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Title: THE VISUAL RESPONSE OF THE PURPLE SHORE CRAB,

HEMIGRAPSUS NUDUS, TO IONIZING AND NON-IONIZING

RADIATION

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Bioelectric responses from the isolated eye of the purple shore crab were measured by the electroretinogram with stimulation by light from wavelengths of 250 to 800 nm, strontium-yttrium-90 beta radiation, 17.5 keV x-radiation, and 3-cm microwaves.

Peak sensitivities to light stimulation were found at 350 and 500 nm. These peaks could be attributable to both the light absorption characteristics of rhodopsin, and fluorescence of eye structures by ultraviolet light. Responses were elicited only with rapid changes in stimulus intensity.

Responses to beta radiation exhibited a peak at the onset of exposure, but no peak at the cessation. Responses to series of stimuli exhibited a decrease in amplitude with each succeeding stimulus, similar to that of visible light. Electroretinographic peaks

did not decline to the baseline before the next response commenced. The results were considered to be due to direct stimulation of the photopigment and secondary stimulation produced by visible fluorescence and possibly Cerenkov radiation. The threshold absorbed dose was near 0.9 mrads.

X-irradiation produced responses similar to those of visible light in that peaks were observed at the beginning and end of stimulation. No peaks were recorded with a gradual change in stimulus intensity. Such responses were thought to be from both direct photopigment stimulation and fluorescence. The x-ray threshold absorbed dose was approximately 50 mrads.

Microwave irradiation resulted in neither direct visual stimulation nor modification of visible light responses during microwave exposure. Due to the low photon energy of this radiation, a direct influence on the photoreceptor mechanism was unlikely, although at much higher stimulus intensities, an interaction between the microwave electric field and nerve membranes might have been possible.

The Visual Response of the Purple Shore
Crab, Hemigrapsus nudus, to Ionizing
and Non-Ionizing Radiation

by

Neil Michael Jordan

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THE VISUAL RESPONSE OF THE PURPLE SHORE
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I. INTRODUCTION

Responses of invertebrates to ionizing radiation, attributable to

its effects on the visual system, have been known since 1896. In the

first recorded experiment, crabs and insects were placed in a box

having one end shielded from the x-ray tube with lead. When the box

was irradiated, the animals moved to the unshielded half. The con-

clusion drawn by the investigator:

> ... thereby one may be led to assume every possibility
> of a sense of sight, for blinded animals do not show
> this capacity (Axenfeld, 1897a, p. 147).

A later experiment by the same author (Axenfeld, 1897b)

confirmed these initial observations. Comparative electroretino-

graphic responses of isolated mammalian eyes to visible light, x-

rays, and radium were recorded by Himstedt and Nagel (1901). More

recent work has, indeed, shown that the eye is sensitive to ionizing

radiation (Kimeldorf and Hunt, 1965). Research with microwaves

also suggests that the nervous system may be sensitive to the influence

of high-frequency electromagnetic fields (Livshits, 1957).

It will be the objective of this paper to examine the relative

visual response of Hemigrapsus nudus, the purple shore crab, to

light of wavelengths 250 to 800 nm, beta rays, x-rays, and 3-cm

microwaves, as measured by the electroretinographic (ERG) response of the isolated eye.

The visual photochemistry of the three phyla which have independently evolved complex visual systems--arthropods, molluscs, and vertebrates--is based on the same basic vitamin A molecule (Wald, 1959). Since most of the research in visual photochemistry has been conducted with vertebrates, much of the introductory material will be based on this, with reference made to arthropod photochemistry where possible. The principles discussed are applicable to arthropods and especially to the decapod crustaceans.

The Electromagnetic Spectrum

Electromagnetic radiation may be considered either as a stream of particles (photons), or a wave in which each particle contains a quantized amount of energy, E, proportional to the frequency of oscillation of the wave, v The relationship is:

$$E = h\nu = h\frac{c}{\lambda}$$

where h is Planck's constant, c is the velocity of light, and λ is the wavelength of the radiation.

The electromagnetic spectrum extends from wavelengths of fractions of a nanometer in cosmic and gamma rays to many kilometers in 60 Hz alternating current. For clarity and perspective, this spectrum is illustrated in Figure 1. Since it is also customary
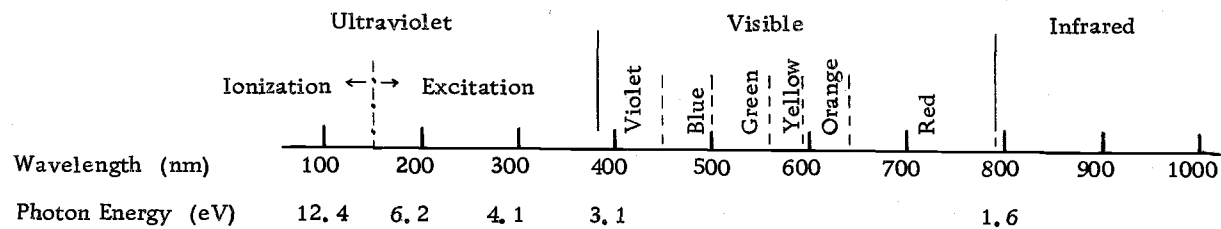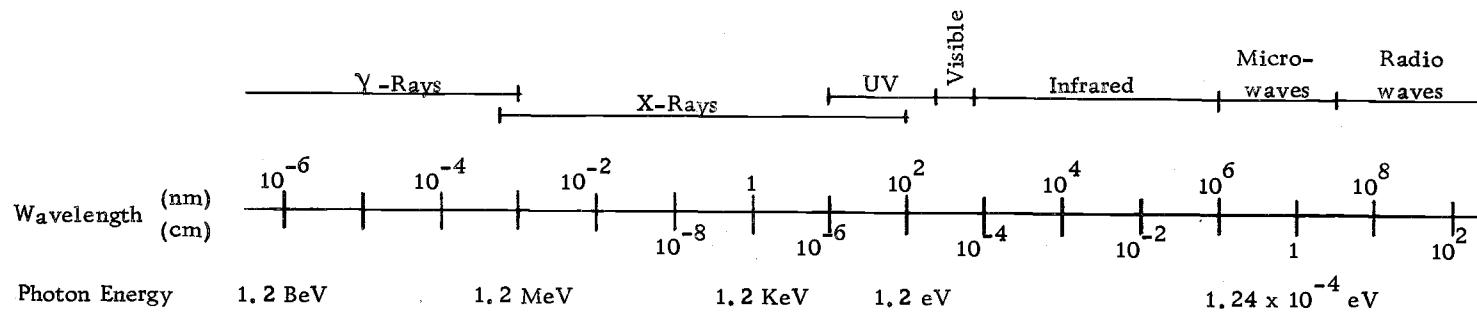
Figure 1. The electromagnetic spectrum, upper scale; the visible spectrum region, lower scale (modified from Jagger, 1967).

to refer to radiation of centimeter wavelengths in terms of frequency, equivalent values for both classifications are listed in Table 1.

Table 1.   Equivalent wavelengths and frequencies for electromagnetic radiation of centimeter wavelengths.

Wavelength (cm)	(MHz)	(GHz)
1000 (nm)	3×10^{11}	3×10^{8}
1	3×10^{4}	30
3	1×10^{4}	10
5	6000	6
10	3000	3
12.2*	2450	2.45
32.9*	915	0.92

*These wavelengths are presently in use for microwave ovens.

It is important to note that there is no sharp demarcation between various types of electromagnetic radiation; the divisions, by convention, apply to various sources or methods of production.

In considering beta radiation, the biological effects are similar to equally energetic electrons ejected by gamma radiation, since most of the ionization produced by gamma rays is due to ejected electrons.

Anatomy of the Compound Eye

The eye of the decapod crustacean is similar in most respects to the compound eye of other arthropods (Figure 2). Each facet of the
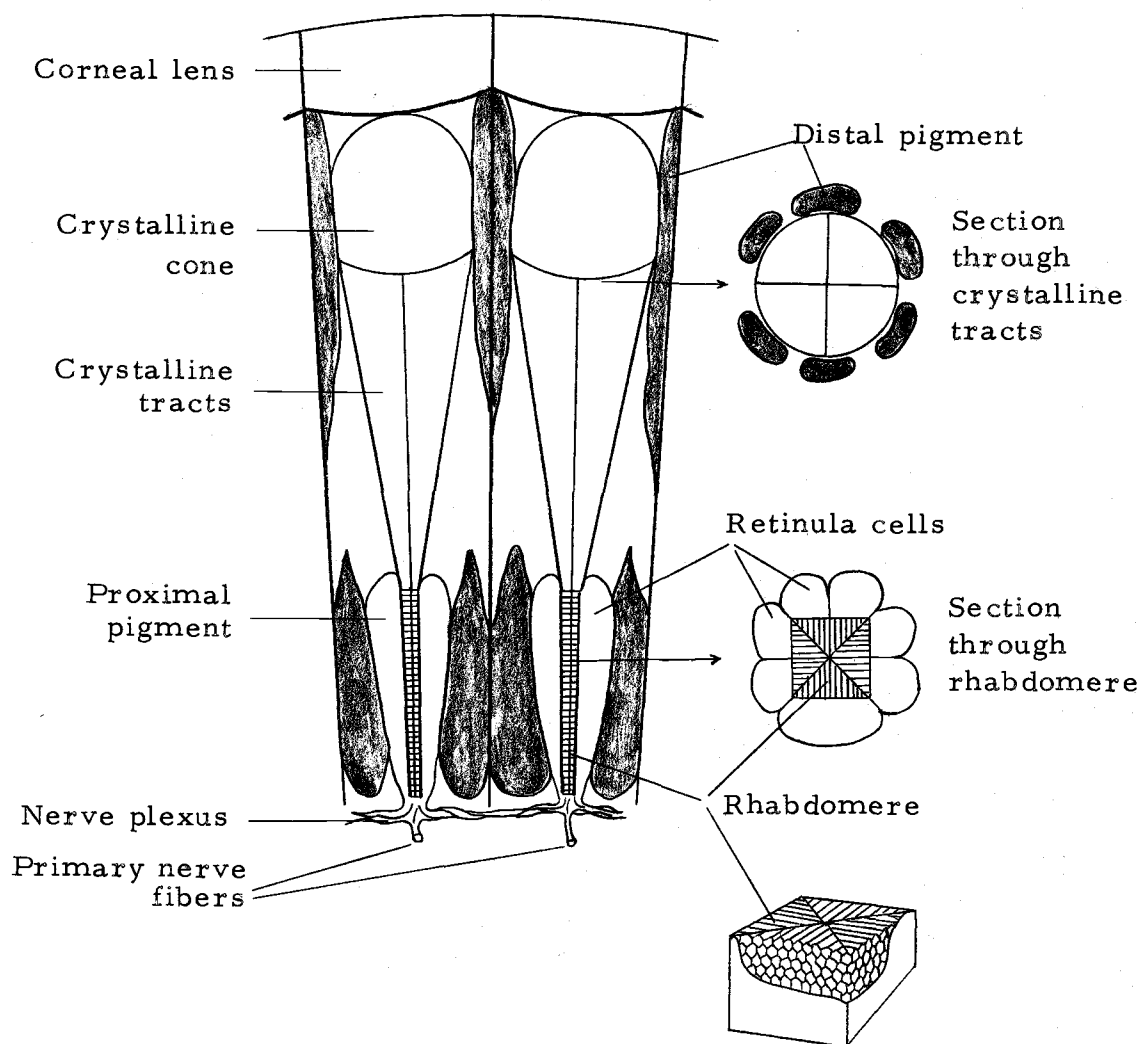
Figure 2. Two ommatidia of the crustacean compound
eye (adapted from Schiff, 1963; Wolken,
1968).

compound eye is termed an ommatidium and is in itself a functional

eye with a visual field of about 15 to 20° (Schiff, 1963). The

ommatidium contains a corneal lens, crystalline cone, and crystalline

tracts which transmit the light onto the retinula cells. These cells

are differentiated into rhabdomeres, the site of interaction between

light and the visual pigment. The photoreceptive unit of the omma-

tidium is the rhabdom, formed from retinula cells and their rhabdo-

meres; the rhabdomere can be considered homologous to the outer

segments of the vertebrate retinal rod. Each ommatidium may be

isolated from its neighbors by the proximal and distal pigments.

The rhabdomere is differentiated into many fine tubules or

microvilli about 50 nm in diameter, with double-walled unit mem-

branes approximately 5 nm thick. These tubules are formed from the

rhabdomere wall membrane and share a common cytoplasm, and con-

tain the visual pigment (Wolken, 1968).

The site of photon-photopigment interaction in arthropods was

identified as the microvilli by Eguchi, Naka and Kuwabara (1962) by

following the embryonic development of the silkworm rhabdom and its

electrical response to photic stimulation; generation of an electrical

response did not occur until the microvilli had been differentiated from

the rhabdomeres.

Experimental evidence from the adult arthropod (Wulff and

Mendez, 1970) also supports this finding. When the retinula cell of

the <u>Limulus</u> lateral eye was hyperpolarized[1], the latent period between

stimulation and production of the receptor potential was increased.

This was not observed when surrounding membranes were similarly

hyperpolarized, indicating that the receptor potential originated in the

rhabdomere membrane system.

In the vertebrate eye, at least, the action of x-radiation has been

localized in the rod outer segment. Using the same procedure as

Eguchi <u>et al.</u>, Dawson (1967) followed the embryonic growth of the

rabbit eye; no response to an x-ray stimulus was recorded until the

outer segments had developed.

Electrical impulses produced by visual excitation travel to the

higher nerve centers by way of the primary visual nerve fibers which

communicate by synapses with the retinula cells (Schiff, 1963). The

nerve plexus at the basement membrane contains nerve fibers which

interconnect the primary visual nerves, and is the site of complex

interactions of nerve impulses (Hartline, 1969), which will be treated

in detail below.

Optical Properties of the Compound Eye

The visual response of the compound eye is governed both by the

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[1]As will be detailed below, the nerve cell membrane will be depolarized when the resting potential is neutralized. Increasing the resting potential by external electrical means (hyperpolarization) delays or prevents normal depolarization of the membrane.

structure of the eye and the position of the pigments between the

ommatidia. In the crustacean eye, which has a characteristic super-

position structure, the photoreceptors are relatively more distant

from the refractive structures than in the apposition eye found in many

insects.

In the light-adapted condition (Figure 3), only the ommatidia

directly beneath the light source can be stimulated because of the

expanded pigment barrier. In a dark-adapted condition with decreased

pigment barriers, one ommatidium can be stimulated by light passing

through the refractive structures of surrounding ommatidia. It can

be seen that this dark adaptation will increase the sensitivity of the

eye at lower light intensities (Wolken, 1968).

Kuiper (1962) and Schiff (1963) found that the crystalline cone and

tract in the superposition eye act not like lenses, but waveguides or

"light pipes. " With a visual angle of 15 to 20°, any object beyond a

short distance from the eye will be seen by several ommatidia, with

each facet not directly in line with the object seeing a slightly dis-

placed image.

Light and Dark Adaptation

In an anatomical or structural sense, light and dark adaptation

are the result of migration of the eye pigments in such a way that the

photoreceptors are exposed to a greater or lesser light intensity. The

Light-adapted   Dark-adapted
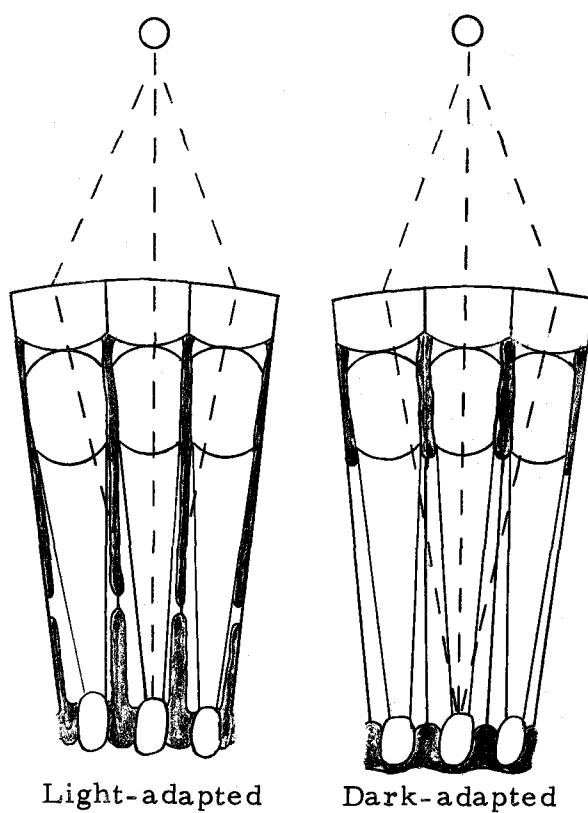
Figure 3.   The compound eye in a light-
            and dark-adapted condition.
            Shaded areas represent the
            shielding pigments (Kuiper,
            1962).

cause of crustacean pigment migration has been attributed to nervous control, hormones, and temperature changes.

Bennitt (1924) found that the intact central nervous system was essential in controlling proximal migration (dark adaptation) but that distal migration occurred even when the eye was excised.

A temperature effect was also noted by this author, in which an increase in temperature produced an effect on pigment migration similar to dark adaptation. This effect was subordinate to that of light, however.

Hormonal control of pigment movement was recognized by Welsh (1930) and in more recent work by Kleinholz (1957) and Bernhard and Ottoson (1960). This may explain the diurnal rhythm of crustacean distal pigments, in which the pigment dark adapts during daylight hours and advances around the ommatidia at night. In such a case, immediate sensitivity to changes in light intensity is controlled by the proximal pigments, while the distal pigments serve to prevent overstimulation of the eye (Kuiper, 1962).

The simple mechanical change in pigment position, however, cannot explain the net electrical output of the eye. Bernhard and Ottoson (1960, 1961) studied dark adaptation in lepidoptera by coordinating electrophysiological recording and examinations of fixed eye sections. Although pigment migration during dark adaptation and a decrease in threshold light sensitivity after several minutes of

darkness correlated well, the initial increase in sensitivity as measured by the ERG was not closely related to corresponding pigment position. In one case, examination of an eye which had produced an ERG dark-adaptation response revealed that the pigment had failed to migrate. The authors stated that resynthesis of a visual pigment might play a role in net sensitivity increase.

Light and dark adaptation, independent of either pigment migration or visual pigment concentration, has been suggested by Dawson (1968), where neuronal inhibition may change the magnitude of the recorded visual signal. Although this is discussed in relation to the vertebrate eye (Alpern, Rushton and Torii, 1969, 1970), it does not seem inconceivable that a similar situation may exist in the compound eye, which also exhibits extensive neural interactions.

Schiff (1963) demonstrated the rapidity of light and dark adaptation in the shrimp. In adapting to darkness the electrical response increased rapidly for the first 10 seconds, then less rapidly for periods up to 30 minutes. In adaptation to a rapid series of flashes, the ERG response dropped to 1/10 the initial value within one second.

Such a change in the ERG response with Hemigrapsus is illustrated in Figure 4. It should be emphasized at this time that these responses, as with those elicited below with ionizing radiation, may not represent adaptation in the context of research by others, but
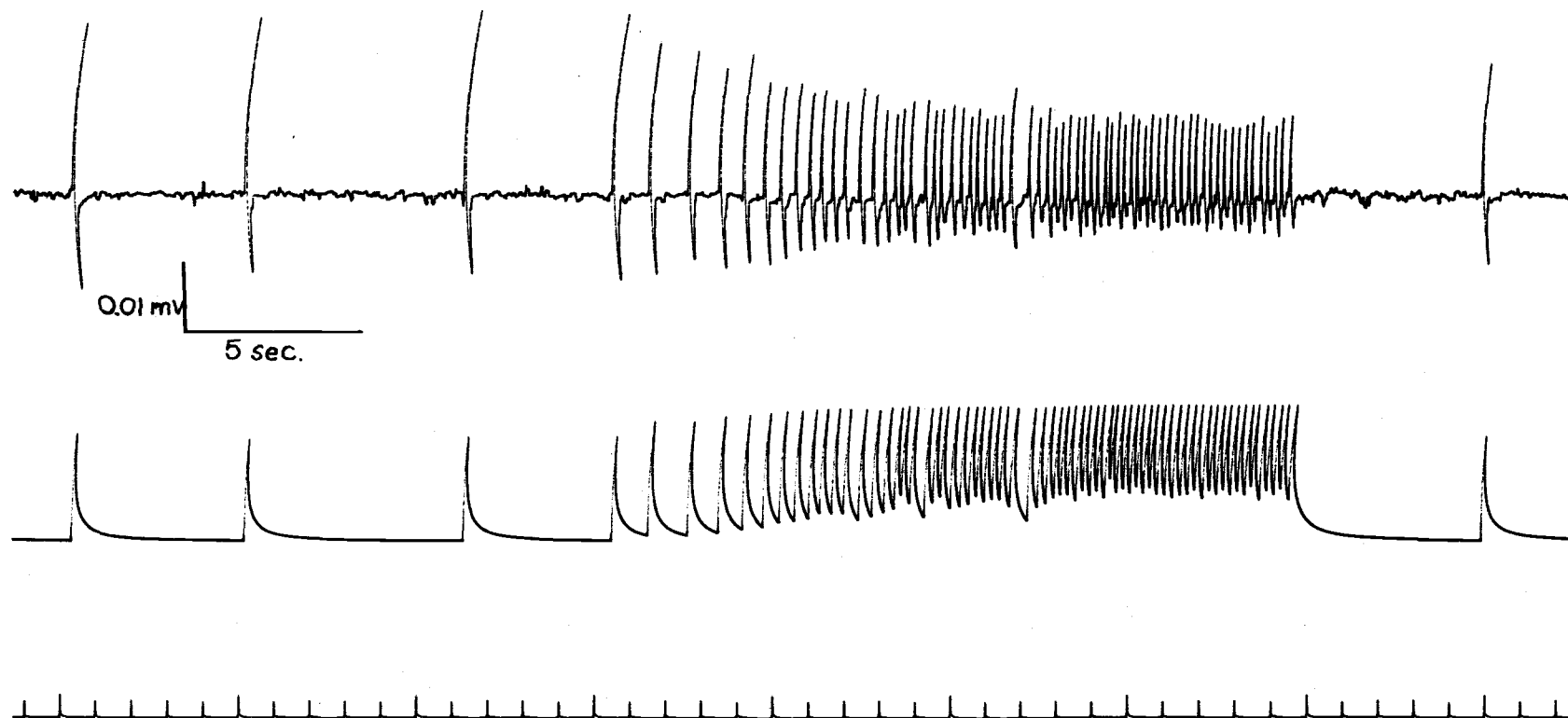
Figure 4. Adaptation in the isolated Hemigrapsus eye. Upper tracing, ERG response with upward deflection indicating cornea-negative potential; middle, light stimulus marker with upward deflection indicating 1/25 second light flashes; lower, time in seconds.

the modification of a series of responses by each preceeding stimulus.
As can be seen in this figure, stimulation with visible light at approxi-
mately five-second intervals produces no change in response
amplitude; the light sensitivity has changed (by one or more of the
above-mentioned processes) as a result of the stimulus and then
returned to the original condition within five seconds. When the
interval between stimuli is decreased to a point where the recovery is
not fully completed, the response decreases.

Visual Pigment Structure

Photoreception takes place at the microvilli membrane surfaces
where molecules of the visual pigment, rhodopsin, are located.
Rhodopsin is composed of a colorless protein, opsin, associated with
the carotenoid chromophore, retinal (retinaldehyde or vitamin A
aldehyde in older literature), in the 11-cis isomer configuration
(Figure 5). Upon stereoisomerization by a visual stimulus, retinal is
converted to the trans configuration (Hubbard and Kropf, 1958; Wald,
1964; Hubbard, Bownds and Yoshizawa, 1965).

Opsin in association with cis retinal takes on an α-helix con-
figuration; the main bonding point between the retinal and opsin is an
ϵ -amino lysine compound. A secondary bonding point is at the ring
end of retinal. Bleaching of rhodopsin results in disruption of the
opsin moelcular configuration, as seen by changes in its absorption
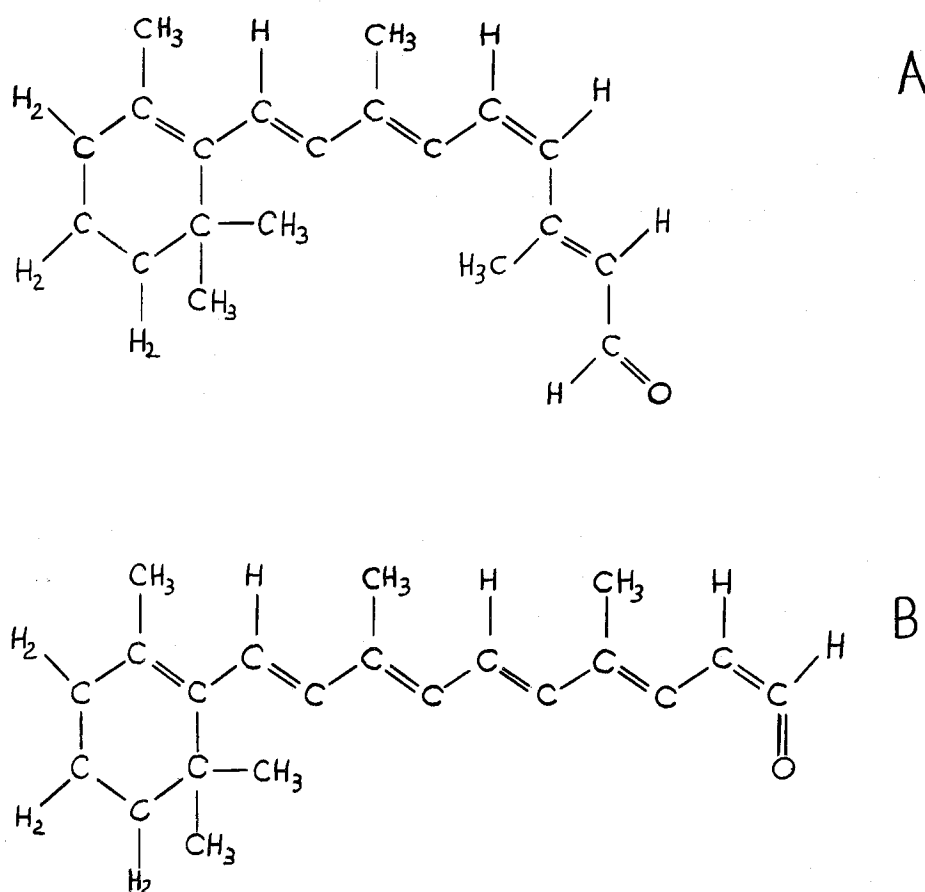
Figure 5. 11-cis retinal before (A), and after (B) stereo-
isomerization (Hubbard and Kropf, 1958; Wald,
1964; Hubbard, Bownds and Yoshizawa, 1965).

spectrum. Such changes are also found in denaturation of secondary and tertiary conformation in other proteins (Hubbard et al., 1965).

Several sulfhydryl groups are associated with opsin (Buckster and Hooper, 1968). Blocking the sulfhydryl groups with p-chloromercuribenzoate (PCMB) or silver ions prevents rhodopsin synthesis, while the addition of glutathione partially protects the groups and permits limited bonding of retinal to opsin.

While the exact structure of rhodopsin is not yet known, Figure 6 illustrates the molecule as based on most recent evidence.

The photopigments of some arthropods may not be rhodopsin (Wolken, 1968). Euphausiopsin, found in some marine crustaceans, for example, has an absorption peak of 460-464 nm. The rhodopsin peak is 496-498 nm. Euphausiopsin bleaches to retinal and then to vitamin A (Boden, Kampa and Abbott, 1961), so the difference in pigments would most likely be in the opsin component.

Photochemical Response to Visible
Light and Ionizing Radiation

It is the cis retinal molecule which absorbs the light photon and initiates the visual process by its conformational change; the initial action of visual importance is the stereoisomerization of cis retinal to the first trans isomer in a series of reactions resulting finally in all trans retinal and opsin.

This process is not exactly known, but Buckster and Hooper
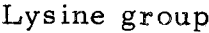
Figure 6. The rhodopsin molecule (Buckster and
Hooper, 1968).

(1968) suggested that after absorption of the photon, the cis retinal

straightens to the trans configuration causing the ring end of the

molecule to extend. The bond at that end is shifted by steps toward the

flexible lysine bond. The retinal is then hydrolyzed and separated

from the opsin. The process is outlined in Figure 7.

In addition to visible light, visual responses obtained from both

beta and x-ray stimulation indicate, but do not verify, that they also

may initiate this stereoisomerization. It is evident that any particle

carrying sufficient energy to trigger the reaction will be able to induce

a photochemical response interpreted as a light sensation (Bornschein,

Pape and Zakovsky, 1953; Lipetz, 1960; Markus, 1964).

Photons having less energy than that required to directly produce

a photochemical response can still produce this stereoisomerization

by the combination of energy carried in the photon with that associated

with the rhodopsin molecule. The wavelength beyond which photons

have insufficient energy is roughly 590 nm (photon energy, 2.1 eV).

Beyond this point, the energy deficit is made up by the thermal energy

of the rhodopsin molecule itself. Thus the stereoisomerization

process beyond 590 nm is temperature dependent (St. George, 1952).

Quantum Efficiency

The quantum efficiency for visible light stimulation was

determined by Hecht, Schlaer and Pirenne (1941) with human subjects;

Vitamin A + opsin $\xrightleftharpoons[\text{DPN}]{\text{DPNH}}$ 11-cis retinal + opsin $\xrightarrow{\text{light}}$ rhodopsin:

DPN

DPNH

DPNH

retinal

opsin

light: at this point photon absorption initiates the visual process

pre-lumirhodopsin

lumirhodopsin

metarhodopsin I:

metarhodopsin II

$+H_2O$

all trans retinal and opsin
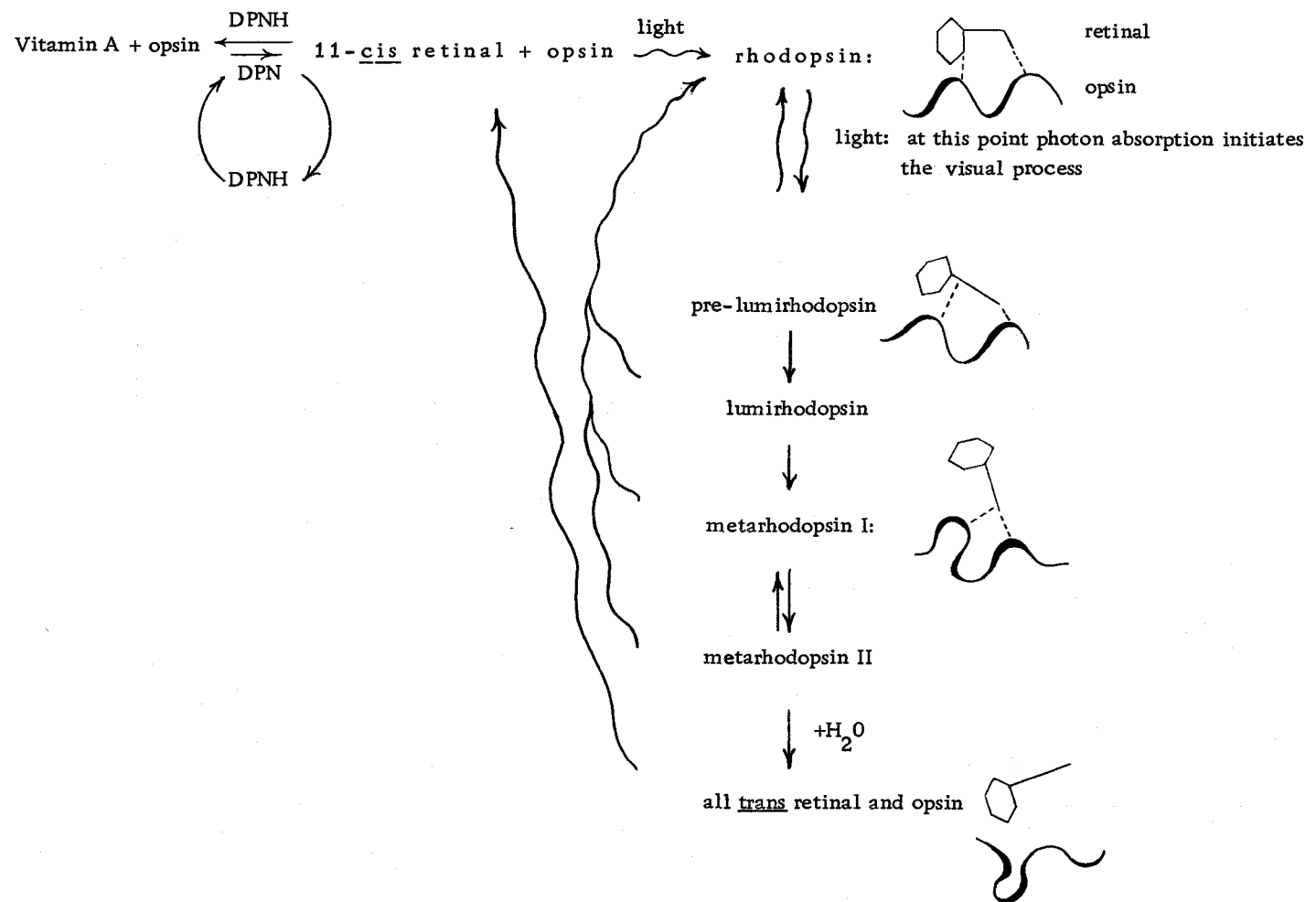
Figure 7. The bleaching process of rhodopsin. Wavy lines indicate reactions caused by light (modified from Hubbard and Wald, 1951; Hubbard et al. 1965; Buckster and Hooper, 1968; Hendricks, 1968).

a photon absorbed by each of five to 14 molecules of retinal could result in visual excitation. Hagins (1965) interpreted this value as the ratio of visual excitations to photon absorptions, giving a maximum quantum efficiency of about 5/14, or roughly 36%. The threshold light sensitivity of the crustacean rhabdom is about the same as that of the human rod (Waterman, 1961).

With 14.5 MeV beta and x-ray stimulation of the human eye, Markus (1964) calculated quantum efficiencies of about 1/10 or 10% for beta radiation and 1/100 or 1% for x-rays. Using 75 keV x-rays, Lipetz (1960) obtained efficiencies ranging from 1/3600 to 1/85, corresponding to 0.07% and 1.2% respectively.

Quantum efficiencies for ionizing radiation may be expected to vary with the energy of the radiation due to differences in relative biological effectiveness (RBE). For example, Baily and Noell (1958) used the destruction of the ERG by x-radiation to determine the following RBE values: 100 kVp, 0.65; 250 kVp, 1.00; and 2 MeV, 1.19.

The reason for the relatively low efficiency of ionizing radiation is due to its high energy, which is much greater than that required to initiate the photochemical reaction. An ionizing photon or particle will expend a large amount of energy which may not directly lead to visual excitation, and the excitation itself becomes a function of the probability of interaction between the ionizing radiation and target

molecule (Lipetz, 1955; Markus, 1964; Dawson, 1965).

Calculation of direct interaction efficiencies is further complicated by the fact that some of the energy expended by ionizing radiation results in visible light production in the eye medium, which may in turn stimulate the photoreceptors. This consequence will be discussed below.

Initial Bioelectric Response

During stereoisomerization, the electrical response associated with the depolarization of the microvillus membranes is generated. The initial bioelectric response occurs with extremely short latency after absorption of the photon and was first identified by Brown and Murakami (1964) in the outer segments of the monkey retina. They termed it the "early receptor potential" (ERP) to distinguish it from later events which require participation of neurons (ERG). The ERG had a latency of about two msec, while the ERP was recorded about 25 μsec after photon absorption (Cone, 1965).

The maximum ERP found by Cone was generated by 500 nm light, near the absorption peak of rhosopsin and the maximum visual sensitivity of the experimental animal. The ERP is very resistant to anoxia and cannot be eliminated without first abolishing the ERG, leading to the suggestion that the ERG is initiated by the ERP. The ERP is the only visual response linearly related to the intensity of the stimulus; each excited rhodopsin molecule makes a quantized

contribution to the ERP and only the depletion of rhodopsin modifies the response, unlike the ERG which can be modified by pigment migration and neural factors (Pak, 1965).

Generation of the Nerve Potential

The ERP is the initial presynaptic impulse which leads to the later ERG. In order to examine the probable mechanism for the production of the initial potential and the succeeding nerve potential, it is perhaps desirable to give a brief description of nerve depolarization and conduction based on the work by Hodgkin (1958).

The nerve cell in the resting state contains potassium ions and excludes sodium ions against a concentration gradient by actively transporting sodium ions outward through the membrane which is relatively impermeable to sodium. This, along with other factors, results in a polarization of the membrane with the outer surface having a net positive charge. Upon excitation, the membrane becomes much more permeable to sodium; the sodium ions flow into the nerve cell followed by an outflow of potassium ions, resulting in a localized transient depolarization of the membrane. This depolarization which sweeps down the length of the nerve fiber is the site of the electrical impulse, or action potential, associated with nerve conduction. This process also occurs in the wall of the microvillus, a specialized nervous tissue.

If the sodium concentration surrounding the nerve fiber is decreased, the magnitude of the response decreases, and conversely.

The initial visual excitation, as reported by Hagins (1965) with the squid, takes place within seven μ of the point of photon absorption and results in an inward flow of sodium in this region. This depolarization has also been found in Limulus by Fuortes (1959).

The link between depolarization and the stereoisomerization of rhodopsin was demonstrated by Pak (1965), in work with frog retinas. When the retina was perfused with Ringer's solution, both the ERP and ERG were elicited by visible light stimulation. Perfusion with choline chloride, which replaces the sodium ion around the membranes, decreased the ERG but had no effect on the ERP. Addition of potassium chloride, which blocks nerve conduction, completely eliminated the ERG, but the ERP remained.

The ERG, then, may be the result of typical membrane ion flow and depolarization; the ERP must be due to some non-ionic or non-neural process in the receptor cell.

Hubbard et al. (1965) explained this by proposing that the initial photon absorption results not in immediate stereoisomerization and depolarization, but only in a shift of charge distribution on the opsin molecule. This is observed in other proteins where one small molecule (cf. retinal) induces a change in its substrate (cf. opsin).

The ERP might possibly be a product of the initial changes in

the opsin molecule, since these are intramembrane rather than trans-membrane or trans-synapse phenomena which are susceptible to ionic disturbance. The ERG immediately following might be the result of the conformational changes leading to membrane depolarization.

The Electroretinogram

The ERG is a measure of the net bioelectric response of the eye due to the initial photochemical and nerve reactions detailed above. The ERG may be considered a visual response of the organism, although behavioral reactions have been noted at light intensities too low to elicit a recorded electrical response (Bullock and Horridge, 1965). Since the ERG is a product of many primary visual nerve action potentials and their interactions at the ganglionic level, the shape of the ERG response depends on the location of the recording electrodes and the functional state of the synapses involved.

One of the first basic investigations of the ERG was made by Hartline (1928) with Limulus. The typical responses to the beginning (cornea-negative "on" response peak) and cessation (opposite-polarity "off" response peak) of illumination were noted, along with the fact that the ERG was identical from recordings in vivo, from the isolated eye, and from the isolated retina.

By inserting electrodes into specific areas of the compound eye, the sources of the ERG can be determined. At the preganglionic or

rhabdomal level, the response of one ommatidium to a flash of light is a simple monophasic action potential typical of nerve cells. If the region being tested includes the interconnecting nerve fibers from adjacent stimulated ommatidia, the result is a typical diphasic ERG response. The gross response from the whole eye is essentially identical to the localized response (Schiff, 1963).

If nicotine, which blocks synaptic transmission, is placed in the eye, the diphasic response is reduced to a monophasic response, showing the contribution of neighboring receptors and higher-order neurons to the ERG (Autrum, 1958).

In detail, the diphasic response may be a product of either self-inhibition in the primary nerve fiber of a single ommatidium, or interaction of excitatory and inhibitory impulses from several adjacent ommatidia, or a combination of these.

In the first case, observed in some receptor cells, the visual nerve axon may branch, with one fiber connected back to the original fiber by a synapse. Each impulse from the receptor will inhibit additional impulses beyond that synapse by producing a hyperpolariz-ing potential at that point. The fiber inhibits its net output by its own discharge, which tends to oppose any change in receptor output beyond the synapse (Hartline, 1969). Gorman and MacReynolds (1969) studied these hyperpolarizing potentials in the scallop and found that the hyperpolarizing cells produced a transient net increase in output

with a decrease in light intensity.

In the second case, impulses from one or more adjacent ommatidia produce the inhibiting hyperpolarizing potentials in each other. A diagrammatic example can be shown:

receptor cells

nerve interconnectors
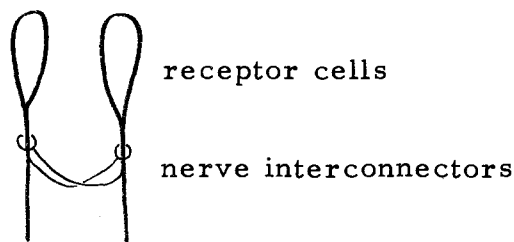
In actuality, the interconnections are more complex and involve many more ommatidia. If both receptors are suddently illuminated by a constant light stimulus, they will initially escape mutual inhibition and will each produce an electrical response. The response from both will decrease shortly thereafter due to mutual inhibition, but will increase again because of a lower inhibition level. This "recurrent inhibition" will produce a damped oscillation in the neural output with any change in light intensity and is a cause of the peaks recorded in the ERG at the beginning and end of a stimulus (Cornsweet, 1969). The magnitude of the oscillation is a function of the light intensity and the number of receptors illuminated, and the frequency is dependent on the distance between mutually inhibiting receptors (Hartline, 1969).

Responses recorded from Hemigrapsus illustrate these points. In Figure 8, it can be seen that the response has two main components: the "on" response peak and the "off" response peak. In Figure 9, the
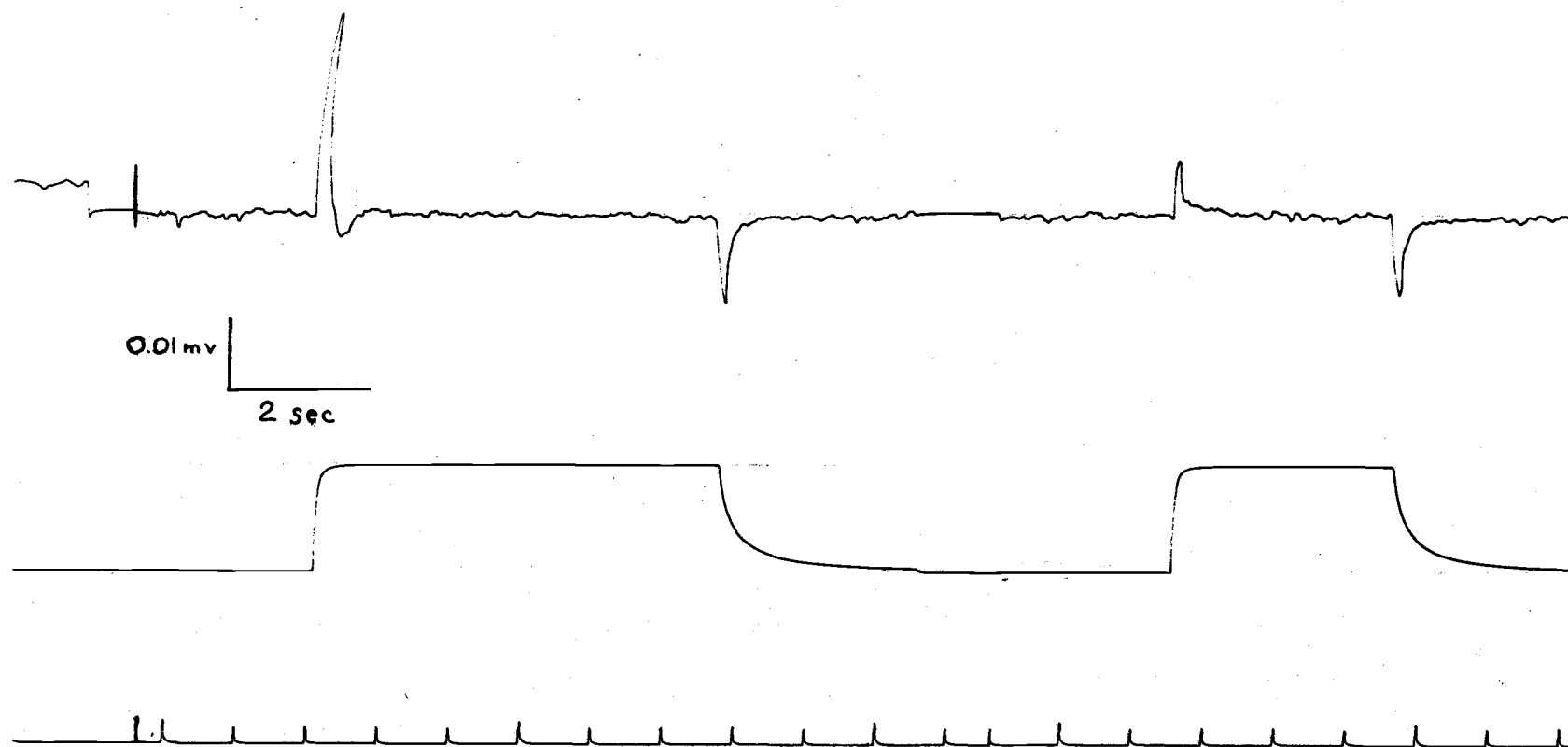
Figure 8. The ERG, showing "on" and "off" response peaks. Upper tracing, ERG response with upward deflection indicating cornea-negative potential; middle, stimulus light marker; lower, time record in seconds. Light stimulus: 500 nm, 5.78×10^3 ergs / cm^2 -sec.
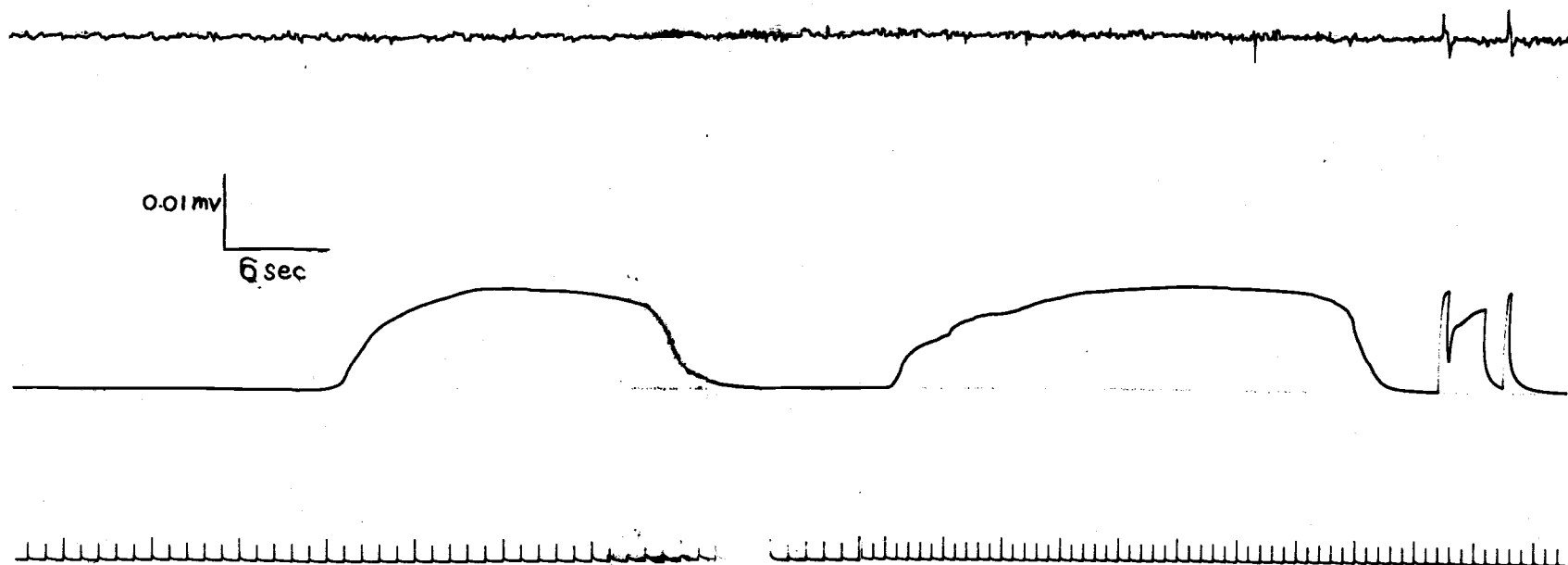
Figure 9. Suppression of the ERG response from a slowly changing light intensity. ERG peaks
are seen at far right when stimulus intensity changes rapidly with time. Light
stimulus: 500 nm, 5.78 x 10^3 ergs /cm^2-sec.

stimulating light intensity has been gradually increased; the ERG is

not seen. These are excellent examples of the visual responses of the

compound eye. Nerve impulses from a constant or slowly changing

stimulus intensity are suppressed, while responses to a sudden change

in light intensity (e. g. , the shadow of a predator) are emphasized.

II. TECHNIQUES AND PROCEDURES

Experimental Animal

The experimental animal used in this research was Hemigrapsus nudus Dana, the purple shore crab, a decapod crustacean in the family Grapsidae (Figure 10). This crab is found among rocks in the inter-tidal region and has a range from Sitka, Alaska to the Gulf of California (Rathbun, 1917). Specimens were collected on the central Oregon coast along a rocky beach south of the Yaquina Head lighthouse near Newport. The specimens averaged 25 to 40 mm across the carapace and had eyestalks approximately 3 to 4 mm long and 1 to 1.5 mm in diameter.

Quantification of the ERG

As explained above, the ERG is a measure of the sensitivity of the eye to visual stimulation, but it can be modified by adaptation, depletion of rhodopsin, temperature, and in the case of the isolated eye, hypoxia and possible hormonal disruption. Therefore, the long-term response of the eye to repeated stimuli can be expected to change, as shown in Figure 11.

Based on this, it was decided to follow each test stimulus with an exposure to a known stimulus, light of 500 nm wavelength, one minute later. By interpolating the bracketing 500 nm stimuli response

Figure 10. Hemigrapsus nudus Dana.
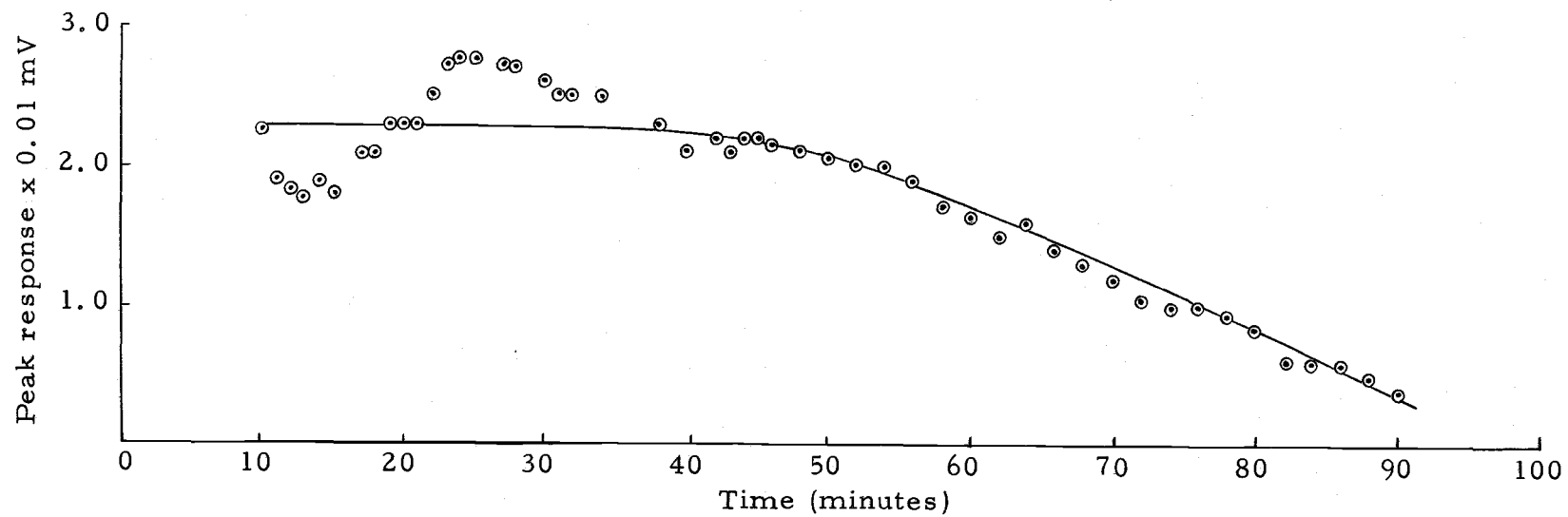
Figure 11. Long term response decrease from 500 nm light stimuli. One flash (1/25 second duration) each minute.

magnitudes to the time of the test stimulus, the relative visual

response compared with the standard stimulus response could be

determined.

The interval between stimuli, although arbitrary, represents a

compromise between the effects of repeated stimuli and the long-term

decrease of the response.

The ERG is composed of several peaks, all of which have been

used in the literature to measure the visual response. The "on"

response is measured from the cornea-negative peak to the transient

positive peak; the peak response, from the cornea-negative peak to

the baseline; and the "off" response, from the cornea-positive peak

to the baseline (Burkhardt, 1962).

In this research, the peak response was used as a measure of the

ERG and visual response, since it was consistently observed and

readily measured.

The amplitude of the peak response to visible light as a function

of stimulus duration is shown in Figure 12. Non-linearity may be due

to the time constants in the amplifying and writing apparatus in the

oscillograph, and the saturation of the ERG at longer flash durations.

The response as a function of stimulus intensity is shown below in

Figure 14, page 38.
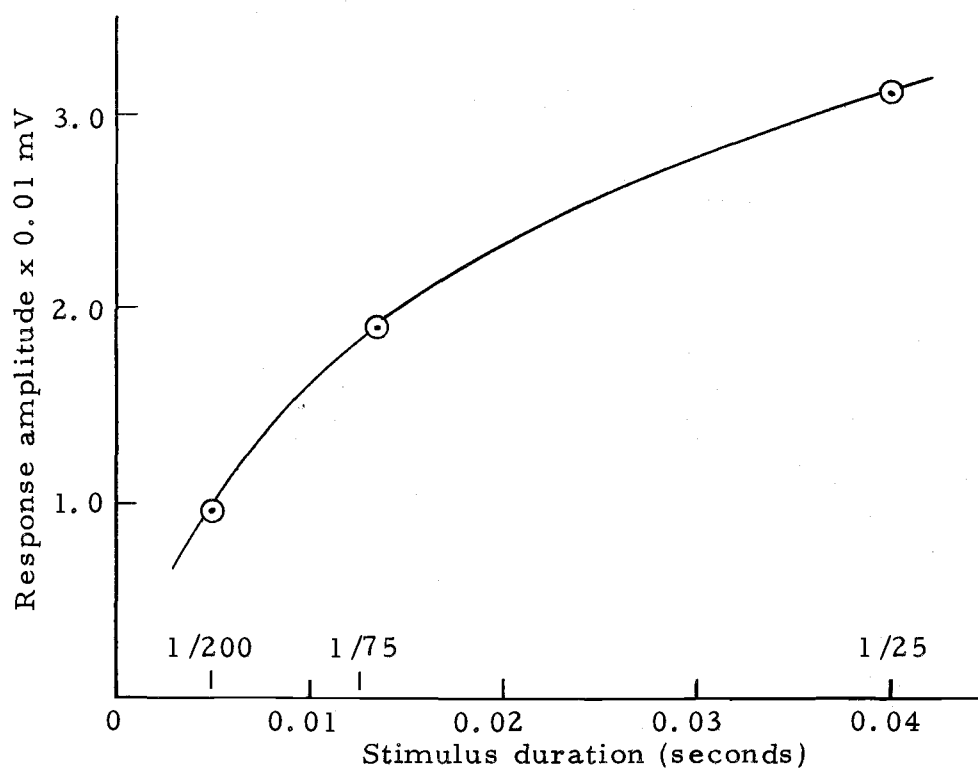
Figure 12. Peak ERG response as a function of stimulus duration. Shutter times are shown in fractions of a second. Light stimulus: 500 nm, 5.78 x 10^3 ergs/cm^2-sec.

Visible Light Action Spectrum
Apparatus and Procedure

To record the ERG, the isolated eye was used in order to minimize large D. C. potentials due to muscle movements, a situation experienced with intact preparations. The isolated eyestalk was placed in a small lucite clamp (Figure 13) and a Grass E-2 platinum reference electrode was inserted to a depth of about 1-2 mm in the severed end of the eyestalk. Adjacent to this was placed a thin cotton wick extending from a small glass pipet holding sea water to keep the eye tissues moist.

A Transidyne No. 410 platinum-clad microelectrode, clamped to the arm of a micromanipulator, was inserted through a pilot hole in the cuticle of the eye to a depth of about 0.5 mm. If the microelectrode was placed too close to the ommatidia which were in line with the light beam, the recorded response was absent or diminished, possibly due to damage of refractive or receptor structures.

The eye preparation was enclosed in a light-tight box with one opening, in front of which was placed a leaf-type camera shutter. The light source was a Bausch and Lomb 150-watt xenon lamp with visible and ultraviolet monochromator gratings. To eliminate overlapping orders of wavelengths, a Corning 3-74 filter was placed between the monochromator exit slit and shutter for wavelengths between 400 and 800 nm, and a Corning 7-54 filter for wavelengths
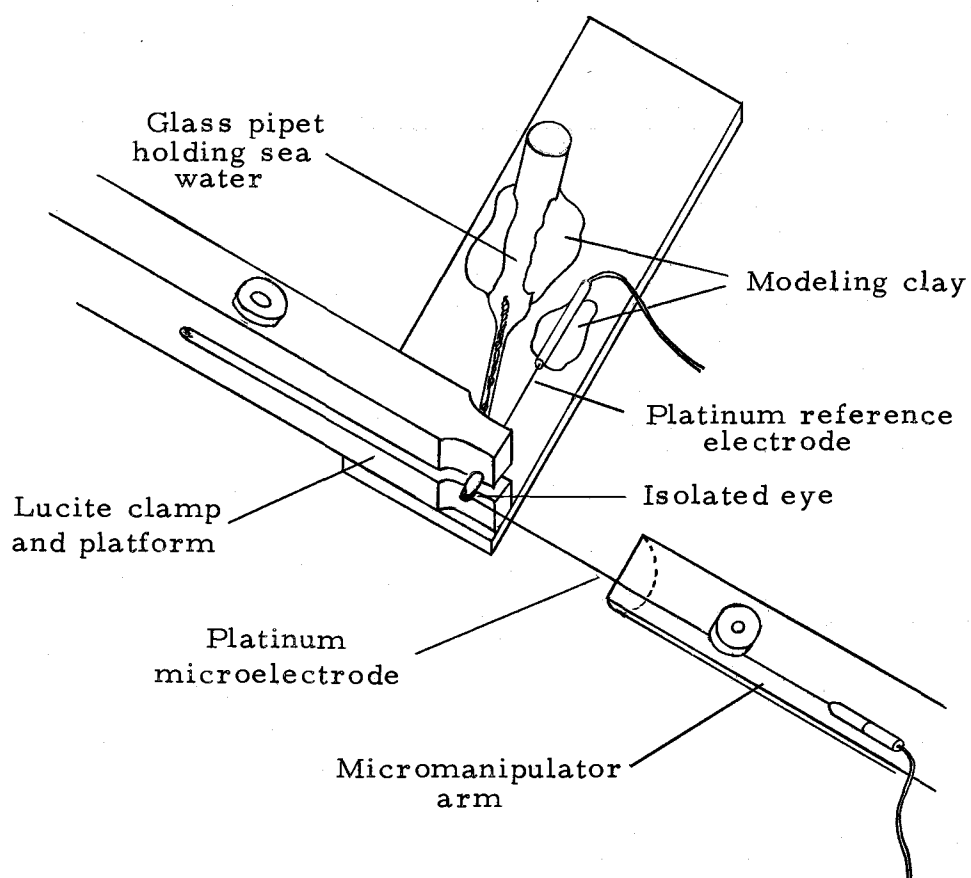
Figure 13. Clamp and electrodes for isolated eye
preparation. Parts exposed to beta or
x-ray beam were painted black.
(Approximately actual size.)

below 400 nm.

Bioelectric responses were recorded on a Grass model 7 polygraph with a model 7 Pl A low-level D. C. preamplifier and model 8 P A ink-writing oscillograph.

If visual responses to several stimuli of different wavelengths are to be compared, the stimuli should be similar in all respects except wavelength. In order to accomplish this, it was decided to use a variation of the procedure of Goldsmith and Ruck (1958). The relative response to each wavelength in question was determined for several intensities at a constant exposure of 1/25 second. The initial light intensity at each wavelength was measured with a YSI model 65 radiometer, and attenuated to levels of 1/2, 1/4, and 1/100 of the initial level with Ealing- TFP neutral density filters. Initial intensities are listed in Table 2.

The responses at each wavelength were plotted as $\log_{10}$ response relative to a standard 500 nm response, versus $\log_{10}$ incident light intensity, producing a family of curves as shown in Figure 14. By drawing a line vertically through the curves at any intensity, the relative response to each wavelength could be obtained.

A quantum energy correction was introduced to normalize the effect of higher and lower energy photons to that of a 500 nm photon (Jagger, 1967). Since photon energy is inversely proportional to wavelength, each relative response at a given stimulus wavelength was

Table 2. Light intensities measured at the eye for grating and filter
combinations used in the action spectrum determination.

Wavelength (nm)	Intensity (ergs/cm^2-sec x 10^3)		
	UV + 7-54	Visible + 7-54	Visible + 3-74
250	0.17	-	-
275	0.47	-	-
300	0.63	-	-
325	0.70	-	-
350	0.60	2.10	-
375	0.80	2.70	-
400	0.60	1.10	0.20
425	-	-	4.00
450	-	-	5.62
475	-	-	6.75
500	-	-	5.78
525	-	-	6.43
550	-	-	6.40
575	-	-	6.00
600	-	-	5.58
625	-	-	5.42
650	-	-	4.80
675	-	-	4.85
700	-	-	4.80
725	-	-	4.70
750	-	-	4.60
775	-	-	4.10
800	-	-	3.65
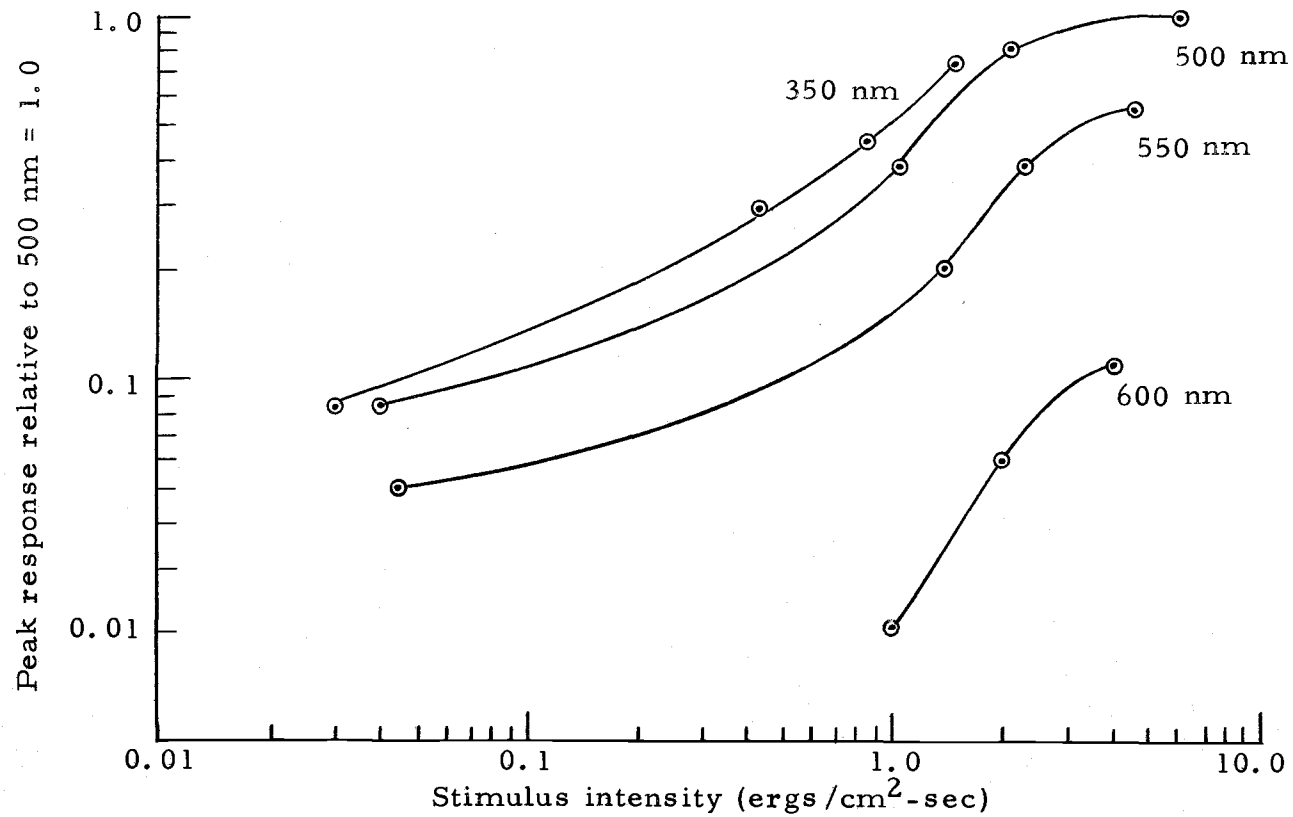
Figure 14. Relative peak ERG response as a function of intensity. Wavelengths 350, 500, 550, and 600 nm are shown. Stimulus duration: 1/25 second. Data are not corrected for quantum efficiency.

multiplied by the factor:

$$\frac{\text{stimulus wavelength}}{500 \text{ nm}}$$

Thus a relative response at a given wavelength may be equated to the response at 500 nm.

A correction for corneal absorption was made, based on data from Wolken (1968), who determined the absorption spectrum of the Drosophila corneal lens. Results including and neglecting corneal absorption were calculated.

In summary, a 500 nm stimulus was followed by a stimulus of a desired wavelength at the intensities mentioned; this was repeated for each wavelength. The resulting responses were plotted on log-log graph paper and action spectra were extracted at intensities of 0.10, 0.30, 1.0, 2.0, and 3.0×10^3 ergs/cm^2-sec. Since the stimulus intensities were reduced from the maximum available at each wave-length, the curves did not overlap for the full range of intensities. In order to obtain a full action spectrum, the relative responses for all intensities covered in a particular wavelength were averaged together. While this may have modified the general shape of the spectrum, the locations of the peak sensitivities were not altered[2].

---

[2] In preliminary experimentation, alternating test and 500 nm stimuli were used and an intensity correction factor was introduced. This also resulted in identical response peaks, although the shape of the spectrum was greatly distorted at high correction factors.

Beta Source and Dosimetry

Beta radiation sources were sealed Tracerlab Medical Applicators containing strontium-90 and yttrium-90 in equilibrium. Two applicators were used, one containing 100 mCi and the other containing 50 mCi of strontium-90. The cup containing the source was capped with a thin metal window; overall dimensions were 1/2" diameter x 1/2" long. The cup was mounted on a 6 3/4" long shaft which allowed the source to be handled.

Excepting bremsstrahlung due to the window and a weak gamma ray produced by yttrium-90 decay, essentially pure beta radiation was emitted. Specifically, these betas were of 0.54 MeV$_{max}$ from strontium-90 ($t_{1/2}$ = 28 years) and 2.26 MeV$_{max}$ from yttrium-90 ($t_{1/2}$ = 62 hours).

Dose rates, as determined below, for the 100 mCi source were 380 rads per minute at 1 cm from the applicator face and 700 mrads per minute at 6 cm, as measured at the eye with the beta exposure apparatus delivering one 1/20 second flash per second. For the 50 mCi source, dose rates were 137 rads per minute and 260 mrads per minute respectively.

Lithium fluoride thermoluminescent dosimeters were used for source calibration and dose rate measurements due to their tissue equivalence, dose rate independence (McCall, Babcock and Fix, 1964), and small size (1.25 mm diameter x 6.5 mm long) which closely approximated the dimensions of the eye.

Lithium fluoride dosimeter calibration factors were based on an earlier dose rate measurement of the 100 mCi source using ultra-thin lithium flouride dosimeters (Banchune, 1969). A series of ten dosimeters were inserted in a small hole drilled in a 3-cm thick lucite sheet and exposed to the 100 mCi source at distances of 1, 2, and 6 cm. Since the dose rate at 1 cm from this source was known, calibration factors were calculated by dividing the known dose rate by the mean dose rate value measured by the dosimeter rods at 1 cm distance. Subsequent measured dose rates were multiplied by this factor in determination of the 50 mCi dose rate and absorbed dose measurements duplicating experimental conditions. All calibration measurements were within an error of 10%.

Normalization factors, or the correction of each dosimeter to the mean response of all dosimeters used, had been determined by Barone (1970), and due to their low average values of 3%, were not included in calculations.

Beta Exposure Apparatus

For presenting the eye with flashes of either beta radiation or visible light, a rotating steel disc with two 5/16" holes drilled at the edge was used (Figure 15). The holes were located opposite each other such that when one hole exposed the eye to the stimulus, the other hole was in front of a shielded light source used to actuate a photocell. The

Photocell light source

Rotating disc with
two apertures

Photocell mounted
on movable arm

Steel face-plate

Eye preparation

Variable-speed
DC motor

Beta source and
light pipe clamp

Beta source
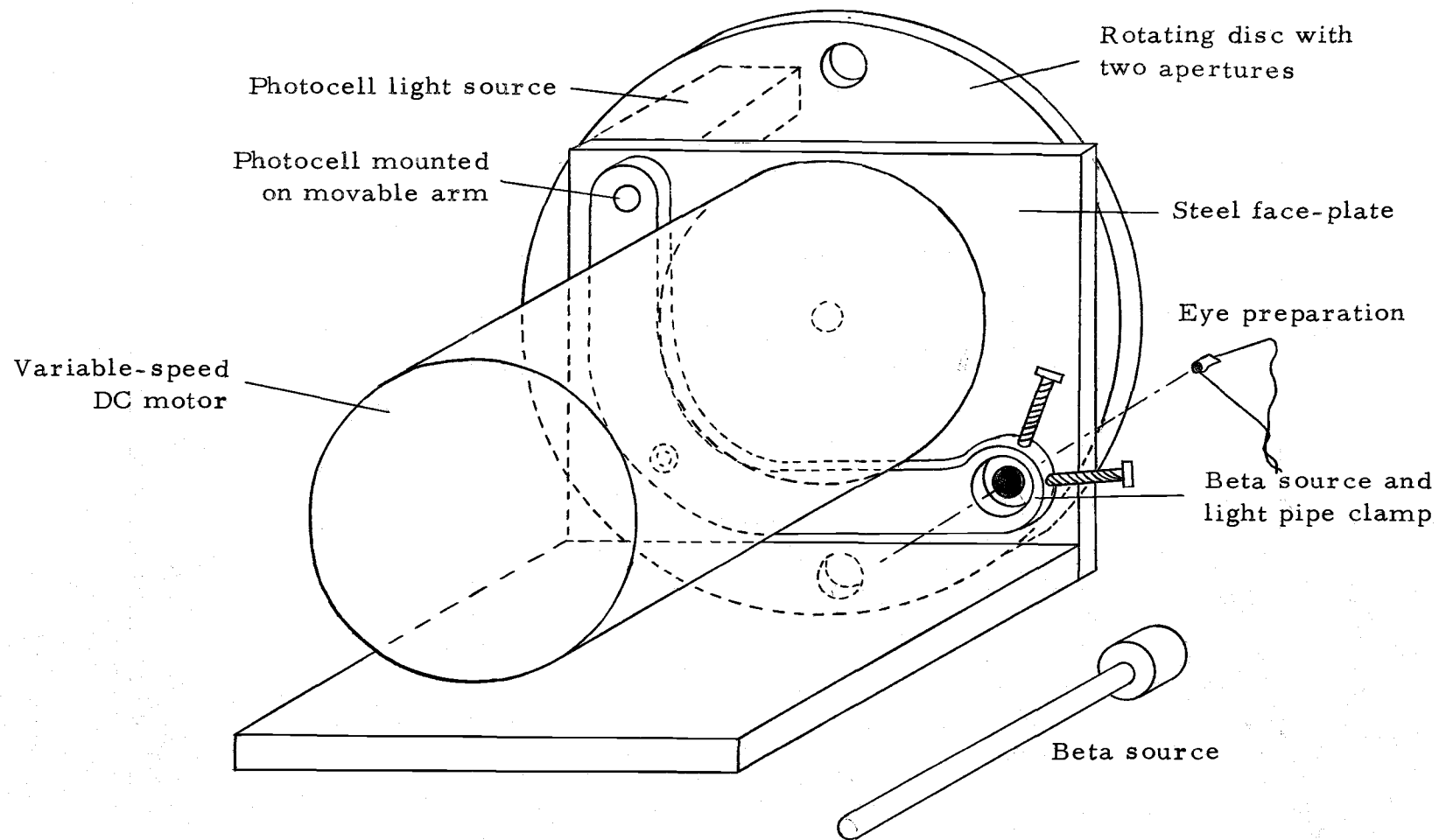
Figure 15. Rotating-wheel beta exposure apparatus.

photocell output was fed into one channel of the oscillograph and served as an exposure monitor. The photocell and stimulus source were mounted on a bracket which, when rotated through a small arc, brought both the photocell and source into their respective positions in front of two holes in the steel face plate.

A lucite eye clamp, described above, held the eye 6 cm from the face of the stimulus source. All parts exposed to the radiation were painted black to prevent any fluorescence from stimulating the eye.

Xenon and tungsten light sources in conjunction with a monochromator provided visible light of 500 nm wavelength. Intensities at the position of the eye were 225 ergs/cm^2-sec for the tungsten source and 817 ergs/cm^2-sec for the xenon source. Light stimuli were directed toward the eye through a light pipe, the end of which was clamped into the source holder.

The rate of wheel rotation was increased in order to decrease the duration and dose presented per flash. At one flash per second, each stimulus was about 1/20 second in duration; a complete summarization of stimulus durations, and beta radiation and visible light incident upon the eye are listed in Table 3. It should be noted that as the rate of revolution is increased for shorter exposures, the number of exposures per unit time increases. Therefore, the net exposure per unit time remains constant.

Table 3. Beta radiation absorbed dose and visible light exposure, measured at the eye.

Flash rate	Stimulus duration	Beta dose per flash (mrads)		Light exposure per flash (ergs/cm^2)	
		100 mCi	50 mCi	Xenon	Tungsten
1/sec	1/20 sec	11.6	3.1	40.8	11.2
2/sec	1/40 sec	5.8	1.6	20.4	5.6
3/sec	1/60 sec	3.5	0.9	13.6	3.7

Beta Exposure Procedure

In determining the relative response of the eye to beta radiation and visible light, three procedures were followed. First, single flashes of beta radiation and visible light were presented in order to compare the magnitudes and shapes of the ERG responses. Next, the course of adaptation to an extended series of beta or visible stimuli was recorded, measured by the ERG response of each flash. Finally, the course of recovery from beta and visible stimuli was observed by using a 500 nm light flash to elicit the ERG at various times after cessation of the adapting stimulus.

X-Ray Source and Exposure Apparatus

A General Electric model D-2 diagnostic x-ray machine served as an x-ray source, operating at 70 kVp and tube currents between 1 and 10 mA. The x-ray tube enclosure was mounted on top of a lead-lined cabinet.

Micromanipulators holding the eye clamp and microelectrode were mounted on a plywood shelf and could be inserted and removed from the irradiation enclosure as a unit in order to permit electrode implantation.

X-ray exposures were controlled with a solenoid-actuated focal plane shutter constructed of lead. The stimulus duration and interval between stimuli could be independently varied by an electronic

control, the circuit diagram of which is given in the appendix. In preparations where electrical interference was of a significant magnitude, the shutter was operated manually.

The isolated eye preparation was enclosed in a small lead box with openings for the micromanipulator arms. The x-ray beam entered through a hole 1 cm in diameter such that only the eye and electrode tips were exposed (Figure 16).

The x-ray beam was monitored by a photoconductive cell, over which was placed a piece of fluorescent screen. This assembly was covered by an opaque plastic cap so that no visible light could escape.

X-Ray Dosimetry

Since x-rays are emitted in a spectrum of energies, the specification of kVp alone does not completely describe the quality of the radiation. For this research, the first and second half-value layers[3] were determined and expressed as:

$$\frac{\text{First HVL}}{\text{Second HVL}} = \text{Homogeneity coefficient}$$

where the homogeneity coefficient is a measure of the energy composition of the beam. With monoenergetic gamma radiation, for

[3] The first half-value layer is the filtration thickness needed to attentuate the incident beam by one-half; the second half-value layer is the additional thickness needed to attenuate the beam by one-fourth.

X-ray tube

X-ray beam path

Lead shutter   Solenoid

Opaque plastic cap
Fluorescent screen
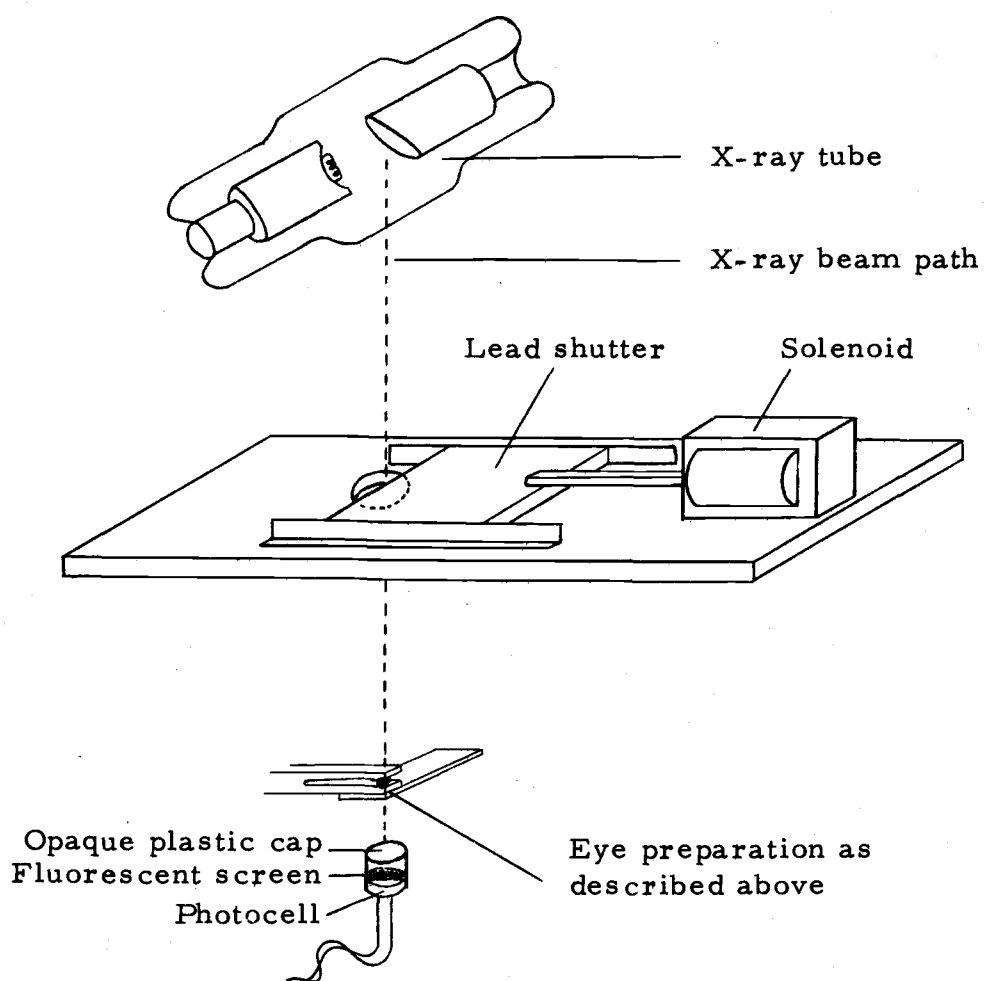Photocell

Eye preparation as
described above

Figure 16. X-ray exposure apparatus. Lead enclosure
around eye preparation not illustrated for
clarity.

example, additional filtration changes only the intensity and not the energy of the incident beam; the homogeneity coefficient is 1.0. With x-rays, the lower-energy radiation is attenuated more rapidly by filtration, leaving a beam made up of higher energy photons. Thus the second half-value layer will be larger than the first and the homogeneity coefficient will be less than 1.0 (I. C. R. U., 1964).

Scattered radiation due to beam filtration will produce inaccuracies in these measurements (Trout, Kelley and Lucas, 1960). At any fixed geometry, however, the effect of scatter is linearly related to the diameter of the irradiated filter. Inaccuracies can be eliminated by determining half-value layers at two different diameters and extrapolating the results to zero diameter. This extrapolated value is termed the unique half-value layer.

Aluminum first and second half-value layers were measured at 70 kVp using diaphragms of 1 cm and 4 cm diameter. The resulting unique first half-value layer was 0.54 mm aluminum; second unique half-value layer, 1.21 mm aluminum; and homogeneity coefficient, 0.45.

The effective energy of the beam was obtained from the first half-value layer by determining the energy of monoenergetic gamma radiation also having a half-value layer of 0.54 mm aluminum (Grodstein, 1957). At 70 kVp with no added filtration, this value was 17.5 keV.

Lithium fluoride thermoluminescent dosimeters, described above, were calibrated with the same x-ray unit at 70 kVp using a Victoreen R-meter and a 25 R thimble chamber. Chamber exposure rates were measured in air and corresponding measurements were made with the LiF dosimeters centered on a sheet of saran wrap stretched over a thin wire loop.

All chamber exposures were corrected for temperature, pressure, and energy response. The corrected values were converted to absorbed dose by the formula (I.C.R.U., 1964):

$$D_{med} = 0.86 \frac{(\mu_{en}/\rho)_{med}}{(\mu_{en}/\rho)_{air}} X$$

where

D_{med} = absorbed dose in tissue (water)

X = measured exposure in roentgens

(μ_{en}/ρ) = mass energy-absorption coefficients for water and air (0.83 and 0.81 respectively).

LiF calibration factors were based on the mean values of 10 chamber measurements and 10 dosimeter measurements. With this reference point, absorbed dose rates at the position of the eye were obtained both directly and indirectly to determine the effect of removing and replacing the preparation support and lead collimator shielding.

First, dosimeters were placed in the eye clamp and irradiated at each of the tube currents used. Each exposure necessitated the removal of the preparation support from the cabinet and replacement

for the next exposure. Second, the mean of one set of LiF dosimeter values taken in the eye clamp was used to convert the in-air thimble chamber exposure rates at each mA value to in situ dose rates. Thus, the first set of values reflected the variation in centering of the eye under the beam port, and the second set served as a control whose values were affected only by the tube current.

As can be seen in Table 4, the dose rates differ by less than 6%.

Table 4. X-radiation absorbed dose rates at the position of the eye. Values are shown as calculated from in-air exposure rates and as measured by LiF thermoluminescent dosimeters.

Tube current at 70 kVp (mA)	Absorbed dose rates	
	Measured (rads/sec)	Calculated (rads/sec)
1	0.27	0.26
3		0.78
4	1.04	1.04
5	1.25	1.30
6	1.49	1.60
8	2.00	1.98
10	2.32	2.42

X-Ray Exposure Procedure

X-ray responses were elicited to determine the shape of the ERG and the effect of each x-ray exposure on the succeeding response in a series of responses. Due to the inertia of the lead shutter, flash durations were limited to a minimum of about 1/5 second. In

addition, the operating limitations of the x-ray machine (at 10 mA,

two minute's operation required a two-hour cooling period) made short

exposure times necessary in order to be able to continue stimulation

of the eye before response deterioration from other factors occurred

(see page 29).

As with visible light, ERG response amplitude as a function of

dose rate was determined.

Stimulus intensities were also gradually changed by slowly

opening and closing the shutter, in order to compare the x-ray response

with that of visible light under these conditions.

Microwave Apparatus and Procedure

A klystron and power supply were used to generate microwaves

of 3 cm wavelength. The power output as measured with a bolometer

was no higher than 1-2 mW/cm^2. The visible light used in conjunction

with microwave stimulation was a filtered incandescent source of 525

nm wavelength; the intensity at the position of the eye was approxi-

mately 8 x 10^3 ergs/cm^2-sec.

The apparatus was mounted on an optical bench and arranged,

as illustrated in Figure 17, to allow the eye preparation to be placed

at such a distance from the end of the waveguide that a maximum

standing wave existed in the region between the source and target. A

grounded copper shutter on an aluminum sheet was placed directly in
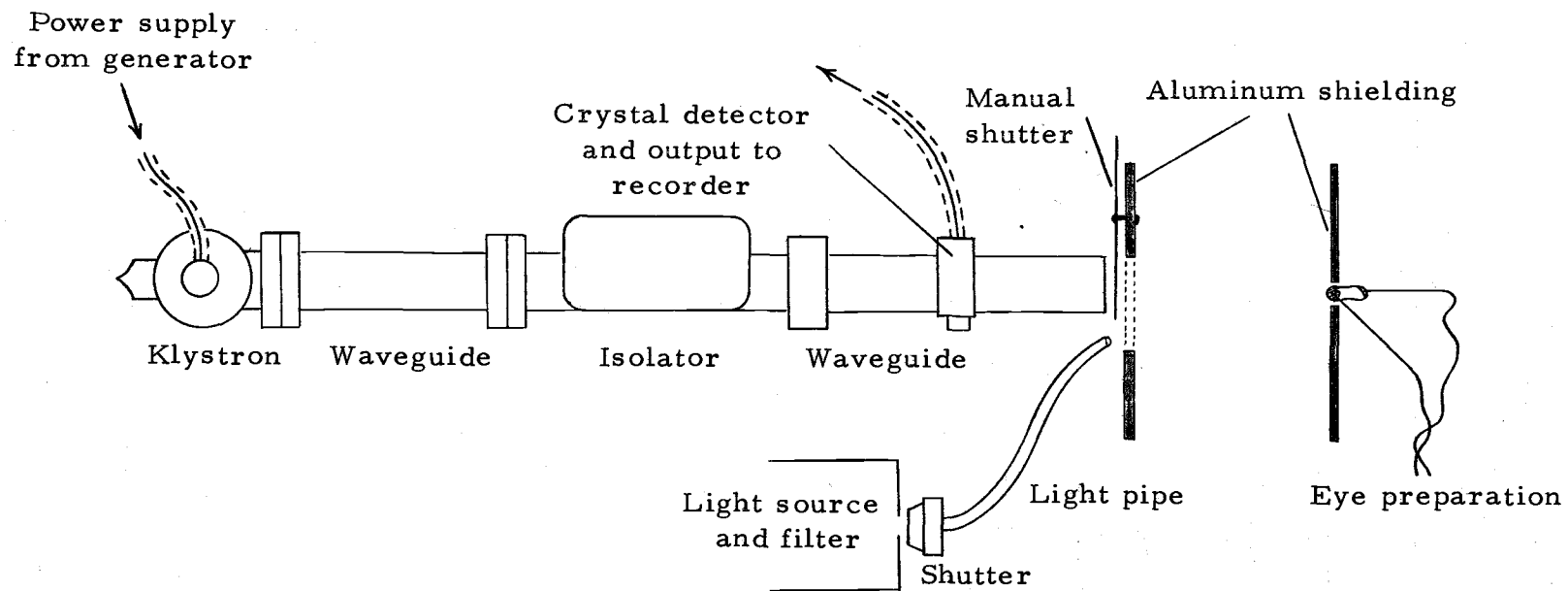
Figure 17.   Microwave-exposure system.   The eye preparation is identical to that
described above.

front of the waveguide. The crystal detector output was fed into one channel of the oscillograph to permit continuous monitoring of relative standing wave intensity, thereby allowing both the adjustment of optimum source-eye distance and recording of shutter position, since any change in the standing wave pattern was seen as a change in monitor output.

The eye was extended through a small hole in a grounded sheet of aluminum behind which all electrode implantations were made, in order to prevent direct interference of the microwave radiation with the electrodes and leads.

For simultaneous exposure to microwave and visible stimuli, a glass fiber "light-pipe" was mounted under the end of the waveguide. The metal shutter obscured only the waveguide, and a camera shutter at the light source controlled light stimuli. By manually operating the metal shutter, microwave stimuli of approximately 1/2 second duration could be given; visible light stimuli were 1/25 second long.

Two experimental procedures were conducted, the first to determine the effect of microwave stimulation alone on the eye, and the second to determine the effect of microwaves on the visible light-induced ERG.

III. RESULTS AND DISCUSSION

Ultraviolet and Visible Light
Action Spectrum: 250-800 nm

The action spectrum obtained with light stimulation from 250 to

800 nm showed distinct peak sensitivities at 500 nm (blue-green) and

350 nm (near-ultraviolet), either considering or neglecting corneal

absorption (Figure 18). Beyond wavelengths shorter than 275 nm and

longer than 700 nm, a response was present but of too low a magni-

tude for accurate quantification. Wavelengths below 400 nm had band

widths of $\pm$ 9.6 nm; above 400 nm, $\pm$ 19.2 nm.

Additional points, which were included in the introduction,

should be mentioned here since they are of importance in interpreting

the responses to ionizing radiation. In all cases, the peaks in the

visible light-induced ERG were rather sharp (Figure 4). Both the

"on" and "off" responses were elicited with rapid increases and de-

creases in light intensity, but neither peak was observed with a grad-

ual change in intensity (Figure 8, 9). The magnitude of the ERG peak

response was linearly related to stimulus intensity at constant stimulus

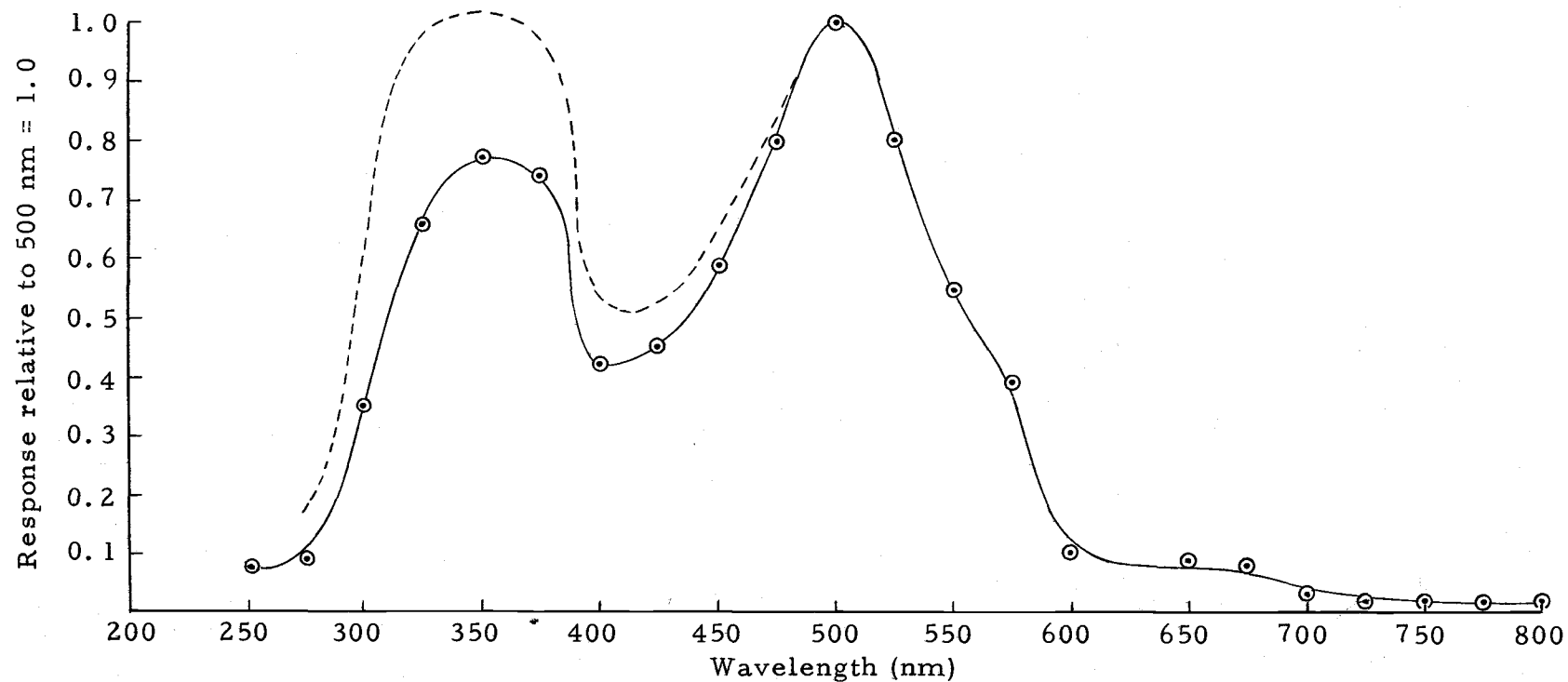duration, but saturated at high intensities. When plotted on logarithmic

Figure 18. Action spectrum from wavelengths 250 to 800 nm. Response peaks are at 350 and
500 nm. Dotted line indicates action spectrum after correcting for corneal
absorption.

coordinates, this resulted in a sigmoid curve (Figure 14).

Beta Radiation

In the attempt to compare the relative magnitudes of beta and visible light ERG responses by stimulating the eye alternately with flashes of these radiations, it was noticed that the light and beta responses elicited from different eyes had little quantitative relation to each other. Relative responses to a pair of stimuli in one eye were not equal to the relative responses to the same intensities in another eye. In fact, an optimal beta response was not necessarily followed in the same eye by an observable light response, even though both stimuli had been shown, in other preparations, to produce a measurable ERG (Figure 19).

If the ERG responses to beta radiation (Figure 19, 20, 21) are compared to those of visible light (Figure 4, 8, 22), two generalizations can be made: first, the beta response lacks the "off" response, and second, the "on" response does not drop sharply like that of visible light. Instead, the beta response appears to decay to the baseline. A combination of these factors may be the cause of the baseline rise especially evident in Figure 21, but observed in even minimal responses during irradiation.

The course of response decrease to a series of beta stimuli (Figure 20, 21) as measured by the amplitude of the "on" response

Figure 19. Beta radiation and visible light stimulation of the isolated eye. First two beta stimuli each deliver 3 mrads in 1/20 second; second two 500 nm light stimuli each deliver 41 ergs/cm^2 in 1/20 second. Upper line, two 0.015 mV beta responses and two very small visible light responses; middle line, exposure monitor; bottom line, time scale in seconds.

Figure 20. Responses to a series of beta stimuli from 100 mCi strontium-90 source. Absorbed dose per 1/20 second flash: 11.6 mrads.
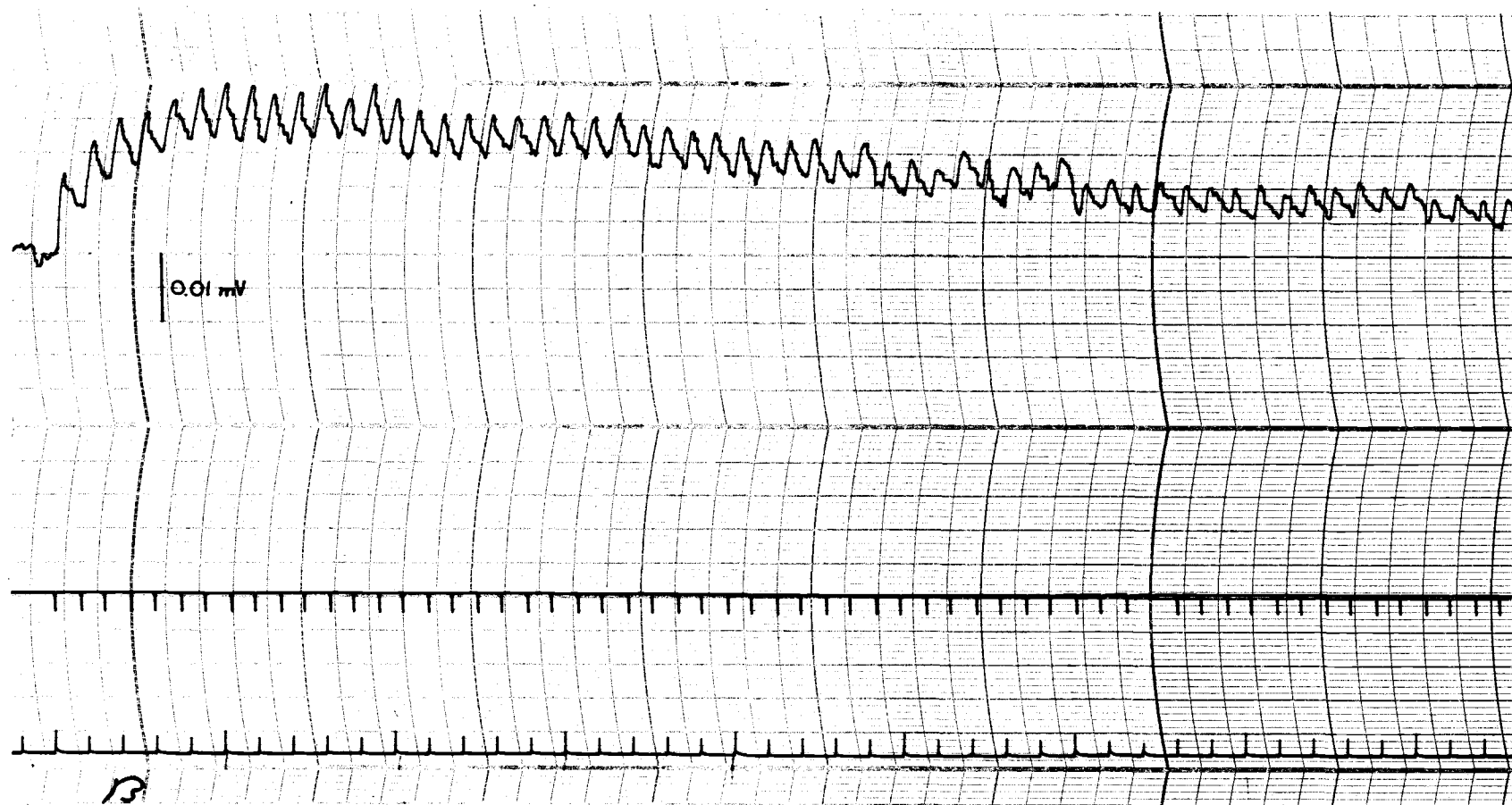
Figure 21. Responses to a series of beta stimuli from 50 mCi strontium-90 source. Absorbed dose per 1/20 second flash: 3.1 mrads.
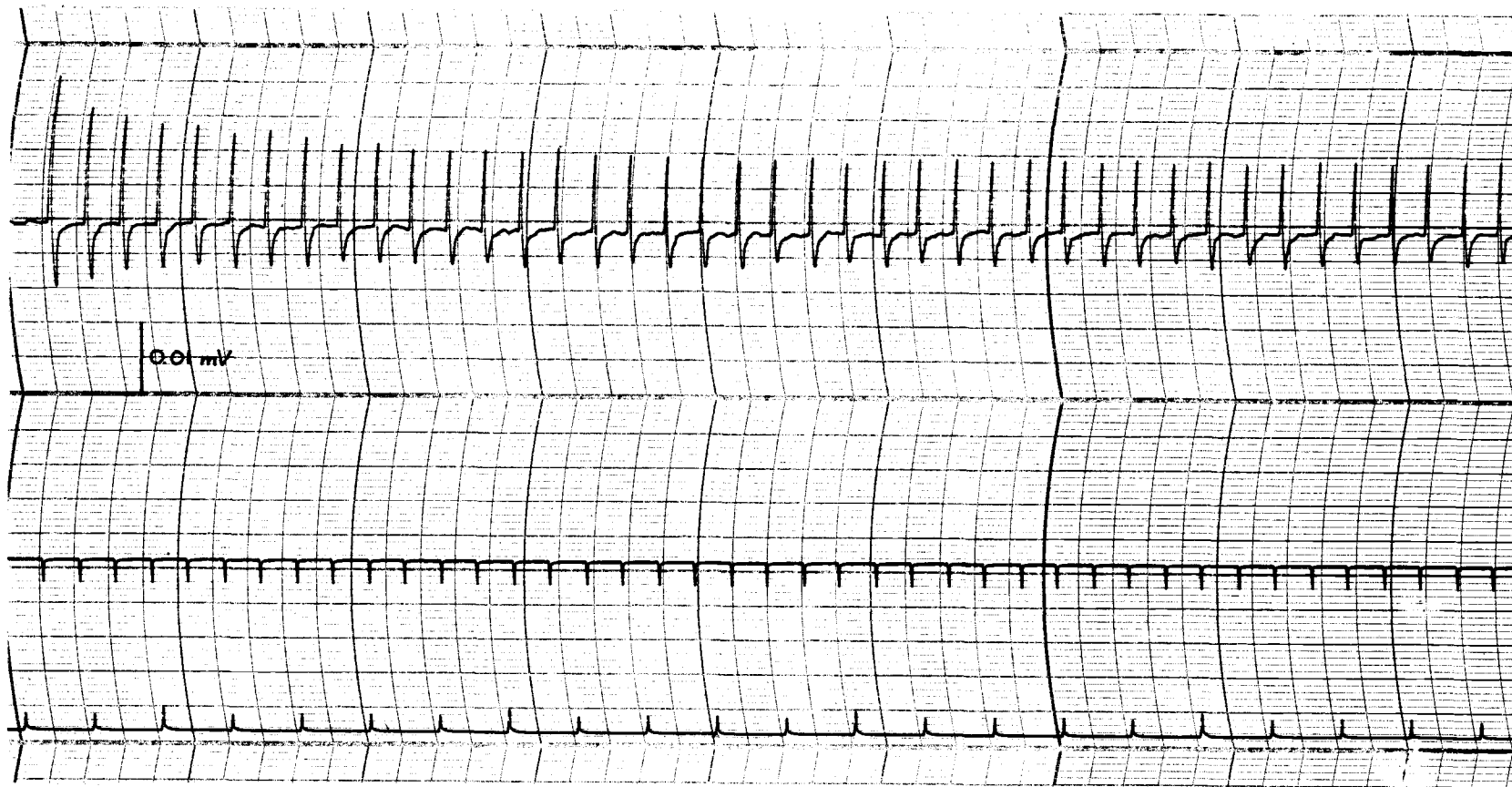
Figure 22. Responses to a series of 500 nm light stimuli. Total exposure per 1/40 second flash: 20.4 ergs/cm^2.

above the tail of the previous response, and results from a similar

series of visible light stimuli, as measured by the peak response, are

summarized in Figure 23.

The response to visible light declined to an asymptote clearly

visible as ERG peaks with amplitudes at least three times the noise

level, even after several minutes of stimulation. The beta responses

gradually merged with the noise and became indistinguishable except

for the baseline rise within a minute.

The points on each curve are averages of several responses with

stimuli presented at one, two, and three flashes per second. The dose

per unit time at the eye was constant with any one of the stimulus

sources due to the inverse relationship between dose per flash and

flashes per unit time.

Recovery of sensitivity to further beta stimulation after the

response had been diminished by either continuous beta exposure or an

extended series of stimuli, was seen only as a slight rise in the base-

line during irradiation, even when beta stimuli were presented up to

20 minutes after termination of a previous beta exposure. Sharp

response peaks to beta stimuli were never obtained after the initial

decrease of the beta response.

Stimulating the eye with flashes of visible light (xenon source),

however, did not affect a subsequent response to beta radiation.

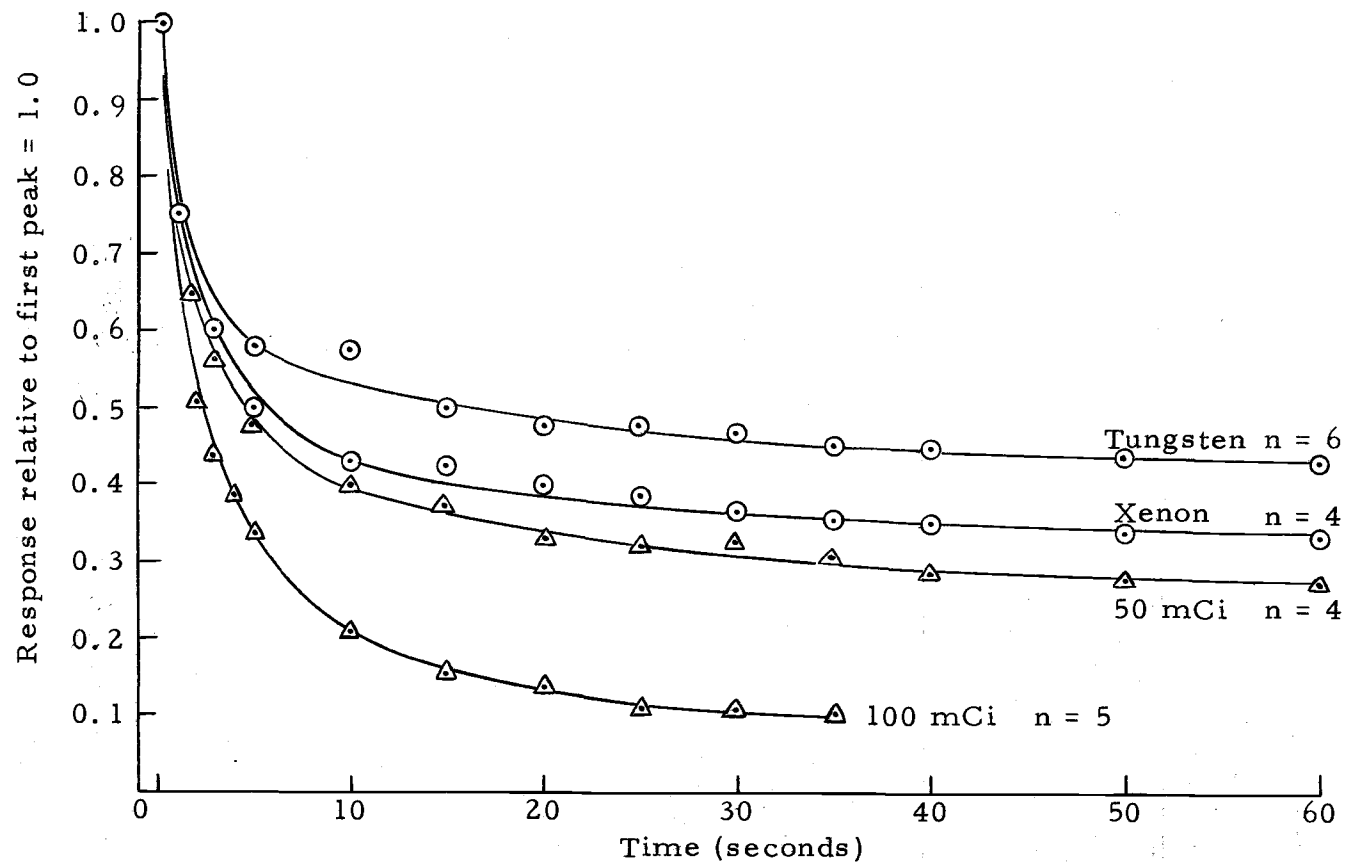Conversely, sensitivity to visible light stimuli was not significantly

Figure 23. Response decrease to beta and light stimuli. Average of responses to one, two, and three flashes per second.

affected by previous exposure to beta radiation. Following a one-minute continuous exposure to beta radiation (about 28 rads), the asymptote of a series of visible light responses was about 0.008 mV. Following a four-minute exposure (110 rads), the asymptote was 0.007.

The minimum beta dose which produced a measurable "on" response was between 0.9 and 1.6 mrad. These doses were presented in a series of 1/60 and 1/40 second stimuli from the 50 mCi source. The 0.9 mrad dose series resulted in only a slight rise in the baseline.

X-Radiation

Responses to single flashes of x-rays (Figure 24) clearly showed the "on" and "off" responses discussed above. In comparing the shape of the x-ray peaks with those elicited with light (Figure 8), it can be seen that the x-ray response is less clearly defined. The responses more gradually return to the baseline, much as in the case of the beta responses previously discussed.

Another similarity between the x-ray ERG and the visible light ERG is found in Figure 25. When the stimulus intensity was slowly increased or decreased, no responses were recorded. Identical results are shown in Figure 9 for visible light.

The decrease on the x-ray responses resulting from either repeated stimuli at two flashes per second (Figure 26) or constant exposure was insignificant.
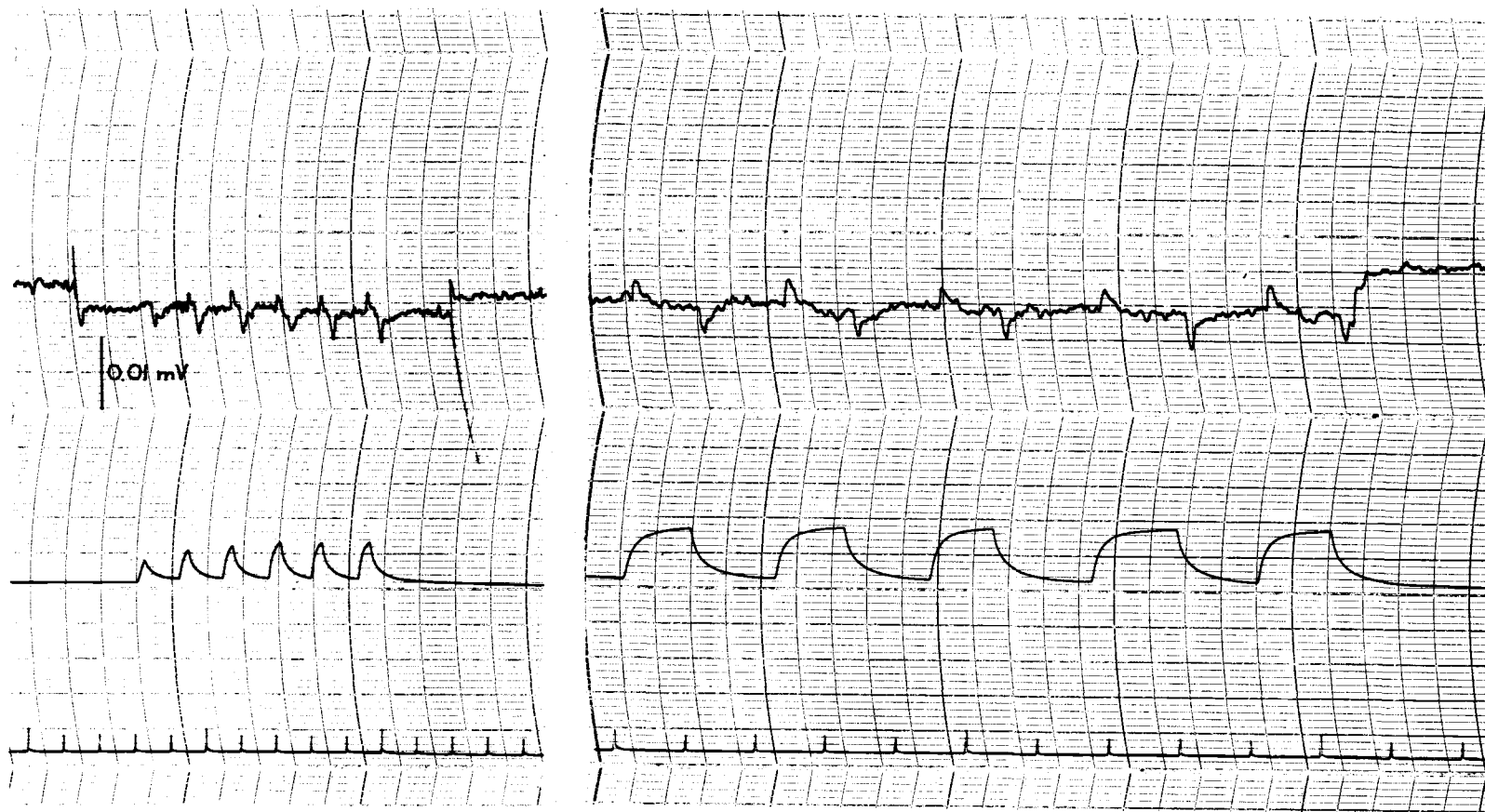
Figure 24. ERG response to x-ray stimuli, showing "on" and "off" response peaks. Dose rate for first set of responses, 1.7 rads per second; second set, 2.0 rads per second. Middle line, photocell exposure monitor with upward deflection showing beam on; bottom line, time record in seconds. Sharp peaks at beginning and end of stimulus series are due to x-ray machine timer.
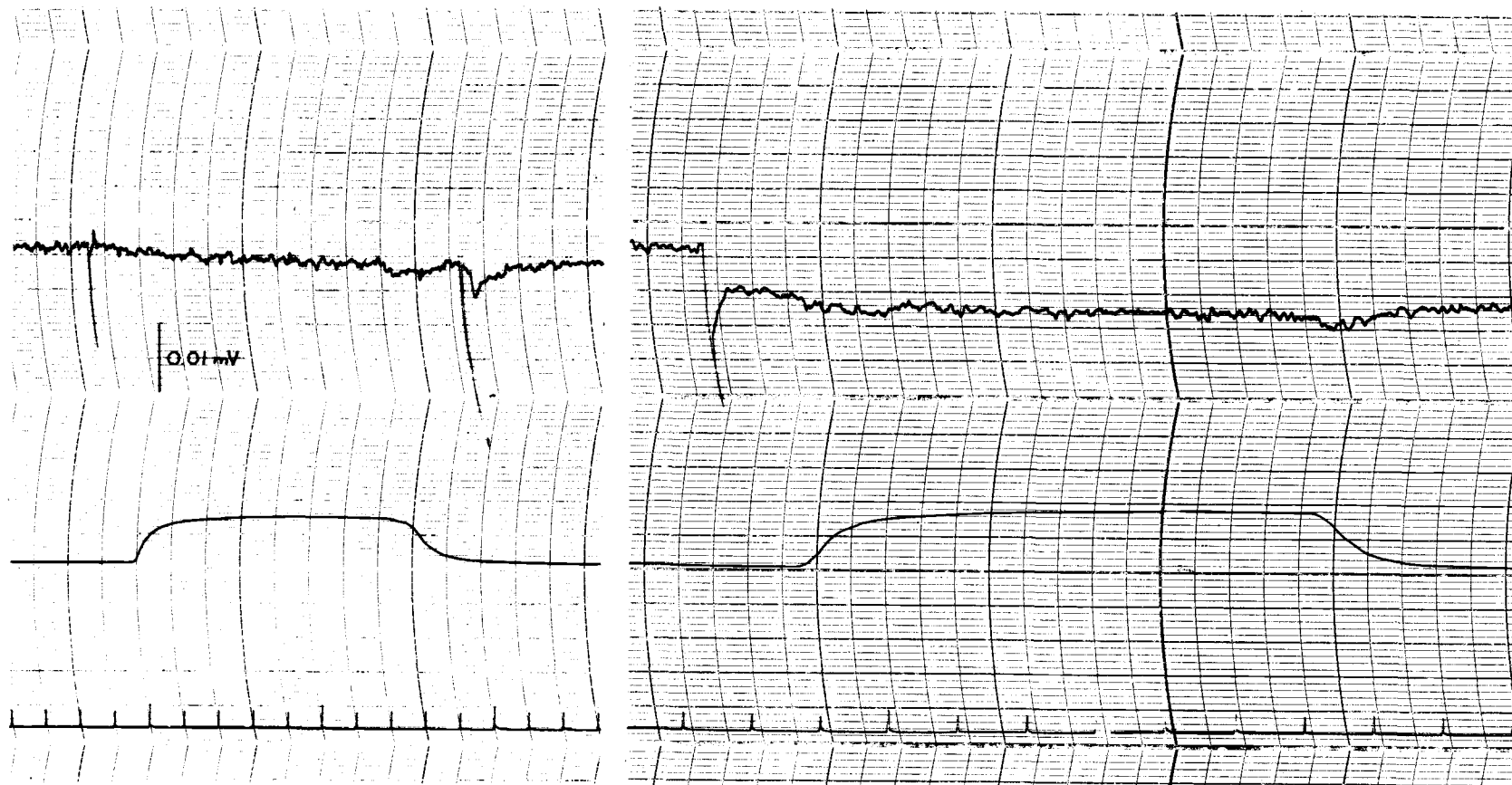
Figure 25. Failure of gradual x-ray intensity change to elicit the ERG. Dose rates: left, 1.7 rads per second; right, 2.0 rads per second. Sharp peaks are artifacts due to x-ray timer operation.
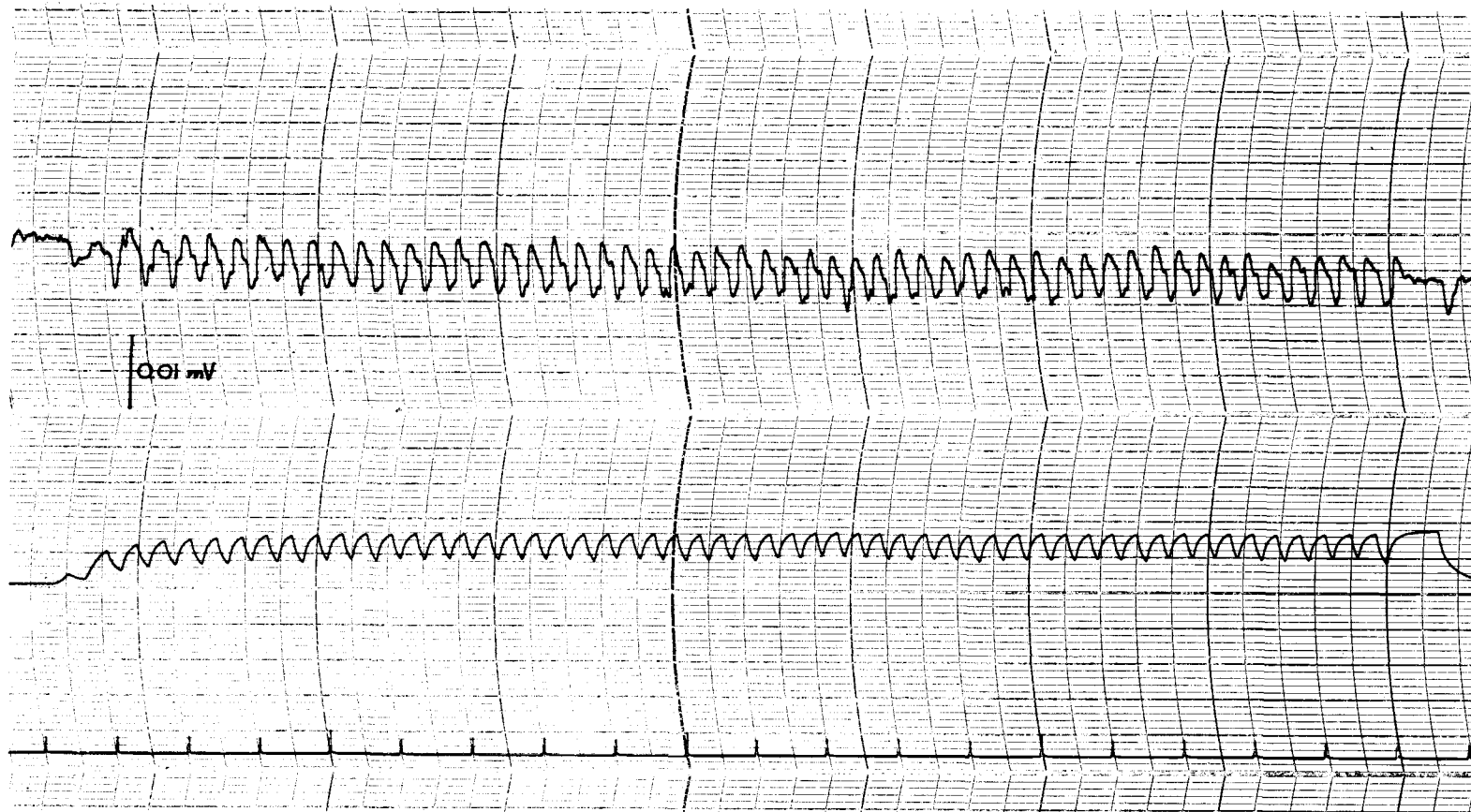
Figure 26. Response to a series of x-ray stimuli. Absorbed dose per flash: approximately 0.4 rads.

The peak response amplitude as a function of absorbed dose per flash (Figure 27) was nearly linear. When the same points were plotted on logarithmic coordinates (Figure 28), the similarity between the visible light and x-ray data could be seen (compare with Figure 14).

At the lowest tube current possible, an absorbed dose of 50 mrads produced a 0.002 mV response. This approached the minimum response detectable with the technique and apparatus used.

Microwaves

With direct microwave stimulation, neither an ERG response nor a change in the dark-adapted visual activity could be detected. There was no observed modification of the visible light response under microwave stimulation when the light flash was presented during microwave irradiation.

Discussion

Visual responses in the ultraviolet region have been found in many invertebrates. The question is not their presence, but whether the existence of more than one peak sensitivity represents different origins of the response, specialized receptor pigments, or simply the result of differential absorption of fluorescence in the eye structures.

Both Schiff (1963) and Carlson, Smith and Stanley (1968)
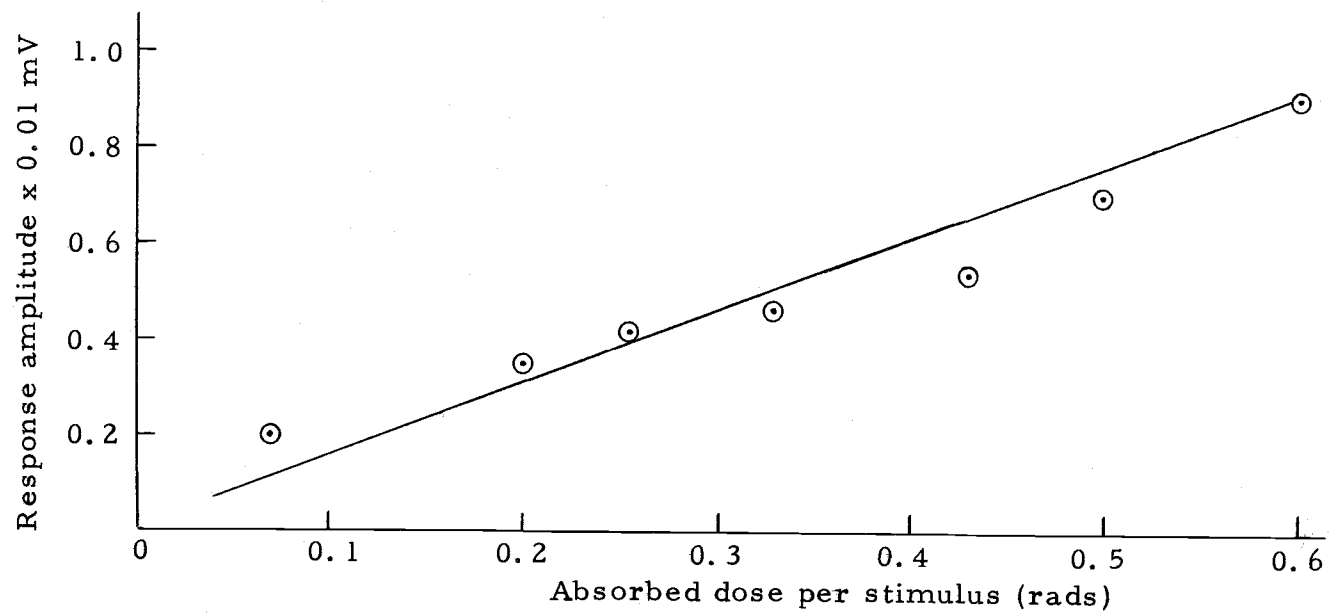
Figure 27. X-ray response amplitude as a function of absorbed dose per flash, on linear coordinates. Stimulus duration: 1/25 second.
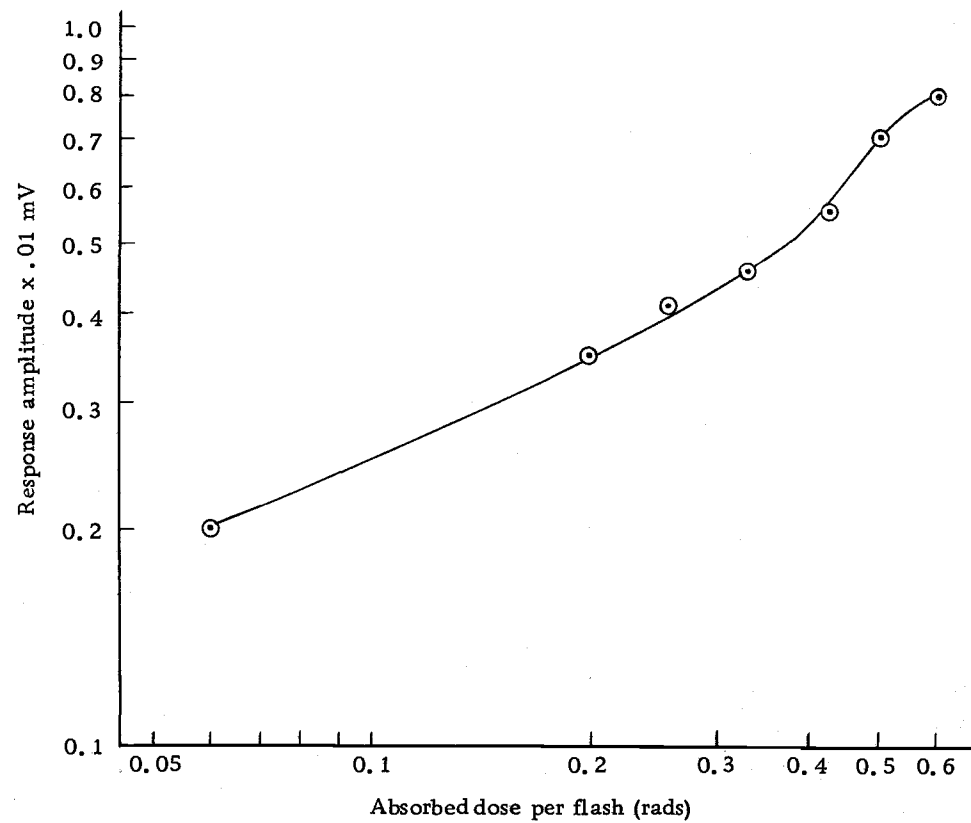
Figure 28. X-ray response amplitude as a function of absorbed dose per flash, on logarithmic coordinates. Stimulus duration: 1/25 second.

suggested that ultraviolet sensitivity may be due in part to direct

stimulation of higher order neurons at the base of the receptors.

Schiff reported maximum visual responses in the shrimp at 365 nm

and 535-555 nm with an intervening minimum at about 450 nm.

Carlson et al. found a maximal response in the moth at 550 nm, lower

peaks at 500 and 380 nm, and very small responses between 270 and

310 nm.

In evaluating direct neuronal stimulation, Goldsmith and

Fernandez (1968) observed no difference in the action potentials in the

housefly due to either wavelengths below 300 nm or to blue-green

light. Such a difference might have been expected if different modes

of excitation were involved.

The active wavelengths for direct neural stimulation in the crab

are 255 and 285 nm (Lieberman, 1967); in neither the literature

reviewed nor the results obtained were response peaks in this region

noted.

Action spectrum peaks can be attributed to specialized receptor

pigments if the visual pigments isolated also show corresponding

absorption peaks. This is the case in mammalian color vision (Wald,

1964) and in some invertebrate visual systems. Bees and flies in

particular exhibit complex action spectra with a pigment absorption

maximum at about 510 nm, whereas the rhodopsin peak is near 498 nm.

In these organisms, such specialized photopigments may not be

rhodopsin, since the protein substrate may not be opsin per se (Wolken, 1968).

In the insect, Notonecta, ERG response peaks were obtained at 375, 575, and 620 nm. By selectively reducing these responses by bleaching them with light of the same wavelengths and noting the effect on the other peaks, it was concluded that there was a trichromatic vision in this genus (Bennett and Ruck, 1970).

A non-rhodopsin pigment, euphausiopsin, has been isolated from the eyes of euphausiid marine crustaceans and its absorption peak (462 nm) correlated well with an action spectrum peak in the 460 nm region (Kampa, 1955; Boden et al., 1961).

The presence of response peaks attributable to specialized photopigments may only be due to experimental technique or data treatment in the case of responses involving more than one receptor. In an experiment using stimuli pairs at 355 and 496 nm matched to give identical ERG's, Burkhardt (1962) found identical adaptation responses in Calliphora with all possible combinations of adapting and stimulating wavelengths. The peaks at the two wavelengths could be explained by a few receptors being strongly stimulated at one wavelength and several receptors being stimulated to a lesser degree at another wavelength.

There is evidence which indicates that eye structure and fluorescence may play a role in shaping the action spectrum. In the

ant (Marak and Wolken, 1965), it was suggested that the behavioral

light sensitivity at 360 and 505 nm, and a smaller peak at 620 nm,

might be due to both receptor sensitivities and lower pigment absorp-

tion in the ultraviolet region.

Schiff (1963) reported definite fluorescence in the eye of the

shrimp, caused by ultraviolet light. Certain pigments between the

ommatidia showed a green fluorescence that illuminated the refractive

structures and penetrated to the rhabdoms; the cornea had a blue

fluorescence. Therefore, at least part of a stimulus presented as

ultraviolet light could have been received by the receptors as blue or

green light.

One source of this fluorescence has been identified by Chance

(1964) in the bee as excitation fluorescence of mitochondrial DPNH[4].

Wavelengths near 340 nm were absorbed and re-emitted as blue-green

light of 410-500 nm. As additional evidence that this fluorescence

played a role in vision, there was a slightly longer latency in the ERG

with ultraviolet stimulation due to the energy transfer in fluorescence.

In examining the action spectrum obtained from Hemigrapsus,

the 500 nm peak most likely represents the response from rhodopsin,

which has an absorption peak near 500 nm. Rhodopsin also has a very

small peak in the 350 nm region (St. George, 1952) and this, along with

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[4]Note in Figure 5 that DPNH plays a role in conversion of vitamin A
to retinal, in mitochondria located near the photoreceptor structures.

fluorescence and structural effects, may explain the 350 nm peak.

When the response to visible light is compared with that of ionizing radiation, it is possible to observe fundamental differences in the actions of these two types of stimuli.

With beta radiation, the existence of a visual response in absence of a response to visible light may be due to the penetrability of ionizing radiation. Since only those ommatidia included in the visible light exposure field are maximally stimulated, and since intact refractive and receptive structures are necessary for an optimal response (see page 34), the response to visible light is strongly dependent on the structure and structural integrity of the eye. An ionizing radiation stimulus, however, is less affected by these factors, since the stimulus is able to penetrate all parts of the eye without optical effects.

To test if this hypothesis was valid, a series of preparations were stimulated with alternating flashes of beta radiation and visible light, with the microelectrode being advanced into the eye by small increments after each pair of stimuli. The first response to be elicited in all cases was from beta radiation. After the electrode had penetrated more, a response to visible light was recorded if the rhabdomal layer had not been separated from the refractive structures due to the slow insertion of the electrode.

The failure to elicit reproducible relative responses in the

compound eye is therefore most likely due to differences in penetration and refraction of ionizing and visible radiation, and the dependence of the light response to optical properties of the eye and electrode placement.

The beta radiation threshold dose (0.9 mrad) is of the same order of magnitude as the strontium-90 threshold dose of 0.25 mR obtained by Smith and Kimeldorf (1964) in the noctuid moth. With x-radiation, this threshold was 50 mrad. This compares with the threshold of 100-212 mR in the frog (Lipetz, 1955) and 0.8 mR in Limulus (Dawson, 1965).

The threshold dose, however, is strongly dependent on stimulation factors. By decreasing the exposure time to 10 μsec and using a small stimulus field, Markus (1964) was able to lower the human subjective sensitivity to 14.5 MeV electrons and x-rays to 0.02-0.03 mR and 0.01-0.02 mR respectively. This decrease by an order of magnitude was attributed to the fact that the threshold dose was observed to drop with decreasing stimulus duration until extremely short exposure times were reached, at which time the retinal area stimulated became the deciding threshold factor.

In the evaluation of visual responses to ionizing radiation, the question of a direct stimulation of the rhodopsin molecule can only be inferred, knowing that the energy of the radiation is more than adequate to produce the stereoisomerization discussed above. That

radiation-generated visible light may play a role in the response is suggested by the shape of beta and x-ray response peaks, the course of response change to series of beta stimuli, and perhaps the linear stimulus-response relationship for x-rays.

It is known that ionizing radiation is capable of producing fluorescence in eye structures. Himstedt and Nagel (1901) observed that both radium and x-rays produced fluorescence in the isolated vertebrate eye. The cause of radium fluorescence was most likely due to the beta component of the radiation (Lipetz, 1955). Rhodopsin itself fluoresces when irradiated, as was determined by a photo-multiplier response with 180 kV x-rays at 45 rads per minute (Avakyan, 1966).

Comparing the energies of the beta particles emitted in the radium decay process (0.4 to 3.26 MeV) with the strontium-90 beta particles (0.54 and 2.26 MeV), it is entirely possible that fluorescence produced in the eye contributed to the beta ERG response. In addition, the x-ray dose rate at tube currents of 3 mA and above was over 45 rads per minute, so rhodopsin fluorescence may also have been a source of visible light.

Cerenkov radiation, in addition to fluorescence, produces visible light in transparent parts of the eye. Assuming an index of refraction of 1.33 for aqueous humor in the human eye, Markus (1964) calculated that the minimum electron energy to produce Cerenkov radiation was

260 keV. Further, the light emission from a 1 MeV electron would

be about 300 quanta between 400 and 800 nm. Secondary electrons

above this minimum energy ejected by ionizations could also produce

this effect.

For the insect, the refractive index of the rhabdom is 1.5

(Mazokhin-Porshnyakov, 1969); assuming that this value holds for the

crustacean eye, the minimum electron energy needed to produce

Cerenkov radiation is 171 keV[5].

It can be seen that the strontium-90 beta energies are above

this value and may have contributed to the visual response. The low

x-ray photon energy (17.5 keV) would not produce this effect, however.

Assuming that visible light produced within the eye, in addition

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[5] The velocity of light in a medium is c/n, where n = refractive index
of the medium. When a particle exceeds this velocity, Cerenkov
radiation is produced. The minimum kinetic energy, in the case of
the rhabdom, can be calculated:

$$\text{Minimum velocity} = c/n = \frac{3 \times 10^{10} \text{ cm/sec}}{1.5}$$

$$= 2 \times 10^{10} \text{ cm/sec}$$

$$\text{Relativistic electron mass} = m = \frac{m_o}{\sqrt{1 - v^2/c^2}} = \frac{9.11 \times 10^{-28} g}{\sqrt{1 - (4/9) \times 10^{20}}}$$

$$= 12.2 \times 10^{-28} g$$

$$\text{Kinetic energy} = (m - m_o)c^2 = (12.2 - 9.1) \times 10^{-28} g \times c^2$$

$$= 27.9 \times 10^{-8} \text{ ergs} \times 6.2 \times 10^{11} \text{ eV/erg}$$

$$= 1.71 \times 10^5 \text{ eV}$$

to a direct stimulation by ionizing radiation, can produce a visual

response, it is possible to discuss the relative shapes of the ionizing

radiation and visible light response peaks.

Visible light, which acts directly on the visual pigment, elicits

ERG responses which have sharp "on" and "off" response peaks

(Figure 8). A gradual change in intensity produces no response

(Figure 9). If direct stimulation of the visual pigment were the only

interaction possible with ionizing radiation, the visual pigment would

be exposed to a sharp increase in stimulus intensity at the beginning

of a flash and a sharp decrease at the end.

If the x-ray results are considered, both the "on" and "off"

responses are seen, but are not as sharp as those from visible light

stimulation.

The existence of such response peaks has been reported.

Lipetz (1955) and Bachofer and Wittry (1961, 1962) elicited these

responses with visible light and x-rays, and noted a similarity between

the waveforms. The ERG peaks recorded by Baldwin, Sutherland and

Habowsky (1963) revealed that the x-ray peaks were broader and

lacked the sharp return to the baseline exhibited by similar visible

light responses.

Cessation of beta irradiation does not result in the "off"

response, and by inference from visible light, the photoreceptors do

not experience a rapid decrease in stimulus intensity. While there is

no experimental proof of this, it would seem that this is due to

secondary sources of visual stimulation, such as fluorescence, which

obscure the sharp decrease in initial stimulus intensity by their

gradually decaying light output.

Comparing these three types of radiation, x-rays seem to fall

between the extremes of visible light and beta rays in terms of direct

and indirect stimulation of the photoreceptors.

The decrease in response to series of beta stimuli (Figures 20,

21), assuming only a direct stimulation of the photopigment, would not

be the result of shielding pigment migration, since the shielding pig-

ments are transparent to ionizing radiation.

The change in bioelectric response to beta radiation with no

change in pigment position was shown by Smith and Kimeldorf (1964)

by determining dark adaptation in the moth eye after an intense flash of

light which produced a physiological light-adapted state but resulted in

no pigment migration. Dark adaptation curves for beta radiation and

visible light were nearly identical.

This observation is supported by Savchenko (1966) who stated

that dark adaptation was mainly a neural process involving an insig-

nificant change in rhodopsin content. Dawson (1968) also proposed

that both light and dark adaptation were due in part to neural inhibition.

A change in rhodopsin concentration as a cause of the response

decrease can be eliminated, at least at the absorbed doses presented

here. Using alpha particles, deuterium nuclei, and x-rays, Lipetz

(1955) attempted to bleach frog rhodopsin. An absorbed dose of 10^7

rep was required.

In fact, x-rays have reportedly stimulated the synthesis of

rhodopsin; both Kang (1962) and Dawson and Wiederwohl (1965)

observed increases in rhodopsin density at exposures up to 1500 rads.

Visual response reduction due to impairment of metabolic

processes has not been demonstrated at doses below 3-6 krads (Noell,

1962; Dawson, 1968).

Since the visual response may be in part due to visible light

produced in the eye, however, the mechanisms which have shown to

affect visible light sensitivity should also affect the response to the

beta stimuli and to ionizing radiation in general. Figure 23 illustrates

this point; the curves for both beta radiation and visible light are nearly

identical.

The responses to a series of x-ray stimuli (Figure 26) decreased

by about 20% in 20 seconds. If the data of Dawson (1969) are inter-

polated, his 63% response decrease in 100 seconds would mean roughly

a 20% drop in 30 seconds. Such a decrease from the initial low

response amplitudes encountered in this research is difficult to

quantify, but the results mentioned above are within this order of

magnitude.

Peak x-ray response amplitude as a function of absorbed dose

presented per stimulus was nearly linear. This relationship was also observed by Bachofer and Wittry (1962) in the frog, at doses below saturation of the response, and by Dawson (1965). This point may be interpreted to support either a direct interaction between x-rays and the visual pigment, or a stimulation by secondary visible light.

The failure of the eye to recover its original sensitivity to beta stimulation, yet show little change in a visible light response, is most likely not from damage to the receptor structures. Even at the lowest dose rate where the total absorbed dose was about 260 mrads, this decrease was observed. Smith and Kimeldorf (1964) were able to maintain a response to strontium-90 beta radiation for about 45 minutes in the moth. Lommatzsch, Fürst and Ulrich (1968) noted the first reversible reduction in the rabbit ERG at 3-5 kR. Referring to Figure 23 as an explanation, the beta radiation curves reach lower asymptotes than visible light. It may be possible to assume that sensitivity of the eye to subsequent beta stimulation had not increased to an extent that responses could be detected above the noise level.

At the opposite end of the electromagnetic spectrum, 3-cm microwaves which have photon energies of about 4×10^{-5} eV produced no visual response. Since the limit of visual sensitivity as determined above was about 800 nm (photon energy, 1.6 eV), it would seem that the microwave photon cannot directly cause stereoisomerization of retinal. The low power output may also have been of importance,

although even a greater intensity of photons of such low energy might not produce visual stimulation.

It is of interest, however, to discuss the probable actions of microwaves on nerve tissues, of which the visual receptors are a part. The influence of microwaves most likely comes not from actions of photons, characteristic of much shorter wavelengths, but from certain thermal and non-thermal effects of the electric fields associated with this radiation.

Thermal effects refer to either general or localized heating of a material, but the net biological effect may not be attributable to this cause alone (Carpenter, 1968).

Non-thermal effects, as the name implies, result in actions not associated with an increase in temperature. The electric field can cause small particles to be aligned along field lines in a "pearl chain" fashion. Resonance of polarized macromolecules has also been postulated, but this is not supported by theoretical treatment. Another consequence may be the rectification of the electric field by nerve membranes to such a degree that the potential created across the membrane is sufficient to depolarize the nerve. Power levels needed to produce such a field strength may also induce thermal effects (Schwan, 1968).

Kamenskii (1964), in a study of the influence of microwaves on neural responses, found that pulsed microwaves produced an

increased excitability of the nerve to electrical stimulation. That is,

a smaller stimulus was needed to depolarize the nerve and induce an

action potential. The refractory period (the short time period during

which the membrane is repolarizing and cannot be stimulated) was

also decreased significantly. Thermal effects were not judged to be

a cause of these two phenomena.

Microwaves have been shown to disturb crayfish and shrimp

ganglionic discharge frequencies, producing first a decrease and then

an increase in discharge rate (Yamaura, 1967). The author assumed

that only the nerve membrane was influenced and theorized that the

high-frequency field caused re-orientation of polar molecules in the

membrane.

It should be added that microwaves have been used as stimuli

for behavioral response studies. Results, although inconsistent, have

been interpreted as being due to both thermal (Bryan, 1966) and non-

thermal effects (Pressman and Rappeport, 1965; Lobanova, 1966,

1968).

In considering the possibility that such low energies could

produce stereoisomerization of retinal, the research by St. George

(1952) must be mentioned. It was found that light photons beyond 590

nm wavelength did not have the energy necessary to induce the photo-

chemical change in rhodopsin. The remaining small fraction of the

total required was supplied as thermal energy from the rhodopsin

molecule. Extending this to microwaves, with even less energetic

photons, the energy must be supplied by rhodopsin (heat) and by the

radiation (heat). However, merely the addition of thermal energy does

not result in stereoisomerization; heating of rhodopsin results in

the release of cis, not trans, retinal and denatured opsin. It is only

through the action of light or other energetic influence that stereo-

isomerization is observed (Hubbard et al., 1965).

At a sufficiently high radiation intensity, field-induced effects

on the nerve membrane structure of the rhabdomere might be analogous

to those observed by Kamenskii (1964).

IV. CONCLUSIONS

The visual response of the crustacean compound eye to visible

light of wavelengths 250-800 nm, as measured by the ERG response,

exhibited peak sensitivities at 350 and 500 nm, corresponding very

closely to the rhodopsin absorption spectrum. Fluorescence, caused

by ultraviolet light absorbed in the 350 nm region and re-emitted as

blue-green light, may have partially accounted for the 350 nm peak.

The ERG was observed only during a rapid increase or decrease

in stimulus intensity, corresponding to the beginning or end of stimu-

lation. These "on" and "off" effects were attributed to the process of

recurrent inhibition in the nerve plexus of the eye.

At stimulus levels above threshold, the magnitude of the response

peaks was nearly linearly related to visible light intensity. A satura-

tion point was reached at higher intensities which produced maximal

ERG responses.

The response decrease to series of light stimuli was very

rapid, with the rate of change being a function of stimulus intensity.

Visible light asymptotic values were reached within 20 seconds after

commencement of illumination. Migration of shielding pigments,

hormonal control, neural inhibition, and rhodopsin bleaching were

indicated as possible causes of this adaptation.

Strontium-90 beta radiation responses were shown to be

relatively independent of the integrity of the eye structures. This was presumably due to the penetrating nature of ionizing radiation, which minimized the influence of intact refractive structures on an optimal response.

The threshold absorbed dose of beta radiation was approximately 0.9 mrads.

Both fluorescence and Cerenkov radiation were implicated as factors in the lack of the "off" response in the beta-induced ERG. This was conceivably due to the slow decline in secondary visible light intensity after the primary beta radiation had ceased.

Beta stimuli, presented in a rapid series, produced a definite rise in the response baseline. The amplitude of each succeeding peak in a series declined rapidly to reach an asymptotic level. Such response changes were nearly identical to those obtained with visible light and were considered to be the result of similar mechanisms, since both direct and indirect visual stimulation were possible.

X-irradiation resulted in responses very similar to those elicited by visible light, in that both "on" and "off" responses were observed; no responses occurred when the stimulus intensity was gradually changed.

The relationship between x-ray response amplitude and stimulus intensity was another point of similarity with visible light.

A 50 mrad absorbed dose produced a near-threshold x-ray

response.

Fluorescence, but not Cerenkov radiation, was considered a possible source of secondary visual stimulation, resulting in a more gradual decline of the response peaks to the baseline. The x-ray response seemed to be less the result of secondary visible light stimulation than direct stimulation of the photoreceptors, when compared to the beta response.

Microwaves produced neither a direct visual response nor a modification of a visible light response, possibly because of the very low energy of the microwave photons used. An effect of microwaves due to interactions between the nerve membranes and the electric field might have been possible at much higher power levels.

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APPENDIX
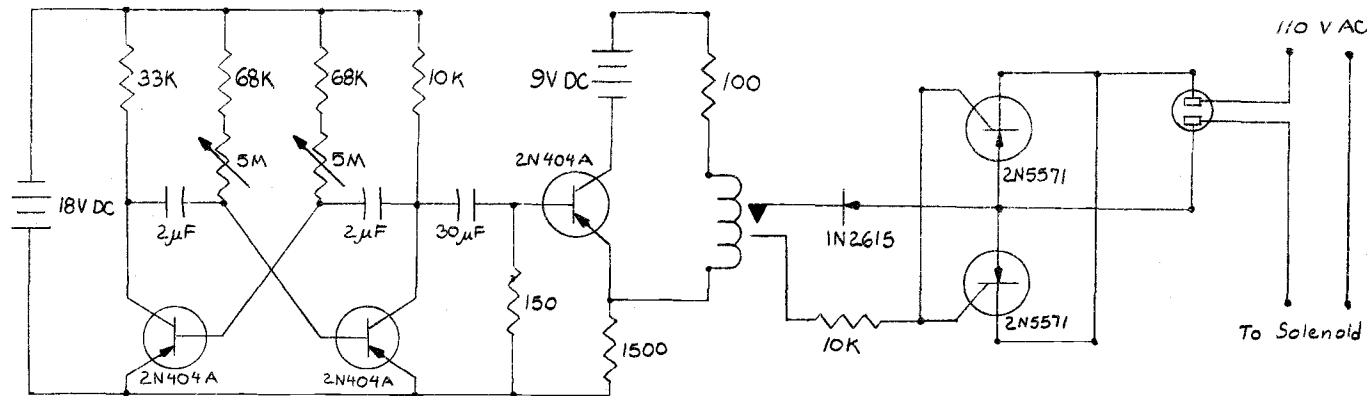
Circuit diagram for electronic shutter control used in x-ray stimulation.