2 were computed using relation 4. The general agreement between data and theory is good. Two discrepancies deserve comment. First, the predicted plateau value for the response magnitude versus log t_f curve for intensity 10° (fig. 2) is higher than the measured plateau values. This discrepancy can be removed if the theory is modified to include a factor for depletion of S, the photosensitive material, during the course of illumination. The inclusion of this factor into the theory is readily achieved. It has the effect of decreasing plateau values at high intensities and also moves the bend in the response curves to the left.

Secondly, there is deviation between theory and data at the low intensity, short flash duration end of figure 2. We believe this discrepancy exists because the retinal action potential of the grasshopper eye in response to low levels of illumination is confused by a second potential wave whose characteristics are not amenable to experimental evaluation from our data (fig. 1).

B. Data and theory pertaining to the latent period

1. The effect of intensity and duration of illumination on the latency of the retinal action potential. The data obtained from one grasshopper maintained in the dark at 20°C are presented in figure 3. The data, represented by the circles and crosses, are the latent periods in seconds plotted as a function of the logarithm of the intensity for different flash

[6] The constants may readily be evaluated as follows: (1) the quantity α is equal to the slope of the linear portions of the curves of response magnitude versus log flash duration of figure 2, symbolically $\alpha = \frac{\Delta E}{\Delta \log t_f}$; (2) the rate constant, k, may be evaluated from the flash duration, t_{fo}, at the intersection of the linear rising portion and the plateau region for intermediate intensities, using the relation $k = 1/t_{fo}$; (3) the quantity $\frac{b}{c_1}$ can be determined from plateau values of the response magnitude for intermediate intensities by using the relation

$$E = \alpha \log \left(1 + \frac{bI}{c_1 k}\right).$$

durations. The latency exhibits a progressive increase as the intensity is decreased (see also figure 1). At any one intensity, the latency increases as the flash duration decreases. The change in latency when the flash duration is decreased from 0.1 second (flash duration greater than the latent period)

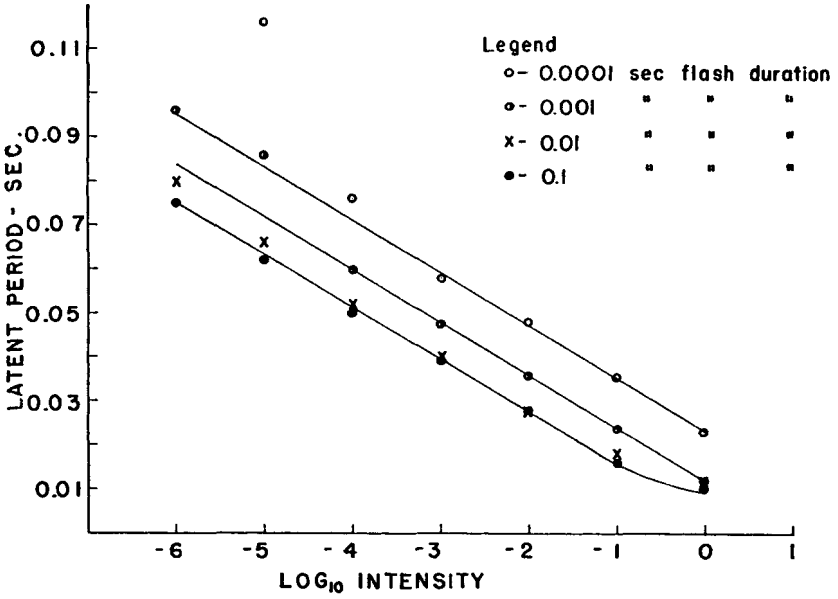

Fig. 3 The circles and crosses represent the latent period of the retinal action potential of the grasshopper plotted as a function of the logarithm of the intensity with the duration of exposure as a parameter. The curves are the theoretical relationship between latent period, intensity and flash duration given by expressions (6) and (7) of the analysis. The theoretical curve corresponding to the experimental points for 0.01 second flash duration (crosses) has been omitted because it is so near the curve for 0.1 second flash duration as to be almost indistinguishable on a graph the size of the figure.

to 0.01 second is very slight. For changes in flash duration from 0.01 second to 0.001 second and from 0.001 second to 0.0001 second the changes in latency are considerably greater. However, the percentage change in latency from exposures of 0.1 second to 0.0001 second is nevertheless very small compared to the thousand-fold change in the exposure.

2. The kinetic model of a process controlling the latent period. A model was formulated to describe the relation between the latent period of the retinal action potential and the intensity and duration of illumination. The model may be described as follows: (1) light, acting on a photosensitive substance S produces a substance or factor P; (2) P accumulates by two processes, one at a rate proportional to the intensity of illumination and the other proportional to the concentration of P. The second process is autocatalytic; (3) the substance or factor P accumulates and, when it reaches a critical concentration, p_c, the electrical response begins, that is, the latent period terminates.

In the formal description of the essential characteristics of the model capital letters designate substances or factors and lower case letters designate concentrations and/or magnitudes. The rate of production of P is given by the following relation:

$$\frac{dp}{dt} = hp + nI \tag{5}$$

where p is the concentration or magnitude of the factor P. The constant h is the autocatalytic rate constant. If we assume that $t = 0$, $p = 0$ then (5) yields for the latent period, t_L, the following relation when the flash duration is equal to or less than the latent period ($t_f \leq t_L$)

$$ht_L = (\ln 10) \log \left[\frac{hp_c}{n(1 - e^{ht_f})} \right] - (\ln 10) \log I \tag{6}$$

where t_f is the flash duration. When the flash duration is equal to or greater than the latent period ($t_f \geq t_L$) then (6) is replaced by

$$ht_L = (\ln 10) \log \left[\frac{hp_c}{nI} + 1 \right]^{7} \tag{7}$$

[7] The constants in these equations (6 and 7) are easily evaluated from the latency data, figure 3. The constant, h, can be evaluated from the slope, m, of the line drawn through the lowest set of points in figure 3, by using the relation $m = \frac{-\ln 10}{h}$. The intercept, I_i, of the same line on the horizontal axis yields the constant $\frac{p_c}{n}$ from $I_i = \frac{hp_c}{n}$.

Although the theoretical development was stimulated by the observation that the latent period was not affected by flash duration from 0.015 second to 1.0 second (Wulff and Pandazi, '51), the theory predicted that latent period should vary with flash duration. The data presented in figure 3, which extend over a greater range of flash durations from 0.0001 second to 0.1 second, do indicate that the flash duration affects the latent period. The continuous lines in figure 3 are computed using equations (6) and (7).

It is noted from figure 3 that the experimental data for short flash durations and low intensities deviate from the linear relation indicated by the theory. This deviation is probably associated with the difficulty of measuring the latency from the tape records at low response magnitudes. As the magnitude of the response becomes small the error introduced in determining the position on the record at which a deviation from the base line occurs becomes larger and tends to yield greater values of the time. This explanation of the discrepancy between theory and data was substantiated by observing that when the gain of the amplifier system was reduced (resulting in smaller deflections on the tape) the deviation from linearity occurred at high intensities and larger flash durations.

The deviation from linearity at high intensities and long flash durations, figure 3, can be satisfactorily accounted for by the appreciable depletion of a light sensitive substance. This modification of the theory has been formulated and applied to the data (lowest curve of figure 3).

DISCUSSION

The evidence which has accumulated bearing upon the peripheral visual process in photoreceptors suggests that the initial event in vision is the absorption of radiant energy by light sensitive unstable pigments, of which rhodopsin is the most familiar example. Following this photochemical event there occur, after the lapse of some time, electrical changes in the photosensitive cells which culminate in the

discharge of nerve impulses. Recently MacNichol et al. ('53) demonstrated that illumination of the lateral eye of Limulus produces a depolarization of sense cells and that the discharge of nerve impulses is associated with the wave of depolarization. One of the major problems in the peripheral visual process which awaits solution is an elucidation of the events which follow the photochemical event and precede the electrical events.

The work of Wald and co-workers which has centered around the photochemistry of rhodopsin, the vertebrate rod pigment, has provided some data which may prove to be important in the coupling processes between the photochemical and electrical events. Wald ('51) has identified two substances, lumi-rhodopsin and meta-rhodopsin, which have a brief existence at temperatures compatible with life. The transient existence of these substances suggests that they may play a role in peripheral vision. More recently Wald and Brown ('52) have demonstrated that rhodopsin, illuminated when in an amperometric titration cell, binds silver ions. Various lines of evidence led Wald to suggest that the observed change in cation concentration was caused by sulfhydryl groups, suddenly uncoupled by the action of light, absorbing cations in the solution being titrated. The time characteristics of the current change were not indicated. An event of this kind, producing an electrical change, might be identified with the model pertaining to the potential magnitude.

The kinetic model presented above consists of two parts, the potential magnitude process and the latency process. The two processes are both initiated by light but, subsequently, there is no obvious relation between the two. It may, indeed, be debated whether two separate processes are necessary to account for the characteristics of the retinal action potential. Our belief that two processes are necessary is based upon the following observations. (1) The starting point for the development of the theory was the difference in the behavior of potential magnitude and latent period with respect to the

duration of illumination. This difference in behavior is further emphasized by the results obtained in the course of experiments reported above. The magnitude versus log t_f data (fig. 2) indicate there is at each intensity a flash duration beyond which the response magnitude will not increase, regardless of the length of flash. This flash duration, called the critical duration (Hartline, '28), does not correspond to the latent period. Our experiments show that there is no fixed relationship between the critical duration and the latent period which holds for all the animals examined. (2) Further, the models have been used to predict results which have been strikingly verified by experiment. The latency model accurately predicted the dependence of latent period on flash duration at constant intensity which was later determined experimentally (fig. 3). (3) The effect of temperature on the characteristics of the electrical response of the grasshopper eye again points to two distinct processes (Fry, Wulff and Brust, '55). In the grasshopper temperature does not affect the response magnitude on the rising portions of the magnitude vs. log t_f curves but the latencies for the same responses are markedly temperature dependent. Our conviction of the necessity of two coupling processes has been considerably strengthened by the temperature effect on the latency and magnitude of the retinal response.

Another prediction made by the theory concerns the effect of temperature on the plateau magnitudes of the response curves. Expression (2) of the potential magnitude model suggests that, if the decay process is temperature dependent, then a rise in temperature should accelerate the decay process and decrease the plateau magnitude. Conversely, a drop in temperature should slow the decay process and increase the plateau magnitude. These predictions have been confirmed experimentally and are presented in the following paper.

The models have thus far stimulated considerable experimentation. In addition, they suggest that the time course of the coupling processes are sufficiently slow to be measurable spectrophotometrically, if the optical densities of materials

are great enough. Experiments are underway to test this idea.

It is probable that the elucidation of the potential generating mechanism in photosensitive cells may increase our insight into generator potential phenomena within the nervous system.

SUMMARY

1. The magnitude of the retinal action potential obtained from dark adapted eyes of grasshoppers is a function of the intensity and duration of illumination. The form of this relation is consistent from one animal to another.

(a) The characteristics of the relation between action potential and intensity and duration of illumination are reasonably accurately described by a theory which assumes that:

(1) The light acts on a photosensitive substance S producing a material C whose concentration manifests itself as an emf across the retina after the lapse of a latent period.

(2) The time rate of production of C is proportional to the light intensity and the rate of depletion, during illumination, is proportional to the difference between the concentration of C at any time and the equilibrium concentration in the dark adapted eye.

(3) The maximum values of the emf generated after the lapse of the latent period are proportional to the logarithm of the concentration of C at the instant the flash ends.

(b) The experimentally determined relation between response magnitude and logarithm of flash duration, for any single intensity, exhibits the following: (i) a slowly rising phase at short flash durations; (ii) a linear region at intermediate flash durations; and (iii) a plateau region as the flash duration increases further.

(1) In the linear region and below, curves for different intensities show that a constant value of response magnitude is obtained for a fixed value of the product of intensity and flash duration. This characteristic is derivable from the hypothesis that C is generated at a rate proportional to the intensity of illumination and that the emf across the retina is proportional to the logarithm of the concentration.

(2) The magnitudes of the response in the plateau region (i.e., long flash durations) are, for equal logarithmic increments of intensity, uniformly spaced at intermediate response magnitudes, compressed together at low response magnitudes and also compressed together at high response magnitudes. The existence of the plateau regions is correlated with the depletion process for substance C. The data are quantitatively described by the theory presented in this paper except for the compression at high response magnitudes. To explain this compression it is sufficient to include in the theory the dependence of the rate of the initial photochemical process on the concentration of the light sensitive substance S, which for high intensities and long flash durations, is appreciably depleted during the flash.

2. The latency of the retinal action potential is, for a single flash duration, a linear function of the logarithm of the intensity over almost the entire range of intensities used in the experiments. The latency is a relatively insensitive function of the flash duration.

(a) The characteristics of the relation between latency and intensity and flash duration are accurately described by a theory which assumes that:

(1) The time rate of production of a factor or state P whose magnitude determines when the elec-

trical response begins is proportional to the intensity of illumination and is also autocatalytic.

(2) When the magnitude of the factor P reaches some critical value the electrical response begins.

(b) (1) The linear portion of the experimentally determined relation between latent period and the logarithm of intensity for a constant flash duration is accurately described by the theory based on an autocatalytic rate process initiated by the light.

(2) The latent period varies only very slightly with flash duration as the flash duration becomes less than the latent period. As the flash duration further decreases the latent period changes more rapidly but still quite slowly compared to the changes in flash time. This characteristic of the latency is quantitatively described by the theory just mentioned.

(3) The experimental data show, for the highest intensities and longest flash durations, that the latent period is no longer a linear function of the logarithm of the intensity. As the intensity increases the latency appears to approach a finite non zero value. This aspect of the latent period is explained by modifying the theory to take account of the appreciable depletion of a light sensitive substance which occurs at high intensities and long flash durations.

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