THE ELECTRORETINOGRAM OF THE SHEEP

A Thesis

Presented to

the Faculty of the Department of Physiological Optics

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By:

Earl Leo Smith, III

May, 1975

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ABSTRACT

The electroretinogram of the sheep was quantitatively and qualitatively investigated to determine the range of amplitudes and implicit times of the major components for the normal animal. The stimulus variables were intensity, duration, frequency and wavelength. The normal ERG responses to the above stimulus conditions were established for light- and dark-adapted sheep. The results indicate that the sheep ERG responds predictably to changes in the stimulus arrangement. The form of the recorded response is basically similar to the ERGs recorded from other mammals with mixed receptor retinae. However, the scotopic fusion frequency found for the sheep (34 fps) is higher than the fusion frequency reported for other species.

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INTRODUCTION

A. History

In 1849, when the resting potential of the eye was discovered by du Bois Reymond, the field of ocular electrophysiology was begun. Subsequently, in 1865 Holmgren (See Einthoven and Jolly, 1908) found that light falling on an excised frog eye altered the potential across the globe. Using a string galvanometer, he recorded positive (polarity given with respect to the cornea) deflections at both the onset and cessation of illumination. In 1873, Dewar and M'Kendrick (see Einthoven and Jolly, 1908) independently rediscovered these electrical changes. More importantly, Dewar and M'Kendrick were the first to record the electrical response to illumination from the human eye. These electrical responses became known as "retinal currents" (Karpe, 1945). This response is now generally known as the electroretinogram (ERG). The ERG in its present context may be defined as a complex transient electrical response to light produced by the summed activity of millions of cells in the retina (Finkelstein and Gouras, 1969).

In 1880, Kuhne and Steiner (see Gotch, 1903) investigated the electrical response of the isolated retina. They were able to record normal ERGs from an excised retina. This was the first evidence indicating that the retina was actually the source of the potential. The response that Kuhne and Steiner recorded consisted of only two parts. The first

part consisted of a positive deflection due to the onset of the stimulus. The second part was also positive, but was caused by the cessation of the stimulus.

According to Gotch (1903), other early investigators attempted to quantify the ERG. However, the recording instruments these early experimenters used were too slow to reproduce the rapid phases of the ERG accurately. This was evident from the range (.0004-1.0 sec) of latent periods which were found in the literature.

The first complete electroretinogram was obtained by Gotch (1903) using a capillary electrometer. He divided the electrical response into three parts. The first portion was the rise in potential produced by a sudden illumination. He termed this the "on-effect". The second portion was termed the "continuous effect". This was a period of gradual change during the continuance of stimulation. On cessation of the stimulus, a second rise in potential was noted; Gotch called this the "off-effect". In addition, he mentioned a small negative deflection of short duration which preceded the "on-effect". He concluded that there were two photochemical substances in the retina; one reacting to light and the other reacting to darkness.

In 1905, Piper and in 1907, von Brucke and Garten confirmed the findings of Gotch (see Einthoven and Jolly, 1908). Both parties clearly demonstrated the preliminary negative deflection visible to Gotch on examining his curves with a magnifying glass. These investigators extended their research over a large number of vertebrate species. They reported a marked similarity in the main features of the ERG in all animals tested.

The first systematic investigation of the response changes caused by stimuli of varying intensity was conducted by Einthoven and Jolly (1908). The form of their recorded responses (Figure 1) led to the supposition that there were three separate response processes in the eye. For the "sake of convenience", they postulated the existence of three separate photoreactive substances. The first substance reacted faster than the other two substances. On exposing the first substance to light, it developed a negative potential; by removing the light, a positive potential appeared. The second substance reacted slower than the first substance. On exposing the second substance to light, it developed a positive potential. The third substance reacted in the same manner as the second substance, except at a much slower pace. The sum of these reactions produced a typical response. The peak of the wave which was evoked by the first substance was denoted by the letter A. With longer stimulus durations a positive off-effect occurred and was labeled A_1. The peaks for the second and third substances were marked B and C respectively. Today the ERG peaks are labeled with lower case letters, "a", "b" and "c"; "d" replaces A_1.

Although various early experimenters disagreed on some details, certain characteristics of the ERG were well established. Hence, the object of many subsequent investigators was to expand the work of previous experimenters with the aid of improved techniques and apparatus. Of milestone importance was the contribution of Chaffee, Bovie and Hampson (1923). They were the first investigators to incorporate an amplifier (a two-stage thermionic amplifier) into their recording system.

Figure 1

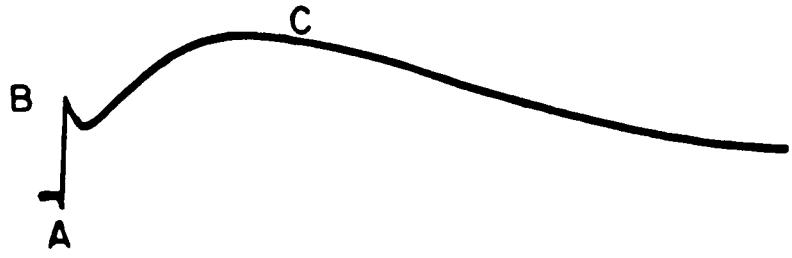


Figure 1. Typical ERG recorded by Einthoven and Jolly (1908). Dark-adapted eye. Green stimulus. Flash duration, 0.01 sec. B-wave amplitude, 170 μv. C-wave implicit time, 16 sec.

Their subsequent research on stimulus intensity and wavelength was carried out with excised eyes, as was most of the previous research.

In 1925, Hartline rediscovered an interesting fact. He found that the indifferent electrode did not have to be confined to the posterior globe but that it could be placed anywhere in the animal's body and still record the electrical responses to illumination. His technique made it unnecessary to operate around the globe in order to apply the electrodes to the eye. In man, one electrode was placed on the sclera or cornea and the other somewhere in the neighborhood of the eye. He suggested placing the indifferent electrode in the subject's mouth. By comparison, this method provided the same qualitative detail as that obtained in recordings from excised eyes. This provided a method for recording the ERG in the intact eye without the usual physiological insults. Soon after Hartline's "discovery", he found that Dewar had described a similar method which he had used to record ERGs from human subjects nearly sixty years earlier.

Cooper, Creed and Granit (1933) incorporated these new techniques into studies with humans. They used Hartline's method of electrode placement and expanded on the work of Chaffee, Bovie and Hampson. This was the first time an electronic amplifier had been used to record the human ERG. They reported average b-wave amplitudes of about 200 μV.

Nonetheless, good clinical records were still difficult to obtain. Much of the difficulty was caused by the cotton-wick electrodes used to make electrical contact with the sclera. These electrodes termed "localized leads" by Karpe (1945) were very sensitive to local nonretinal

potential changes and required a steady fixation which few subjects could maintain. Changes in the contact surface introduced potential changes, especially following a blink. Therefore, it became necessary to develop a more satisfactory method of recording. Independently, Riggs in 1941 (see Riggs, 1954) and Karpe (1945) derived similar solutions; both investigators used contact lenses to solve the problem. After insertion, Karpe filled a scleral contact lens with saline solution through a special bottle neck opening which was then closed with a plug containing a "chlorinated" silver wire. This produced a large surface of contact which eliminated most local potentials and disturbances from eye movements. One of the biggest advantages of contact lens electrodes was that the recorded ERG increased in amplitude. Karpe, repeating the work of Cooper et al. (1933), reported a mean b-wave amplitude of 360 μV (Cooper et al.; 200 μV) with values as high as 510 μV. Riggs also used a saline filled contact lens; the chlorided silver electrode was wedged into a 3mm hole at the corneal bulge of the lens. This is now the most clinically accepted electrode technique.

Another revolution with respect to active and reference electrodes began in 1950. Several investigators used microelectrodes for intraretinal recordings of ERGs. The majority of our knowledge about the cellular origins of the ERG components has been obtained with microelectrodes (Brindley, 1970).

It is apparent that our knowledge of the ERG has proceeded hand-in-hand with the developments in electrophysiology and progress in electrical recording techniques in general. The most recent advances have come in

the area of computer science. Jacobson, Stephens and Suzuki (1962) and Armington, Gouras, Tepas and Gunkel (1961) were the first group of investigators to incorporate averaging computers in their recording system. Armington et al. used an analog averaging computer to record ERGs from patients with retinitis pigmentosa. Jacobson et al. compared recorded responses from penwriters and oscilloscopes to the ERGs recorded with a digital averaging computer. Both groups concluded that computers of average transients were ideal for enhancing the signal-to-noise ratio. Signal averaging allowed for greater ease and accuracy in interpretation of the ERG.

For the most part the ERG has been used as a research tool. But since Karpe (1945) laid down the groundwork for clinical electroretinography, the ERG has become an increasingly valuable tool in the diagnosis of various retinal disorders.

B. Components of the Electroretinogram

Studies by Granit

The complexity of the ERG indicated that it consisted of a number of separate components. To analyze the complex response into its components and to identify the type of retinal cell that generated each component has always been of prime interest to the investigators of the ERG. One of the most recognized analyses to date is that of Granit (1933). He followed the hypothesis laid down by earlier investigators (Einthoven and Jolly, 1908; and Chaffee, Bovie and Hampson, 1923) that the complex appearance of the ERG was derived from some interference

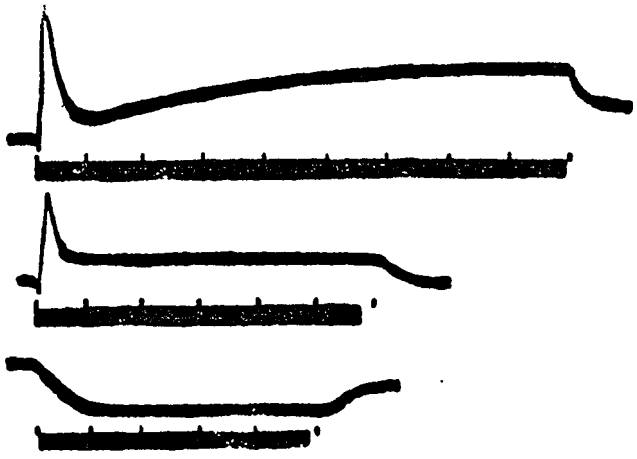
phenomena between several simple electrical potentials with different magnitudes, polarities and latencies. In order to isolate these simple components, Granit assumed that the cells which generated these potentials were selectively sensitive or resistant to certain agents. He found two ways of fractionating the ERG; by administering ether or by causing hypoxia by interfering with the blood supply.

Increasing the depth of anesthesia of a cat, removed in discrete steps, three components of the ERG (Figure 2A). These components were labeled in the order of their disappearance (Figure 2B). Process I (PI) was the first to be affected; it contributed to a portion of the c-wave. Granit showed that PI was essentially a high stimulus intensity component. This meant that for a given level of anesthesia, only the high stimulus intensity response was affected. In the low stimulus intensity response, PI was non-existent and so the ERG, in particular the c-wave after removal of PI reacted to ether in the same manner as the b-wave. The combination of the b-wave and the remainder of the c-wave was the second component to be affected by anesthesia and was termed Process II (PII). After removal of PI and PII only a negative response was recorded. The negative response was called Process III (PIII). It likewise was found to disappear with further narcotization.

Hypoxia produced by cartoid artery occlusion selectively affected PII. Dimunition of PII in this manner enhanced the off-effect. Therefore, Granit attributed the off-effect to PIII. But since it was found to occur only in the presence of PI or PII, Granit concluded that it was an interference phenomenon caused by the rise of PIII.

Figure 2

A



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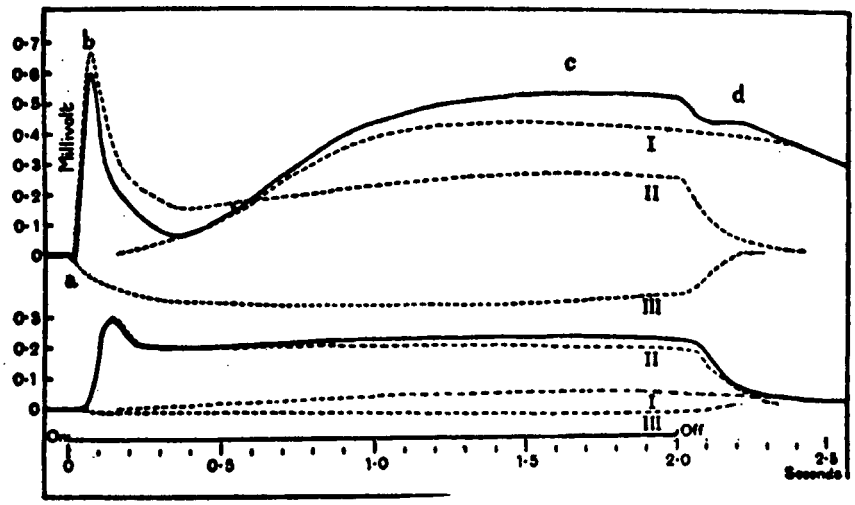


Figure 2. A. ERG components recorded during progressive narcotization. Top curve represents the normal ERG prior to narcotization. The middle and bottom tracings represent the recordable ERG after removal of PI and PII respectively.

B. Analysis of the predominantly rod ERG of the cat at two stimulus intensities. Stimulus intensity; upper 14 mL; lower 0.14 mL. Duration; 2.0 sec. The components are shown as broken lines. (From Granit, 1933).

However, in a later study, Granit and Riddell (1933) provided evidence indicating that the analysis established with the cat's eye (Figure 2B) could not be generalized to all animals. Using the frog, Granit and Riddell analysed both light-adapted and dark-adapted ERGs (Figure 3). The chief changes brought on by light-adaptation were: disappearance of the c-wave, reduction of the b-wave amplitude, and an increase in the size and rate of rise of the off-effect. It was obvious to Granit and Riddell that the analysis obtained with the cat could not be applied to the light-adapted frog. It was apparent that the chief difference was between the frog's responses in the light-adapted and dark-adapted states. The change in the negative component (PIII) was the most striking difference between the two states of adaptation. They reasoned that light-adaptation involved a fundamental change in the response of the eye as a whole. Granit and Riddell postulated that the cone system dominance during light-adaptation was responsible for the difference.

In a subsequent investigation, Granit and Therman (1934) correlated the ERG and impulses in the optic nerve. This study was directed at the differences between light-adapted and dark-adapted ERGs of the frog. They reported that the positive component, PII, was associated with the discharge of impulses in the optic nerve. The negative component, PIII, was connected with inhibition of impulses in the nerve. The component, PI, was not associated with the discharge or inhibition of impulses. More importantly, Granit and Therman found that the amount of PIII associated inhibition increased during light-adaptation. They postulated that the

Figure 3

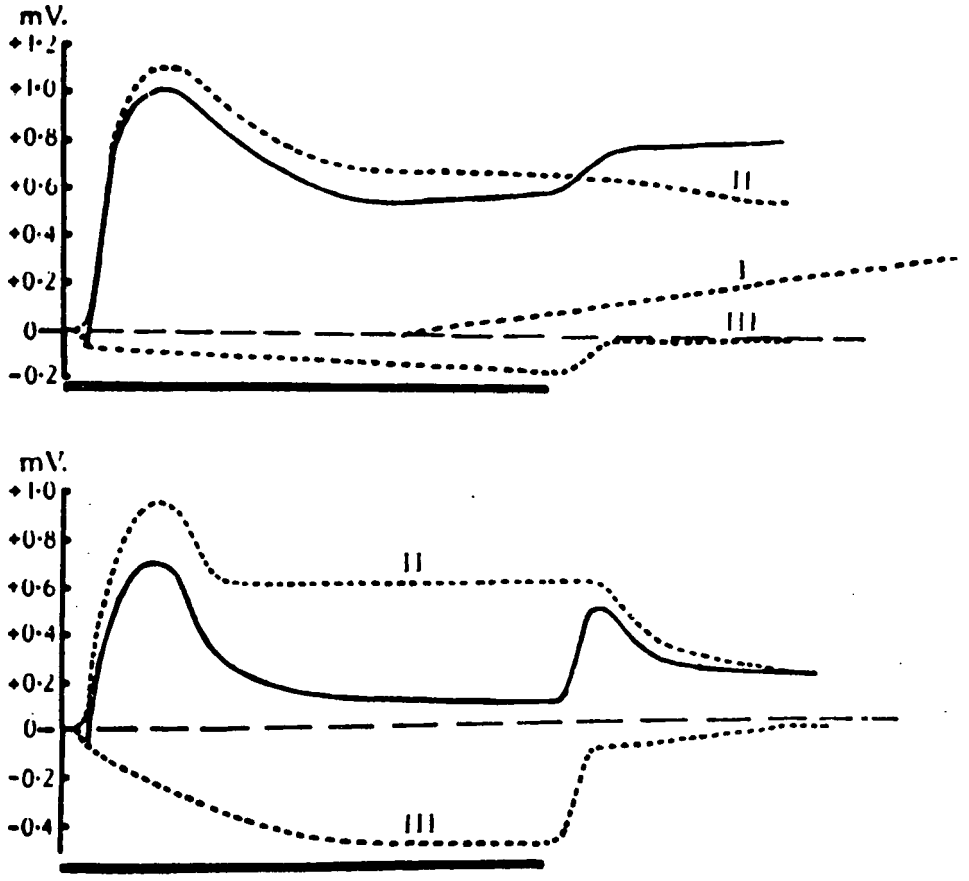


Figure 3. Analysis of the light-adapted (lower) and dark-adapted (upper) frog ERG. The light-adapted analysis is considered to represent the cone type ERG. Stimulus duration 2.0 sec. (From Granit and Riddell, 1934).

increase in inhibition was caused by the increased dominance of the cone system during light-adaptation. It was further speculated that the type of ERG recorded was determined by the receptor system which was dominant.

To verify this hypothesis, Granit (1935) studied the ERGs from several different species of animals (pigeons, cats, rabbits, frogs). The relative number of rods and cones found in the retinae varied between the different species. By using intermittent stimulation, Granit was able to distinguish two distinct types of retinal responses. The two responses were called the E-type (Excitatory) and the I-type (Inhibitory). The E-type response was dominated by the component PII. The I-type response was dominated by the component PIII. In all cases the I-type response was associated with anatomically cone dominated retinae or light-adapted mixed retinae. On the other hand, the E-type response was always associated with anatomically rod dominated retinae or dark-adapted mixed retinae.

The main question that then had to be evaluated was whether E- and I-retinae were analogous to the terms rod- and cone-retinae. Granit presented evidence both for and against equating of the above terms. According to Brindley (1970), Bowhm, Sigg, and Monnier (1944) demonstrated that exceptions do exist. Nevertheless, the terms are used interchangeably by some authorities.

Subcomponents of the ERG

Though Granit's general scheme was accepted for some time as the normal or typical form of the retinal response, it has been recognized that the precise configuration of the ERG was somewhat more complicated. In 1942 Motokawa and Mita (see Brown, 1968) reported a subcomponent of the b-wave in man which they designated the x-wave. It was described

as a fast positive wave which occurred prior to the main portion of the b-wave. Since the x-wave was most easily elicited with red light, they believed that it was produced by the cone system. Adrian (1945) independently rediscovered the x-wave. He investigated the effects of stimulus wavelength, intensity, frequency and retinal location in both light-adapted and dark-adapted eyes. He also concluded that the cone system produced this early subcomponent ("photopic component") of the b-wave. The scotopic system was credited as producing the slower more prolonged portion of the b-wave. Armington (1953) further emphasized the photopic nature of the x-wave. He reported that its relative spectral sensitivity was in agreement with the ICI photopic luminosity function.

The discovery of the two b-wave components has led to confusion in the literature concerning nomenclature. Today it is preferable to refer to these two components as the photopic (b_p-wave) and the scotopic b-waves (b_s-wave) (Johnson, 1958).

Armington, Johnson, and Riggs (1952) discovered a similar complexity in the human a-wave. Until their investigation, the a-wave has been regarded as simple, unitary and primarily photopic. Armington *et al.*, using higher intensities of stimulation than those available to Adrian (1945), recorded a-waves which could be analysed into two components. The component with the shorter implicit time was best observed with red light and was tentatively designated the photopic a-wave. The later component (scotopic a-wave) increased in amplitude throughout dark adaptation and demonstrated a relative spectral response equivalent to the scotopic b-wave. These results identified the later component as scotopic in nature.

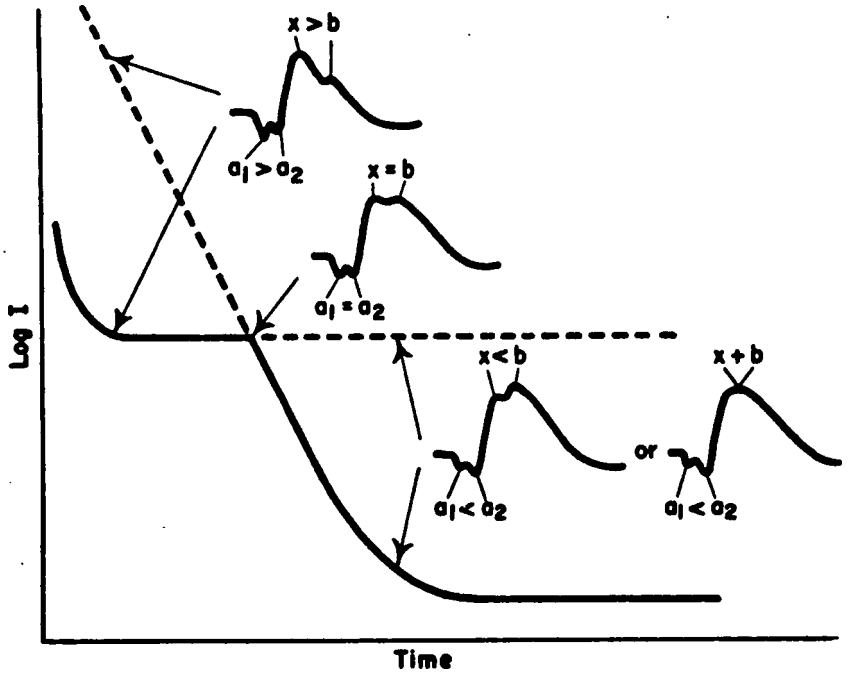
The above conclusions have been contested recently. Brunette (1969) studied the implicit times and amplitudes of the a-wave during dark adaptation. Brunette suggested that a positive deflection of constant latency and implicit time interacting with a single a-wave could explain the double nature of the a-wave. Brunette's hypothesis failed to explain the different relative spectral sensitivities of the subcomponents of the a-wave and, in general, has not been widely accepted.

Auerbach and Burian (1955) further investigated the photopic/scotopic relationships in the ERG. The major portion of their study was directed at the effects of light-adaptation and dark-adaptation. Much of their results and the concept of the bipartite nature of the a-wave and b-wave are summarized in Figure 4. These schematics emphasize the concept that the ERG is the composite of two superimposed responses which have slightly different latencies.

Multiple components have also been noted in the b-wave of the ERG from animals with pure cone retinae. Crescitelli (1961) recording from the antelope ground squirrel reports finding at least two subcomponents of the b-wave. He noted that these components (b_1 and b_2) responded differently to changes in the stimulus intensity. However, b_1 and b_2 failed to respond differently to changes in the stimulus wavelength and showed no difference with regard to the speed of recovery from light-adaptation. The subcomponents found with the antelope ground squirrel differed greatly from the photopic-scotopic components found in man. As Crescitelli pointed out, these two systems can not be considered analogous.

Figure 4

A



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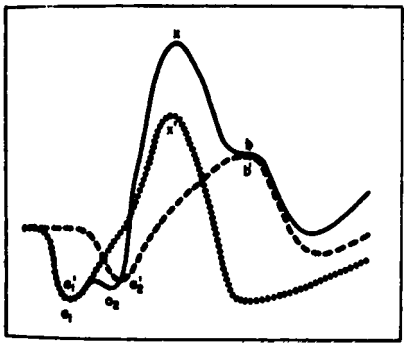


Figure 4. A. Schematic showing the relative contributions of the photopic and scotopic systems to the human ERG at various levels of adaptation.

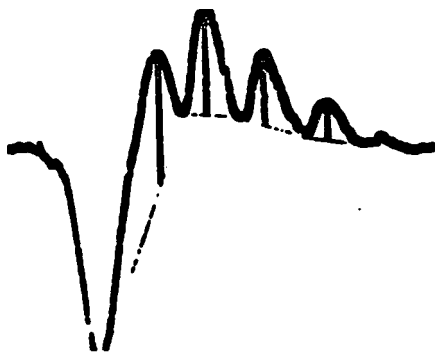
B. Schematic analysis of the contributions of the photopic and scotopic systems to the ERG. (From Auerbach and Burian, 1955).

Another minor component which affects the precise configuration of the ERG is the oscillatory potential of the b-wave. Cobb and Morton (1954) first described these potentials in ERGs from human retinae stimulated with high intensities of white light. Similar oscillations have since been found in many species. These rhythmic components have been designated the e-wave (Algvere, 1968). Figure 5A shows these oscillations from the human retina. Algvere (1968) determined the conditions under which the potentials were most readily recorded. He reported that a moderate state of dark-adaptation, an intense light source (5×10^4 photopic cd/m^2), a stimulus frequency of 2 flashes per sec (fps) and using an amplifier with a relatively short time constant (15 msec) enhanced the recordability of the oscillatory potentials. However, regardless of the stimulus parameters of the adaptive state, the interval between the oscillations remained relatively constant. The wavelets usually had a period of between 5 and 7 msec. The amplitudes of the oscillations are difficult to evaluate and as a result there is some confusion in this respect in the literature. The reader is referred to Algvere (1968) for a detailed treatment of oscillatory potential amplitudes.

With the advent of microelectrodes for intravitreal and intraretinal recording, the ERG has been found to be even more complex. Brown and Murakami (1964) described another minor component which occurred prior to the a-wave. This component was found by recording intraretinally. The response was maximal at the level of the receptors; hence, Brown and Murakami postulated that it was generated by the receptors. Consequently,

Figure 5

A



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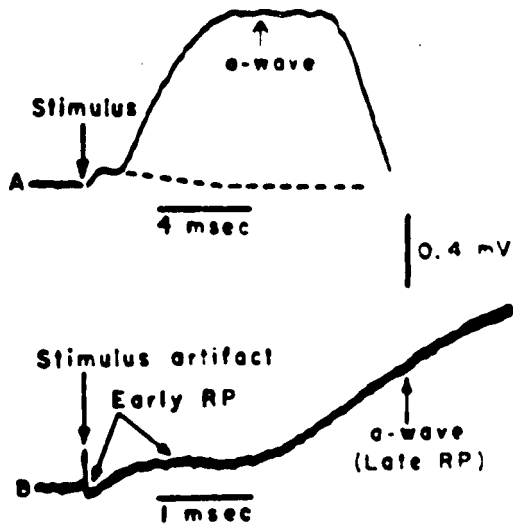


Figure 5. A. Oscillatory potentials of the human b-wave. (From Algvere, 1968.)

B. ERP recorded from the monkey eye. Dotted line indicates the time course of the ERP following removal of the a-wave. (From Brown and Murakami, 1964).

it was labeled the "early receptor potential" (ERP)(Figure 5B). The ERP had two subcomponents, RI and RII. RI was positive and had a shorter latent period than RII, which was negative. The ERP was isolated from the a-wave (late receptor potential) by ischemic anoxia; at which time it demonstrated a duration of 5-10 msec (dotted line Figure 5B). Cone (1964) investigated the ERP using more conventional ERG methods. He reported that the amplitude of the ERP varied directly with the amount of visual pigment bleached. Also, he showed that its action spectrum agreed with the absorption spectrum of the visual pigment. Arden, Ikeda and Siegal (1966) studying the effects of different levels of light-adaptation, suggested that part (RII) of the ERP was related to photo-reversal of visual pigments. ERP responses have been recorded in rods, cones, the pigment epithelium and green leaves (Duke-Elder, 1968). It is generally considered to be a phototransducer potential coupled with the photochemical events in the receptors.

Subcomponents of the corneal-negative PIII have also been reported. Murakami and Kaneko (1966) proved that the PIII of cold-blooded vertebrates consisted of two parts, which were labeled "proximal" and "distal" PIII. Indications of subcomponents of the mammalian PIII were found as early as 1947. However, Hanitzsch (1973) was the first to localize mammalian PIII subcomponents. By incorporating sodium aspartate into a perfusion solution Hanitzsch was able to isolate three separate subcomponents of PIII in the rabbit retina. Two of these subcomponents corresponded to the proximal and distal PIII described by Murakami and Kaneko (1966). The third subcomponent was slow to develop, of long duration and was found proximal to the receptor potential.

By investigating the effects of reduced stimulus intensity, Brown and Wiesel (1961a) isolated a "d.c. component" within the ERG. For an intravitreal active electrode the response was positive; its duration roughly approximated the duration of the stimulus. Further study by Brown and Wiesel (1961a) revealed that the b-wave and d.c. component, the combination of which was equivalent to Granit's PII, were generated by different structures. Brown (1968) suggested that the d.c. component contributed to the falling phase of the d-wave. He further proposed a component analysis (Figure 6) to replace Granit's scheme. Brown's scheme included two analysis models, one for predominantly rod retinae and one for pure cone retinae. Brown's component analysis appears to be universally applicable. The newer texts (Krill, 1972) have adopted Brown's model.

More recently, Knave, Moller and Persson (1972) using stimulus intensities below the b-wave threshold proposed a reinterpretation of the light-adapted (cone dominated) ERG component analysis (Figure 7). Their results suggested that the leading edge of the a-wave reflected the initial steep part of the cone receptor potential. The b-wave was the result of the interaction between the receptor potential and the positive d.c. response. Hence, there was no positive transient component corresponding to the isolated b-wave. This particular analysis was important to the present study since sheep were used as the experimental animals, both in the present study and by Knave _et al_.

Figure 6

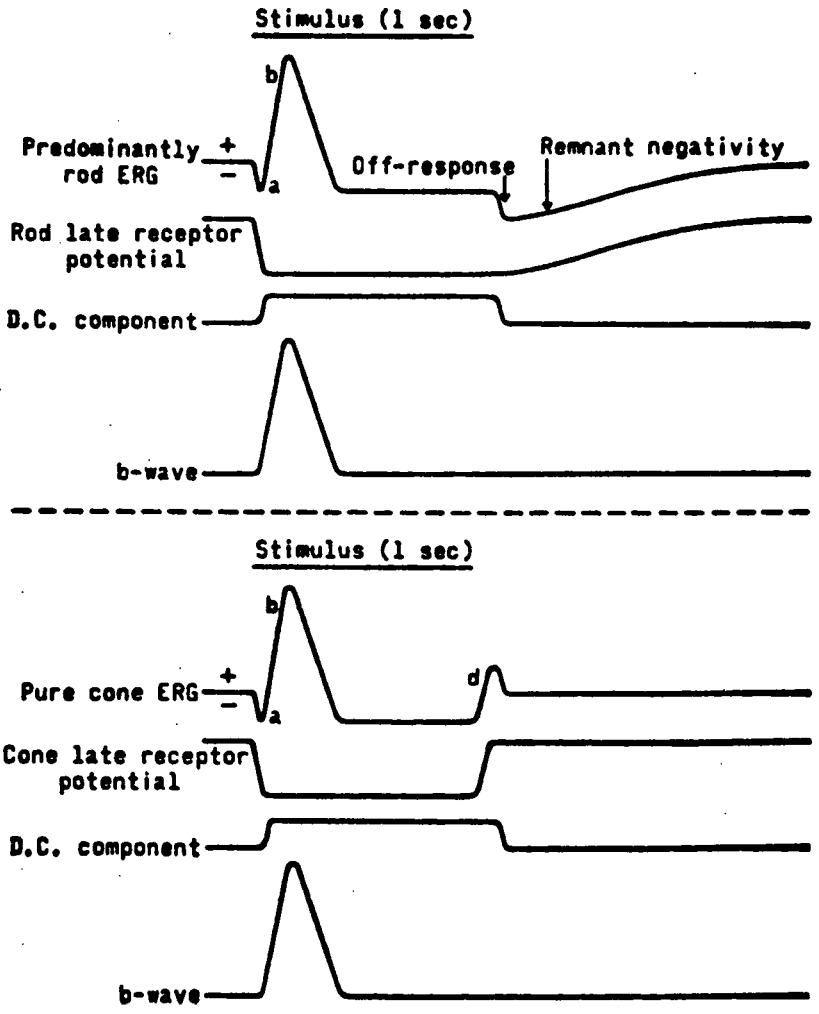


Figure 6. Analysis of the predominantly rod ERG (upper) and pure cone ERG (lower). To facilitate comparison the c-wave was omitted from the rod response and the time course for the a-wave and b-wave was broadened. (From Brown, 1968).

Figure 7

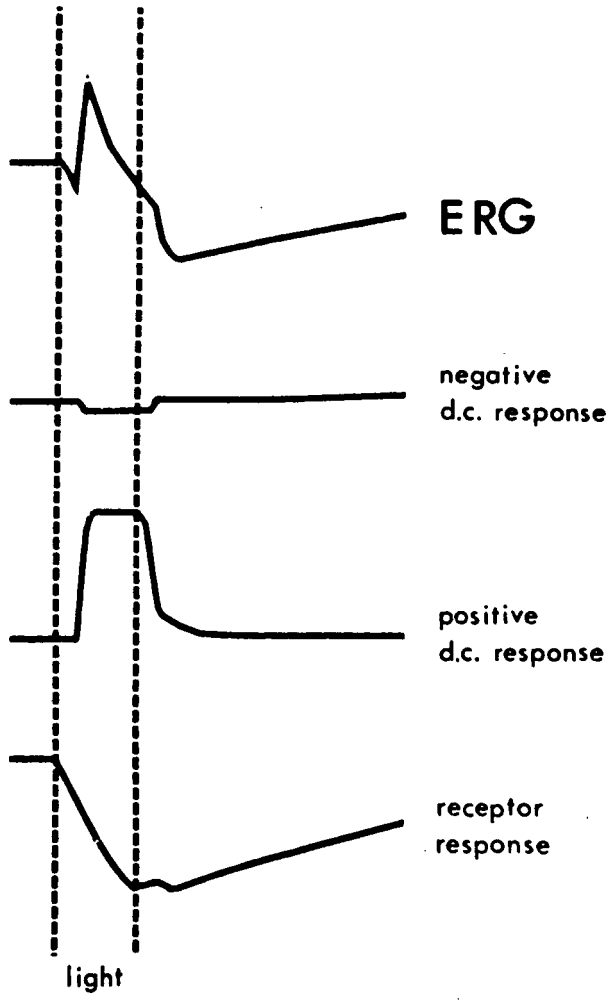


Figure 7. Component analysis for the light-adapted sheep eye. (From Knave, Moller and Persson, 1972).

C. Cellular Origin of the ERG Components

Before the ERG can be used optimally as a tool for visual research or clinical diagnosis, the structures generating the various components must be identified. A number of investigators, using microelectrodes to record the intraretinal response to illumination,have studied the problem. Brown and Wiesel (1961a) introduced methods for intraretinal recording in the intact eye. They established the criterion that each component should demonstrate its maximum amplitude as the electrode passed the structure that generated it. More importantly, they compared and related the ERG components recorded by conventional methods with those recorded "locally" by an intraretinal electrode (local ERG, or LERG).

Further, Brown and Wiesel (1961b) determined the amplitudes of the a-, b- and c-waves as a function of the depth of the electrode in the retina. The maximum amplitudes for both the a- and c-waves were found just on the retinal side of the pigment epithelium. The maximum amplitude of the b-wave was found in the region of the inner nuclear layer. The d.c. component was isolated in the inner nuclear layer although its appearance was shown to precede the b-wave. In the same experiment, Brown and Wiesel reported rare intracellular recordings believed to be single cells of the pigment epithelium. The records revealed a response that appeared to be an intracellular analog to the c-wave. In view of the findings of Noell (1953; 1954) who had reasoned that the c-wave was generated by cells of pigment epithelium, Brown and Wiesel concluded that the a-wave and c-wave were generated by the receptors and the pigment epithelium respectively. However, according to Brindley (1970) the spectral sensitivity of the c-wave corresponds to the absorption spectrum of rhodopsin.

Hence, the receptors must initiate or provoke the pigment epithelium to produce the c-wave. The exact cellular origin of the b-wave, however, was still unknown.

Later investigations expanded on these findings. Brown and Watanabe (1962) confirmed the receptor origin of the a-wave. Murakami and Kaneko (1966), using cold-blooded vertebrates, achieved the clearest isolation of the a-wave with a coaxial microelectrode. They indicated that the PIII component which was responsible for the a-wave was generated at two different retinal levels. The portion designated "distal PIII", was isolated in the bipolar layer. Brindley (1970) states that Hashimoto, Murakami and Tomita (1961), again with coaxial microelectrodes, isolated the bipolar cells as the generating structure for the b-wave. However, more recently, Miller and Dowling (1970) have recorded an intracellular analog to the b-wave from the Müller cells suggesting that the b-wave was generated by glial cells. Hence, there is still controversy concerning the exact cellular origin of the b-wave. Steinberg, Schmidt and Brown (1970) were able to obtain stable intracellular recordings from pigment epithelial cells. Consequently, they positively identified these cells as the origin of the c-wave, further confirming the work of Brown and Wiesel.

The origin of the minor components has also been studied. As stated previously, the early receptor potential has been located in the receptor cells, more specifically the outer segments. Ogden (1972) investigated the amplitudes of the oscillatory potentials as a function of electrode depth. His results indicated that the wavelets were generated in the inner plexiform layer.

Thus it appears that each component of the ERG is the result of the summed activity of many cells of a given type. Because the ERG is easily recorded, it provides the researcher or clinician with a tool which reveals the electrical activity from a known class of retinal cells. Specifically, the electrical activity of the receptor cells is reflected by the a-wave; electrical activity of the inner nuclear layer is reflected by the b-wave, and the electrical activity of the pigment epithelium is mirrored by the c-wave. Since biopotentials, in general, are dependent on the physiology of the generating cells, it can be said that the ERG reflects the physiological state of certain cell types in the retina. Abnormal physiological states are generally reflected by decreased amplitudes and/or lengthened temporal relations.

D. Factors Affecting the ERG

With the conventional method of recording the ERG several factors which may influence the response have been identified. Stimulus parameters which affect the ERG are: intensity, spectral composition, duration, size and position, and frequency.

Intensity

The form, latency and amplitude of the ERG may be affected by the stimulus intensity. In general, the components of the clinical ERG (a- and b-waves) increase in amplitude as the strength of the stimulus increases. This relationship has been described as showing a direct proportionality between the amplitude of the component and the logarithm of the stimulus intensity. As with other functions adhering to the Weber law, the relationship levels off at both extremes of the intensity scale

(Johnson, 1958). In fact, it has been shown (Knave, Moller and Persson, 1972) that the intensity-amplitude function for the b-wave has two saturation levels for the high intensity ranges. Latency decreases with increasing stimulus intensity; the function levels off as it approaches an inherent minimum for each component. The form of the recordable ERG changes with the intensity of the stimulus. For example, at extremely low intensities only the d.c. component will be recorded. By slowly increasing the intensity of the flash in small increments, the other components will be elicited in the following order: b-, c-, and finally the a-wave (Brown, 1968). The appearance of the photopic and scotopic components are dependent on the intensity of the source. Auerbach and Burian (1955) investigated this relationship; part of their results have been given in Figure 4.

Wavelength

The effect of different wavelength stimuli was investigated early on by Chaffee and Hampson (1924). They reported that the amplitude of the ERG (b-wave in this case) changed in a manner that could be predicted from the spectral sensitivity of the eye. Many investigators have studied this phenomenon; two of the most quoted studies were performed by Armington (1953) and Riggs, Berry and Wayner (1949). They are noted for their determination of the photopic (Armington) and scotopic (Riggs, et al.) spectral sensitivity functions. In general, it was found that the relative sensitivity to blue light was greater than anticipated. Boynton (1953) has shown that the increase in sensitivity to a blue stimulus was caused by the increased light scatter by the ocular media of these short

wavelengths. The form of the ERG is altered by different wavelength stimuli. These changes have been described earlier in the discussion of the b_p-wave.

Duration

Johnson (1958) states that the duration of the stimulus does not affect the latency (interval to first deflection) of the ERG. Implicit time (interval to maximum amplitude), however, may be altered as a function of stimulus duration, especially with a low intensity flash. With longer durations, the implicit time increases. If the stimulus duration is less than a certain critical value (30 msec light-adapted; 100 msec dark-adapted) and the ERG amplitudes are below the maximum response amplitudes, then the amplitude of the ERG varies as a function of the intensity and duration of the flash. The relationship found between stimulus intensity and duration is: $(I)(T) = K$ where K is a given submaximum amplitude of an ERG component (Johnson and Bartlett, 1956). The waveform is also influenced by the duration of the stimulus; in order to record the full ERG waveform (a-, b-, c-, d-wave) the stimulus duration must be greater than 0.5 sec (Johnson, 1958).

Size and Position

The size and position of the light source may affect the amplitude and waveform of the ERG. The influence of this parameter may be explained in terms of alterations in the effective amount of light reaching the retina. In other words, changes in the size and angle of view affect the amount of light entering the pupil. Boynton (1953) pointed out that for a small source ($<12°$) and a given amount of retinal illuminance the

amount of light scatter produced by the ocular media tended to mask the effect of these factors. In support of his argument, he cited the findings of Boynton and Riggs (1951). They reported that small stimuli imaged on the fovea, optic disk and a portion of the peripheral retina yielded equivalent b-wave responses. Nonetheless, Adrian (1945) reported that central stimulation enhanced the photopic response. For larger light sources (>12°) Johnson (1958) credited Boynton and Riggs (1951) for demonstrating that the relationship between size and intensity may be expressed by the formula $IA^k = C$ (A = area; C = constant submaximum amplitude ERG; I = intensity; k = constant exponent).

Frequency

As the interval between two successive stimuli is decreased (i.e., increase the stimulus frequency) there comes a time when the response to the second flash is initiated during the first ERG. The second flash will modify the existing ERG. The amount of alteration depends upon how soon the second flash follows the first flash. Increasing the frequency causes a reduction in the amplitude and distortion of the waveform because of the interaction between two responses. If the frequency is high enough, the individual responses are eliminated and the retina reacts as if it were continuously illuminated (Davson, 1963). Adrian (1945) demonstrated that the critical frequency for the photopic response was greater than that for the scotopic response. (Scotopic, 20-25/sec; photopic, 80/sec) It is generally considered that flicker rates greater than 20 fps elicit only cone responses (Krill, 1972).

Subject and Procedure Variables

Subject variables also affect the ERG. Of prime importance is the state of adaptation. Dark-adaptation augments the retinal response to light. All the major components of the ERG increase in amplitude as the sensitivity of the retina increases; the a_p- and b_p-waves increase to a lesser degree than the scotopic components. Under the appropriate conditions, dark adaptation curves, similar to those determined using psychophysical methods, may be obtained from the b-wave amplitude changes. Dark adaptation brings about an increase in latency and implicit time which can not be explained by the mere shift to scotopic dominance. The changes in form of the ERG with dark adaptation have been shown previously (Figure 4, Auerbach and Burian, 1955).

According to Bittini, Nicoletti, and Ronchi (1957) there is a statistically significant difference between the ERGs recorded from males and females. Females have the larger average response.

The full adult ERG in humans does not develop until after the first year of life. It remains relatively stable until the age of 50, at which time the amplitude decreases (Krill, 1972). The refractive error of the subject can influence the amplitude of the response. This factor is related to the axial length of the eye and not the quality of the retinal image. Hyperopic eyes in general have a shorter axial length than myopic eyes; they also demonstrate larger b-wave amplitudes (Krill, 1972). Abnormalities in the optics of the eye which limit the amount of light reaching the retina (e.g. cataracts) may affect the ERG. Pupil size is also a factor in that it may limit the amount of light entering the eye.

Karpe (1945) reported that this problem may be eliminated by using a mydriatic. Further, it is known that artifacts caused by blinking, eye movements and iris contractions may influence the recordable ERG (Karpe, 1945).

Certain procedural variables may also alter the recordable ERG. The type of active electrode is important. "Localized leads" tend to pick up artifacts more readily than do contact lens electrodes. The position of the active electrode must also be considered. Karpe (1945) suggested that the mean position of the contact electrode is the main methodical error in evaluating the representative b-wave amplitude of a series of responses. He stressed the need of insuring a fixed normal position in order to make the averages of different response series comparable.

Honda and Nagata (1972) investigated the effects of long- and short-acting barbiturates on the ERG. Their study showed that barbiturates enhanced the a- and b-waves of the rabbit ERG. But under the influence of phenobarbital sodium higher stimulus intensities provoked only a simple negative deflection. The effects of an ultra-short acting barbiturate (thiopental) on the sheep ERG was studied by Knave and Persson (1974a,b) and Knave, Persson and Nilsson (1974). They report that small to moderate (5-20 mg/kg) doses result in an enhancement of the a- and b-wave amplitude. Larger doses (>20 mg/kg) caused a further increase in the a-wave amplitude; however, the amplitude of the b-wave decreased.

E. The Purpose of the Present Study

Because the large domestic animals (sheep, swine, cow and horse) require special handling facilities for electroretinographic studies, the use of the ERG as a clinical tool in veterinary medicine has been limited primarily to small animals (cats and dogs). However, due to the visual side-effects of drug therapy, nutritional deficiencies, feed additives and accidental poisoning, a means for assessing the functional integrity of the visual system of the large domestic animal is needed. The ERG provides a means for evaluating the functional integrity of a portion of the visual system. The purpose of the present study is to quantitatively and qualitatively investigate the ERG of one specie of large domestic animals, the sheep. The results will aid in defining a range of amplitudes and implicit times for the normal animal. These results may then be used as baseline data for future toxocological studies.

CHAPTER II

PROCEDURE AND APPARATUS

A. Apparatus

A schematic of the stimulating and recording apparatus is shown in Figure 8. Stimulation could be produced by either of the two light systems shown. For procedures requiring a stimulus of short duration, photostimulator I (PSI) was used. The visual stimulus for PSI was generated by a xenon strobe (Grass PS22 Xenon Photostimulator). The xenon strobe was encased on one side by a hyperbolic reflecting surface and on the other side by a clear plastic diffusing screen. In addition, a filter mount painted flat black was secured to the strobe housing. The filter mount was used to hold one of three colored plastic filters (blue, yellow-green, red; Grass Instruments Color Filters) or a neutral density filter. The diameter of the effective aperture was 13 cm. The neutral density filter absorbed all visible wavelengths equally. The color filters used in conjunction with PSI had wide bandpass characteristics. The peak filter transmissions were 470 nm, 530 nm, and 665 nm (See Appendix, Figure 1 for spectral characteristics). The diffuser passed all wavelengths of light in the visible spectrum although it did favor the shorter wavelengths slightly. The intensity of the stimulus could be varied in five discrete steps (see "Calibration" section, p.33 for exact intensity values). The frequency of stimulation was continuously variable with a maximum of 60 fps.

Figure 8

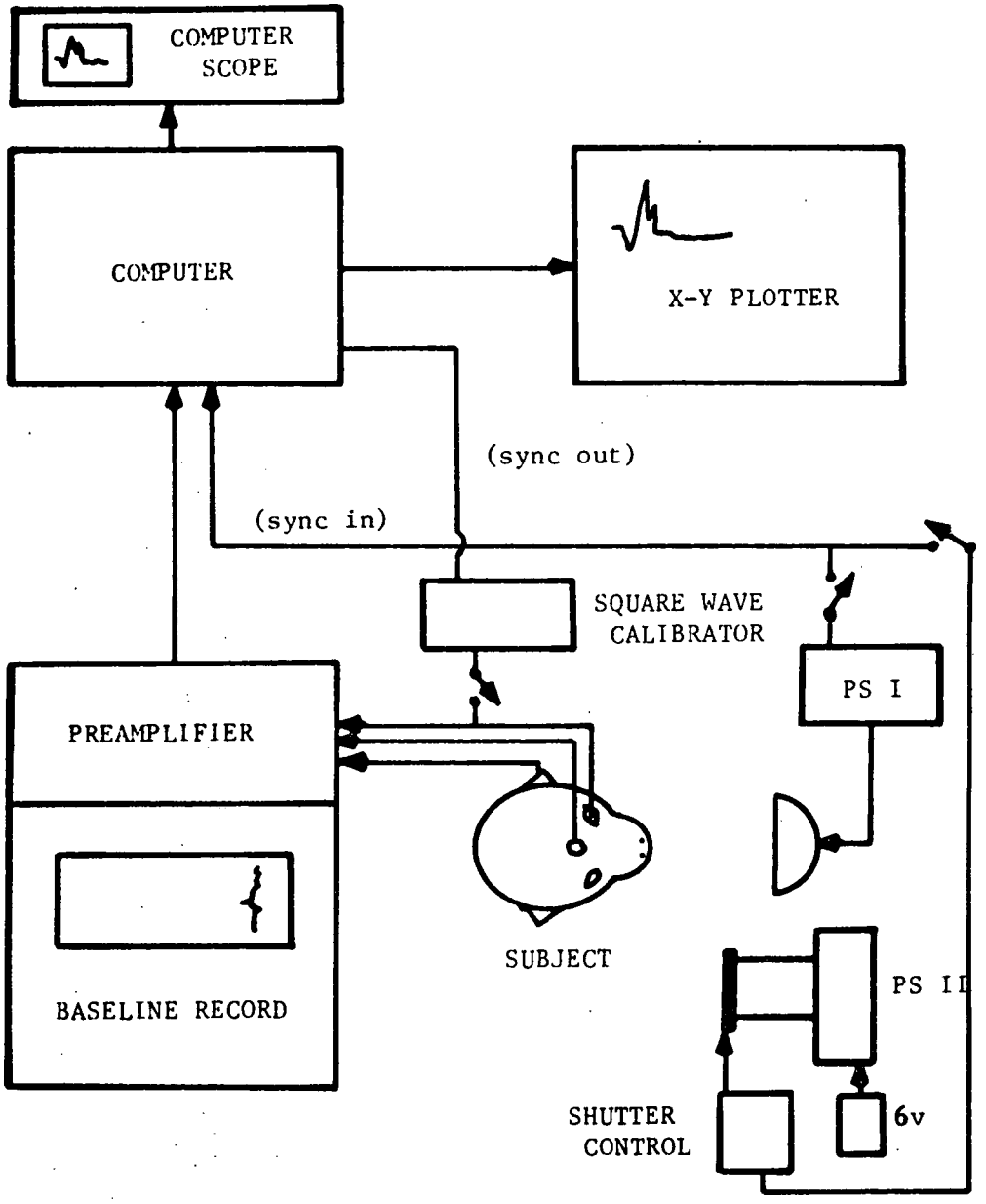


Figure 8. Schematic of the stimulating and recording systems.

Photostimulator II (PSII) provided flashes of longer duration. PSII delivered stimuli from a 6.0 V, 18 amp tungsten bulb, powered by a Cenco A.C. power supply. The source was enclosed in a metal housing with baffled air ducts. A 2.3 cm aperture and a frosted glass diffuser were placed 5 cm from the filament of the bulb. An electronic shutter (Continental type 4-20P) controlled by a Uniblitz Model 300 control unit was fitted 16.5 cm from the diffuser at the end of the viewing tube. The diameter of the shutter aperture was 2.5 cm. The two apertures in series acted as a collimator. At a distance of 25 cm from the shutter, PSII produced a uniform circle of light 4.5 cm in diameter. The area of uniform illuminance was clearly distinguishable from the surrounding penumbra. The duration of the stimulus exposure could be manipulated through the shutter control. The two systems, PSI and PSII, were used independently and were easily interchangeable. Both stimulus systems provided a trigger signal for the averaging computer. Thus, the interval of data imput was synchronized with the photic stimulation.

Three different electrodes were used in recording the ERG (Appendix, Figure 2). The active electrode was incorporated into a plastic scleral contact lens. The proper shape for the lens was determined from a mold of a sheep's eye. The electrode was gold packed around a copper wire. The gold formed part of the inner surface of the contact lens. The gold and copper wire were positioned on the central side of the corneal-scleral flange of the lens. Prior to insertion, the inner surface of the contact lens was filled with a conducting medium (2% methylcellulose, 0.9% NaCl). This electrode provided good electrical connections and kept corneal insult

to a minimum. The reference electrode was secured in place with rubber contact cement; it was placed on the forehead between the two eyes. The ground electrode (gold; Grass, E346) was attached to the animal's ipsilateral ear. It was held in place by a plastic clip. Electrode paste was used with the reference and ground electrodes to ensure adequate electrical connection.

The signal was amplified by an EEG differential A.C. preamplifier (Gould Brush, model 13-4218-CO) and direct writing recorder (Brush recorder model 1200). The sensitivity control of the preamplifier was adjusted so that a 20 mV pulse created a 1 mm deflection on the recorder paper. The preamplifier band pass filters were set at 0.1 Hz (low cut-off) and 1 kHz (high cut-off) to provide an adequate frequency response range for the ERG. The direct writer recording provided a baseline record (background noise) which was used to monitor the level of anesthesia. The chart speed of the recorder was normally set at 0.05 mm/sec when monitoring the level of anesthesia; however, the speed could be increased (maximum 200 mm/sec) in order to view an individual ERG.

The amplified response was also fed into a signal averaging computer (Nicolet Series 1070). The computer enhanced the signal by eliminating the effects of random electrical noise. Furthermore, many recording variables could be manipulated via the computer controls. These variables are as follows: number of responses averaged; dwell time (amount of time per memory address); filter time constants (high frequency only); and gain. The number of average responses varied with the state of adaptations. Four signals were averaged for the dark-adapted subject;

sixteen were averaged for the light-adapted animal. The dwell time was set at 100 μsec for short duration flashes (100 μsec). Thus the total time of data input was 0.4096 sec (for 4096 memory addresses). For longer stimulus durations (1 sec), the dwell time was set at 1 msec. However, only half of the available memory was used; hence the total time of data input was 2.048 sec. The filter time constant was 0.02 Hz for all conditions. The gain was also held constant at ± 4x. An oscilloscope (Tektronix, model R5103N) was connected to the computer to provide a display for the computer memory. An X-Y plotter (Omnigraphic, model 2000) was also coupled with the computer to provide permanent records.

B. Subjects

The experimental subjects were 15 normal sheep (25 eyes), 6 were males and 9 were females. The age varied between 12 and 36 months. The weights varied between 36.4 kg and 56.8 kg. The animals were selected from the flock at the USDA Veterinary Toxocology and Entomology Research Laboratory, College Station, Texas. All animals were in good health at the time of experimentation.

C. Procedure

Prior to an experimental session, the animal was deprived of food and water for 24 hrs. An indwelling intravenous catheter (jugular vein) was used for the administration of the anesthetic (sodium pentabarbitol, 65 mg/ml). Since the anesthetic was administered intravenously, a speci-

fied dose per unit weight was not given. Instead the anesthetic was administered slowly until the desired state of anesthesia was reached (plane 2, surgical). The second plane of surgical anesthesia was easily differentiated from the first plane because of the lack of gross eye movements (Hall, 1966). Since any eye movement greatly affected the baseline record of the recorder, the direct writer provided an excellent monitor of the stage of anesthesia. The depth of anesthesia was maintained by the administration of small amounts of anesthetic (1-2ml) when the baseline recording indicated an increased amount of activity. Cycloplegia was induced with a subcutaneous injection of atropine (0.5 ml, 15 mg/ml). The animal was intubated to insure an adequate air passage for breathing and was placed in sternal recumbency and secured to a series of restraining bars. The forehead and ears (reference and ground electrode position) were shaved and prepared with detergent and alcohol. The reference electrode was held in place with rubber contact cement; once secured, the electrode was filled with electrode paste through two holes on the top surface. The ground electrode was clipped to the ear; liberal amounts of electrode paste were used. The lids were retracted and held in place with nylon sutures. Occasionally, it was necessary to suture the eye in the proper position. In such cases a topical anesthetic and antibiotic were applied after the recording period. The contact lens electrode was inserted. It was then checked for centration and the presence of air bubbles. When PSI was used, it was placed 50 cm from the animal's eye approximately on the pupillary axis. For PSII the shutter was placed 25 cm from the eye also on the pupillary axis. The electrodes were connected to the preamplifier system via shielded cables. Before continuing, the background noise level was checked.

If the signal was exceedingly noisy or contained 60 Hz activity, the electrical connections and contact lens were checked to eliminate the problem.

Due to the lateral position of the eyes, only one eye was investigated at a time. In order to look at the most clinically used components (a- and b-wave), the ERGs produced by the short duration stimulus (PSI) were studied in 10 animals. For a given animal, a series of light- and dark-adapted ERGs were recorded; the light-adapted series was always completed before the dark-adapted series. Each light-adapted ERG recorded was the average response of 16 individual ERGs. The effect of varying the intensity of a white stimulus was examined first. The five different intensities provided by PSI were used. The highest intensity was employed first; the rest were utilized in the order of decreasing intensity. The stimulus was presented once every second. There was a delay of 2-3 minutes between trials; this was the time necessary to print out the ERGs on the X-Y plotter. The effect of varying the frequency of a white stimulus was determined next. The intensity of the flash was held constant at the maximum value. The stimulus frequencies studied and their order of presentation were 10 fps, 20 fps, 30 fps and 40 fps. The effect of different wavelength stimuli were investigated last. The three colored filters described earlier were used in the following order: blue, green and red. The stimulus intensity was set at G16; the stimulus frequency was 1 fps. Upon completing the light-adapted series the animal was dark-adapted for 30 min. Each dark-adapted ERG was the average of four individual responses. To keep the light-adaptation to a minimum, the stimulus

was presented once every minute. Only two stimulus parameters, intensity and wavelength, were tested under dark-adapted conditions. The intensity and wavelength of the stimuli investigated and their order of presentation were the same as those for the light-adapted series. Once the dark-adapted series was completed, the animal's other eye was prepared and the same procedure repeated. For five of the animals the entire experimental procedure was repeated fourteen days after the first experimental session to provide a check on repeatability.

To examine the full ERG waveform a stimulus (PSII) with a longer duration (1 sec) was used. The ERG responses of five animals were recorded for both light-adapted and dark-adapted conditions; only a single intensity of white light was investigated. For the light-adapted animal, the stimulus was presented once every three to four seconds; sixteen ERG responses were averaged. Upon completion of the light-adapted trial the animal was dark-adapted for 30 min. To minimize adaptation, the stimulus was presented once every minute. Four responses were averaged. Only one eye was examined for each animal.

D. Calibration

The calibration of the intensity of a single flash from PSI was accomplished with a radiometer (EG&G). The detector head was placed 50 cm from the clear diffuser. Both source and detector were aligned on an optical bench. A circular aperture was placed 10 cm from the diffuser, and this provided full illumination of the surface of the detector head. The aperture allowed the use of a calibrated neutral density wedge and

balance to reduce the intensity falling on the detector surface. The neutral density wedge and balance were positioned directly behind the circular aperture. A sheet of black velvet was secured in the plane of the aperture to insure that only light passing through the aperture and ND wedge could reach the detector. Caution was taken to eliminate all stray light within the room. A clear plastic carrier was positioned in the source's filter holder during all measurements. Measurements were taken in terms of energy (watts/cm^2) for all four filters (red, blue, green, clear) at the five different intensity settings. Three readings were taken for each measurement, the mean of which was recorded.

It was desirable to produce flashes of equal energy for all four filters (equal energy spectrum). Hence, it was necessary to use auxillary neutral density filters (Kodak Wratten Gelatin Filters) in conjunction with the three filters which produced the higher energy flashes. The neutral density wedge was ideal for determining the value required for the auxillary ND filter. It was found that the red filter provided the lowest level of energy per flash. To derive the proper ND value for the green, blue and clear filters the value of the ND wedge was slowly increased in small increments until it had reduced the energy per flash to equal that for the red filter. The correct ND wedge value was found for all five intensity settings. Selection of the final value for the auxillary ND filter was made with the highest intensity flash. However, the value varied less than 0.2 log units over the five intensities used. The ND values determined for the blue, green and clear filters are 0.9, 0.8, and 1.3 respectively. The final energy level per flash for the five intensity settings were:

G16, 1.24×10^{-9} watts/cm^2; G8, 4.9×10^{-10} watts/cm^2; G4, 2.7×10^{-10} watts/cm^2; G2, 1.73×10^{-10} watts/cm^2; and, G1, 8.3×10^{-11} watts/cm^2.

The duration of a single flash from PSI was measured with a fast responding photodiode (EG&G, model SGD 100 Photodiode). The diode was positioned 20 cm directly in front of the xenon strobe. The output of the diode was displayed on an oscilloscope, and photographed with a polaroid camera. Three responses were recorded at the G16 intensity. The flash duration was measured at the half-amplitude level (Appendix, Figure 3A). The duration measured from all three responses was the same; it was found to be 100 μsec. The electronic shutter for PSII was timed with the same photodiode. The diode was placed 20 cm from the shutter and again the output was displayed on an oscilloscope. The rise time for opening the shutter was 6 msec; the closing time was also 6 msec. The total duration of the system was adjusted for a 1 sec exposure (Appendix, Figure 3B).

The irradiance of PSII was calibrated with a circular Eppley 16 junction thermopile. It was placed on an optical bench 25 cm from the shutter. To increase stability the thermopile was enclosed in a box with its interior painted flat black; all sources of stray light were eliminated from the room. A circular aperture approximately 5 cm in diameter was put in the box in line with the source. Upon illumination, the entire lamp black surface of the detector was filled with light. The thermopile was coupled with a Keithley 150B microvolt/ammeter. The output from the

thermopile was read out in μV on the microvolt/ammeter. The voltage output was converted into irradiance values (watts/cm^2). Prior to opening the shutter of PSII the microvolt ammeter was zeroed. The shutter was then opened and left open until a stable reading was recorded from the meter. The average energy level for 3 measurements was 3.34×10^{-8} watts/cm^2.

The level of background illuminance of the experimental area was measured with a SEI Photometer. Measurements were taken from a milk glass surface of known reflectance. The reflecting surface was placed at the approximate position of the animal's eye. The readings were taken from a distance of one meter. For light-adapted conditions the level of illuminance was 195.8 lux.

A square wave calibrator (Grass SWC-1B) was incorporated into the recording system to produce signals of a known amplitude and duration. This allowed calibration of the recording instrumentation and hence the recorded ERGs. Calibration records were made after each experimental session using the same instrument settings as used to record the ERG. The recording instrumentation remained stable throughout the entire experiment.

CHAPTER III

RESULTS

The ERG of the sheep was investigated in a qualitative and quantitative manner. Quantitatively, the ERG was described in terms of amplitudes and implicit times. The aspects of the ERG which were quantitatively considered were the a- and b-waves. The amplitude of the a-wave was defined as the potential difference (μV) between the baseline and the lowest point or trough of the a-wave. The b-wave amplitude was the potential difference (μV) between the trough of the a-wave and the highest point on the b-wave. The implicit time was the interval (msec) from the presentation of the stimulus to the maximum deflection of a given ERG wave. Only ERGs produced with PSI were studied in this manner. The stimulus conditions evaluated were the five intensities of white light (G16, G8, G4, G2, G1) and a single intensity (G16) of red, green and blue light. Both light-adapted and dark-adapted conditions were evaluated.

A. Stimulus Intensity

The typical responses elicited by different intensities of white light are shown in Figure 9. The left column of the ERGs were recorded under light-adapted conditions; those in the right column represent dark-adapted ERGs. The value of the stimulus intensity increases from the lower to the upper curve. The expected alterations which were noted in the introduction are generally demonstrated in these intensity series.

Figure 9

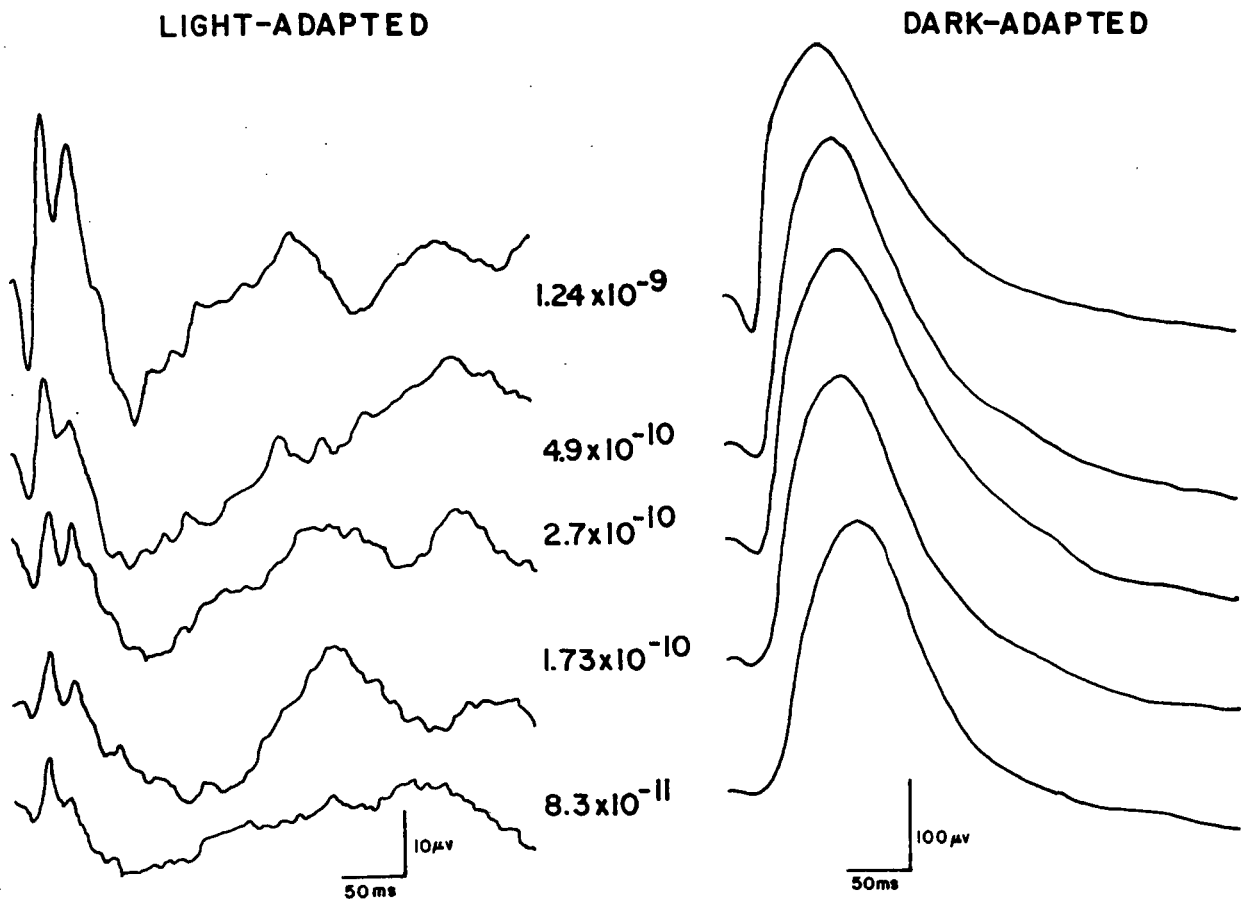


Figure 9. Light-adapted (left column) and dark-adapted (right column) sheep ERGs. White stimulus; intensity varies, decreasing from top to bottom as indicated. Stimulus duration 100 sec. Calibration: light-adapted 10 sec. Calibration: light-adapted 10 v, 50 msec; dark-adapted 100 v, 50 msec.

For the light-adapted series each component (a-, b_p-, and b_s-wave) increases in amplitude as the intensity of the stimulus increases. At the lower intensities, however, the a-wave amplitude measurements are sometimes uncertain (e.g., G4) because of a gradual departure from the baseline of the a-wave. The implicit times remain relatively stable despite changes in the stimulus intensity. The form of the responses is altered due to the changing interaction of the photopic and scotopic b-waves. In some cases, the amplitude of the scotopic component of the b-wave exceeds that of the photopic component.

In the dark-adapted series similar changes are observed with only minor differences. For example, the amplitude of the b-wave for stimulus intensity G16 (651 μ V) is smaller than the response for G8 (723 μ V). This over-emphasizes the levelling-off effect found at the extremes of the intensity scale. The b-wave implicit times decrease with increasing stimulus intensities; the a-wave implicit times remain relatively constant. Subtle changes in waveform also occur. With increasing intensity, the effects of the photopic b-wave become more obvious. Although the individual b_p-wave was not isolated, its contribution results in a faster rise time for the b-wave (see G16 response).

Table I shows the means, standard deviations, and ranges for the amplitudes and implicit times of the b-waves for the various white stimulus intensities. The data are from ten sheep (20 eyes) for both light-adapted and dark-adapted conditions. In the light-adapted responses in which the scotopic b-wave component is larger than the photopic component, some alterations in measurement are made. If the b_p-wave is obvious, the

TABLE I

Stimulus Condition and Intensity		b-wave Amplitude (μV)			b-wave Implicit Time (msec)		
		$\bar{X}$	S.D.	Range	$\bar{X}$	S.D.	Range
Light-Adapted	G16	24.2	8.3	12.6 - 38	29.1	4.2	24.2 - 38.91
	G8	15.6	4.5	8.4 - 21.5	30.4	3.2	23.75- 35.84
	G4	12.5	4.5	5.0 - 17.2	30.4	4.1	25.6 - 37.88
	G2	10.3	4.1	4.0 - 17.0	30.9	2.1	28.67- 37.43
	G1	8.1	2.7	1.9 - 12.9	31.3	5.2	27.03- 51.81
Dark-Adapted	G16	454	121	252 - 664	86.3	8.3	75.7 -104.5
	G8	453	141	248 - 800	93.7	8.6	79.9 -112.2
	G4	419	131	243 - 693	97.9	10.1	84 -122.9
	G2	403	128	209 - 659	106.4	10.8	93.2 -129
	G1	352	133	146 - 627	109.7	8.9	96.3 -122.5

implicit time and amplitude are measured with respect to the b_p-wave even though the b_s-wave actually represents the peak of the b-wave response. However, if an obvious b_p-wave is not present, measurements are taken with respect to the b_s-wave. The trends seen in Figure 9 are mirrored in the mean values for the entire group of animals. In particular, the b-wave amplitudes for both the light-adapted and dark-adapted conditions vary directly with changes in the stimulus intensity.

To further illustrate this, the mean amplitudes of the b-wave (some investigators consider the b-wave amplitude as the amplitude of the ERG) are plotted versus the log relative stimulus intensity. Figure 10 shows data for the dark-adapted condition (circles). Included in Figure 10 is the curve representing the findings (triangles) of Riggs and Johnson (1949); their records are from the dark-adapted human eye. Since Riggs and Johnson used a wider range of stimulus intensities than employed in the present study, only a portion of their data is shown. Arbitrarily, the 4.66 log stimulus intensity for Riggs and Johnson is aligned with the G1 stimulus intensity of the present study.

Riggs and Johnson show that the b_s-wave amplitude is linearly related to log I. The relationship is linear for the lower stimulus intensities, leveling off at the higher intensities. A trend analysis (Winer, 1962) was applied to the lower intensity findings (G1, G2, G4, G8) for the sheep. Due to the amount of variability between subjects (see the standard deviations, Table I) a significant linear component is not indicated. As a result, a single linear function could not be determined and the points are simply connected. Nevertheless, the

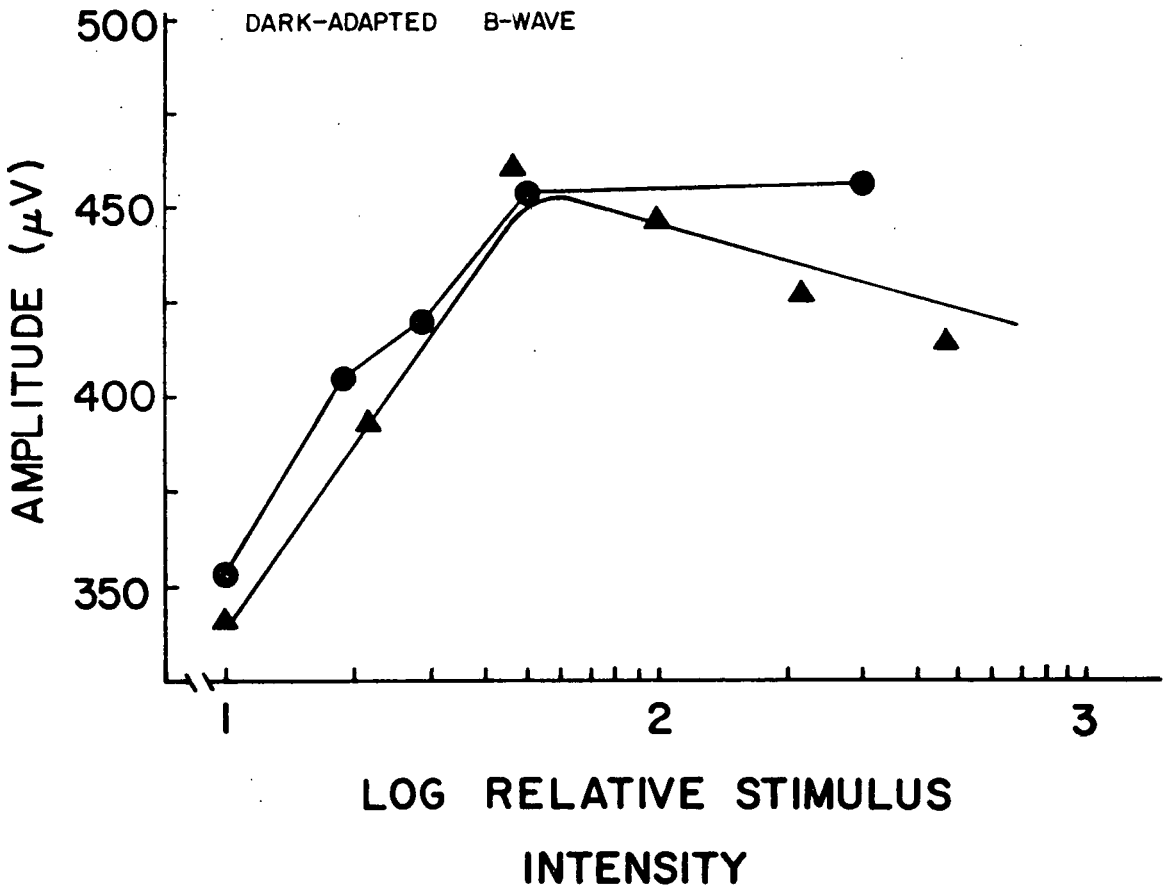


Figure 10. Mean dark-adapted b-wave amplitude plotted against log relative stimulus intensity. Sheep, circles; human, triangles. For the human data: white stimulus, duration 0.04 sec, 7.5° diameter. (From Riggs and Johnson, 1949).

trends for the two sets of data are in close agreement. It should be noted that the data from Riggs and Johnson show a decrease in amplitude with the extreme high intensities. The same trend was observed earlier for the curves in Figure 9, although it was apparently masked in the averaged data.

In Figure 11 the averaged data for the light-adapted animal is shown (circles). The amplitude of the b-wave is plotted against the log relative stimulus intensity. For comparison, a portion of the human data of Crampton and Armington (1955) are also shown (triangles). The curves are adjusted so that the lowest stimulus intensities for each study coincide on the abscissa.

The b-wave amplitude appears to be linearly proportional to log I of the stimulus. A trend analysis (Winer, 1962) of the sheep data indicates a significant ($p < .01$) linear component (Least-squares linear equation: $y=11.2x+7.6$). Both sets of data exhibit a gradual increase in b-wave amplitudes with increases in stimulus intensity. This should be contrasted to the data for the dark-adapted eye where a steeper and more sigmoid plot is found. Comparing Figures 10 and 11, it is found that the sheep b-wave amplitude found under dark-adapted conditions is approximately 19 times greater than the light-adapted b-wave. Crampton and Armington reported only a 5-fold difference in the human response. Several factors may have contributed to this discrepancy. The function for the light-adapted b-wave is known to show an upward turn at very high intensities; apparently intensities greater than those used in this

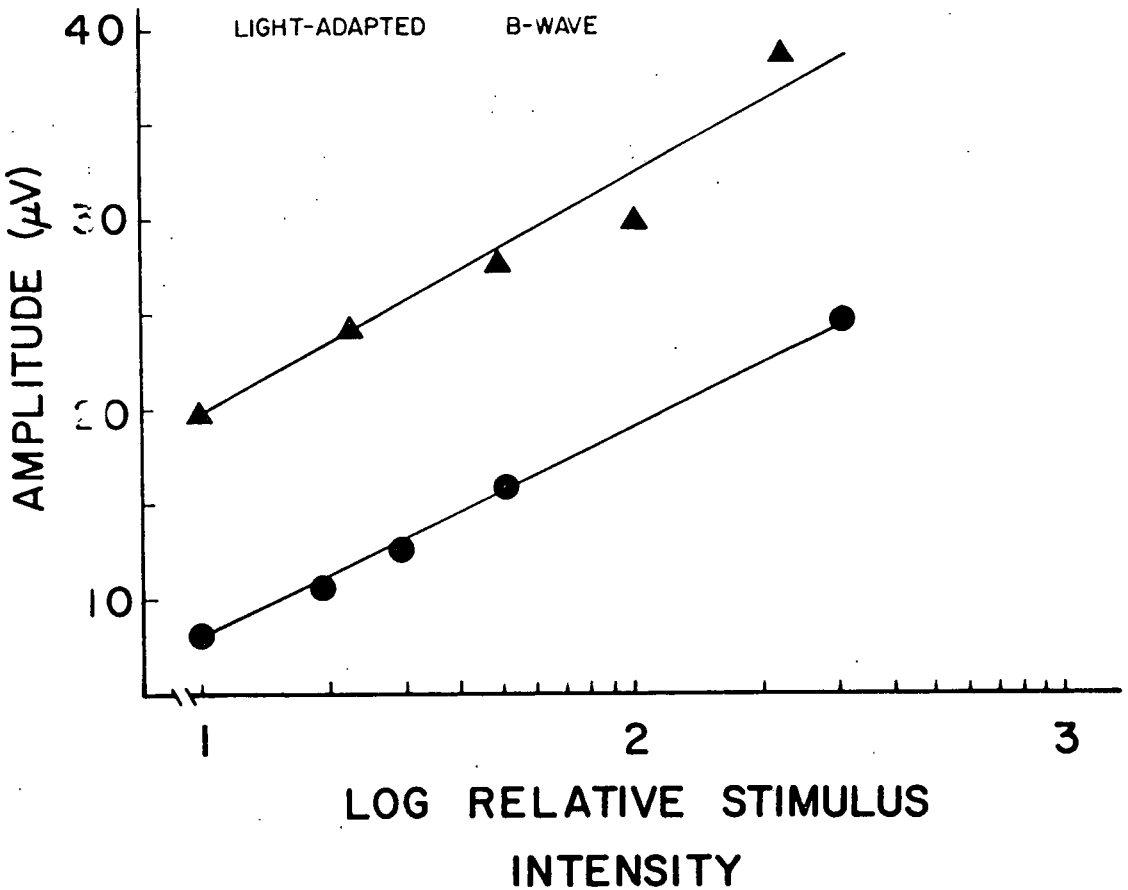


Figure 11. Mean light-adapted b-wave amplitude plotted against log relative stimulus intensity. Sheep, circles. Human, triangles. For the human data, red stimulus, 26°4' diameter, duration 0.01 sec, level of adaptation 2 Ft-l. (From Crampton and Armington, 1955).

study. Since the light-adapted function does not level off at high intensities as does the dark-adapted function, the higher the intensity of the stimulus the smaller the difference in amplitudes. However, if one compares the difference in light-adapted and dark-adapted amplitudes elicited with intensities found on the linear portion of the light-adapted function, the data from Crampton and Armington show 7.5 times greater dark-adapted amplitudes. The larger part of this difference is most easily explained as an interspecies difference.

Figure 12 illustrates the mean b-wave implicit times plotted as a function of the log relative stimulus intensity (sheep, circles). A trend analysis (Winer, 1962) indicates a significant ($p<.01$) linear component for the dark-adapted implicit times (Least-squares linear equation: $y=16x+108.3$) but not for the light-adapted implicit times. Data from Johnson and Bartlett's (1955) study of the human eye are also shown (triangles). Both the light-adapted (open points) and dark-adapted (filled points) curves are arranged on the intensity axis so that the maximum intensities for both studies coincide. All curves with the possible exception of the light-adapted sheep data show that the implicit time varies logarithmically as a function of intensity. Increasing the stimulus intensity decreases the b-wave implicit time; the human ERG being more affected by changes in the stimulus intensity than the sheep ERG. The graph also demonstrates that the sheep ERG is more affected by the adaptation level than the human response. The increase in implicit time following dark adaptation is much greater than would be anticipated from a simple shift of dominance from the photopic to the scotopic system.

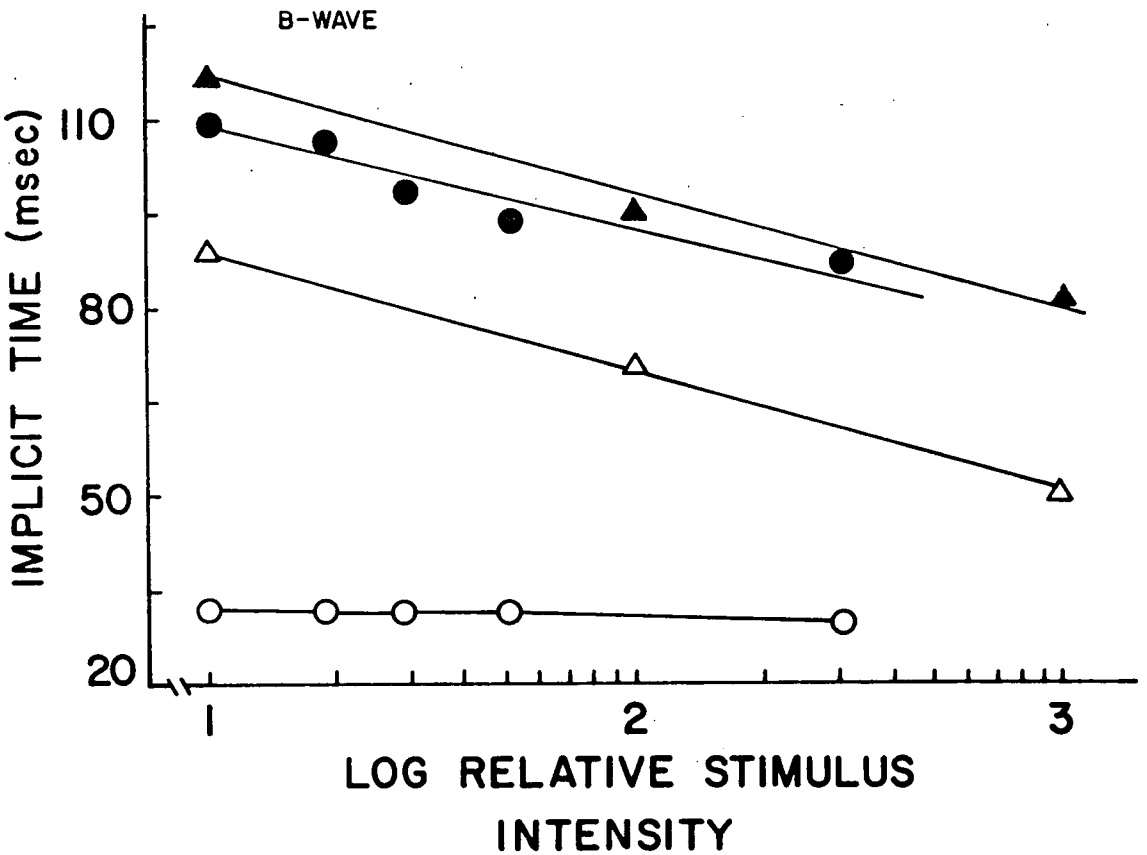


Figure 12. Mean light-adapted and dark-adapted b-wave implicit time plotted against log relative stimulus intensity. Sheep, light-adapted open circles; dark-adapted filled circles. Human, light-adapted open triangles; dark-adapted filled triangles. For the human data white stimulus, 7.5° diameter, level of adaptation 0.02 ft-l. (From Johnson and Bartlett, 1955).

This is most easily seen by comparing the implicit times for the b_s-wave under light-adapted conditions to the dark-adapted b-wave implicit times shown in Figure 9. If there was a simple shift in dominance, the b_s-wave would have the same implicit time under both adaptive states.

The means, standard deviations and ranges for the a-wave amplitudes and implicit times are given in Table II. Data is shown for the five stimulus intensities of white light for the light-adapted and the dark-adapted conditions. Notice that the a-wave is treated as a single entity. It is not possible to consider the photopic and scotopic components individually because a clear separation is rarely achieved. Nonetheless, the a-wave amplitude behaves in a predictable manner with respect to changes in the stimulus intensity. In Figure 13, the amplitudes for both the light-adapted (open circles) and dark-adapted (filled circles) a-waves are plotted as a function of the log relative stimulus intensity. A trend analysis (Winer, 1962) indicates that both the light-adapted and dark-adapted data have significant linear components (Least squares linear equation: $y=2.6x+1.7$ for the light-adapted condition; $y=34.4x+6.9$ for the dark-adapted condition). Along with these curves is plotted the data from Burian (1954) for the dark-adapted a-wave (triangles). The highest intensity used by Burian is compared with the highest intensity used in the present study. The general shapes of the two dark-adapted curves are quite similar. It appears that there is a rapid increase of the a-wave amplitude lasting over an intensity range of approximately 1.5 log units. Moreover, it has been reported (Johnson, 1958) that the intensity-amplitude relationship does not approach a response maximum. (Some investigators

TABLE II

Stimulus Condition and Intensity		a-wave Amplitude (μ V)			b-wave Implicit Time (msec)		
		$\bar{X}$	S.D.	Range	$\bar{X}$	S.D.	Range
Light-Adapted	G16	5.6	3.0	1.0 - 13.4	15.3	2.0	12.8 - 19.8
	G8	3.3	2.0	0.5 - 7.3	14.2	2.8	8.2 - 20.3
	G4	3.0	1.7	0.0 - 5.5	15.3	2.4	11.5 - 21.0
	G2	2.5	1.2	0.4 - 4.0	15.7	2.1	13.4 - 21.0
	G1	1.7	1.1	0.0 - 5.0	15.3	2.0	11.0 - 19.5
Dark-Adapted	G16	58.8	27.3	231 - 128	25.1	3.4	22.1 - 31.3
	G8	29.6	14.9	4.7 - 52.2	26.3	2.3	21.8 - 30.6
	G4	21.9	13.9	7.8 - 67	25.3	2.6	20.8 - 30.6
	G2	13.9	7.7	5.8 - 37.2	26.4	4.7	21.3 - 41.2
	G1	10.4	5.6	2.4 - 20.9	26.0	6.1	15.8 - 46.7

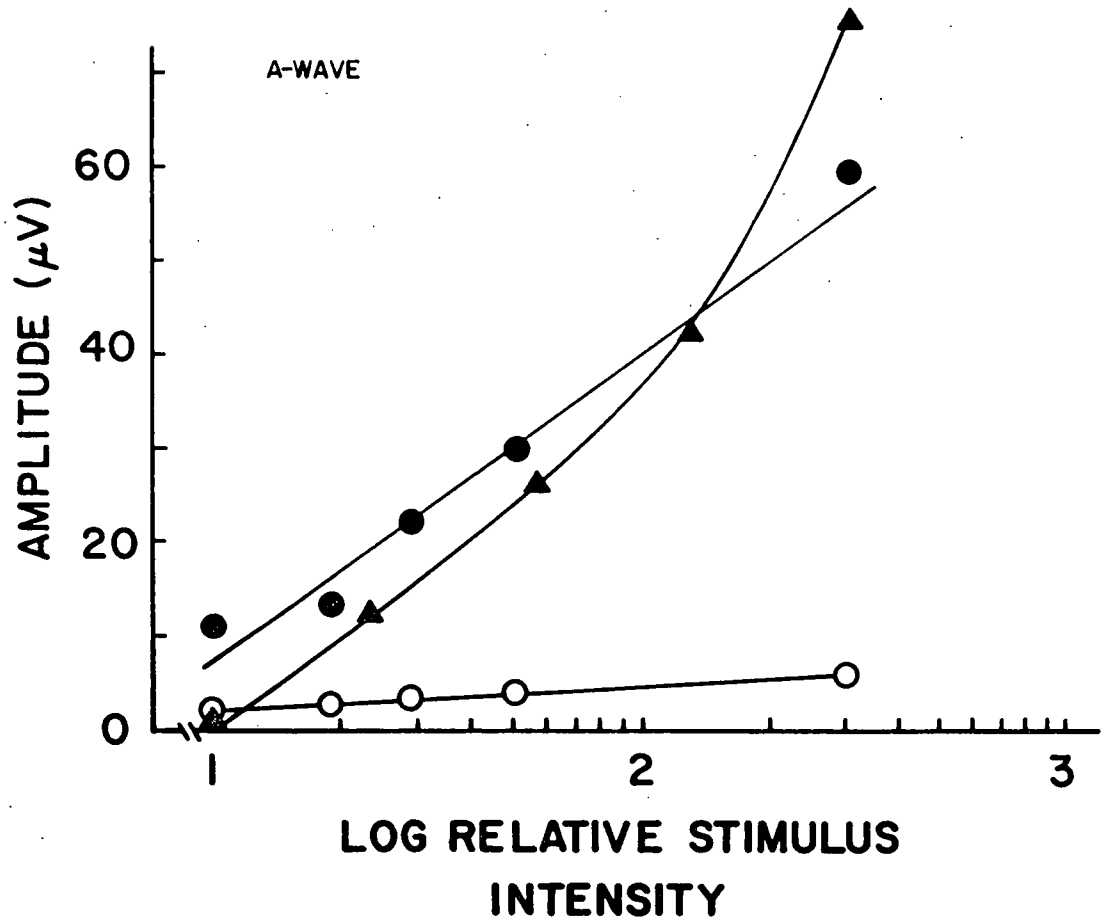


Figure 13. Mean light-adapted and dark-adapted a-wave amplitudes plotted against log relative stimulus intensity. Sheep, light-adapted open circles; dark-adapted filled circles. Human dark-adapted triangles. For the human data white stimulus, duration 30 msec. (From Burian, 1954).

have hypothesized that the increasing a-wave function accounts for the leveling-off of b-wave function. Johnson, 1958). The range of stimulus intensities over which large a-wave amplitude changes are seen is considered to be preceded by a range of lower intensities at which only a b-wave may be recorded.

The light-adapted a-wave function demonstrates a gradual increase in amplitude with increases in log I of the stimulus. This is comparable to the form of the light-adapted b-wave function (Figure 11). The slope of the a-wave curve is more gradual in comparison to the b-wave curve. However, due to the gradual departure from the baseline (e.g., Figure 9, G4) of the a-wave, the amplitude-intensity relationship may not appear consistent in some individual sheep. The difficulty is determining the proper baseline from which the measurement is to be taken.

The light-adapted (open circles) and dark-adapted (filled circles) a-wave implicit times are graphed in Figure 14. A trend analysis (Winer, 1962) indicates that there is not a significant linear component for either the light-adapted or dark-adapted data. Hence, the mean implicit time values are simply connected by straight lines. Although systematic changes can be seen for some individual intensity series, the descriptive data for the entire group is not remarkable in this repect. To add perspective, the implicit time values for the dark-adapted human a-wave (triangles) determined by Krill and Lee (1963) are included in Figure 14. The function found by Krill and Lee is fixed on the abscissa so that the positions for the lowest stimulus intensity for the two studies coincide. The human responses show a more rapid change within approximately the

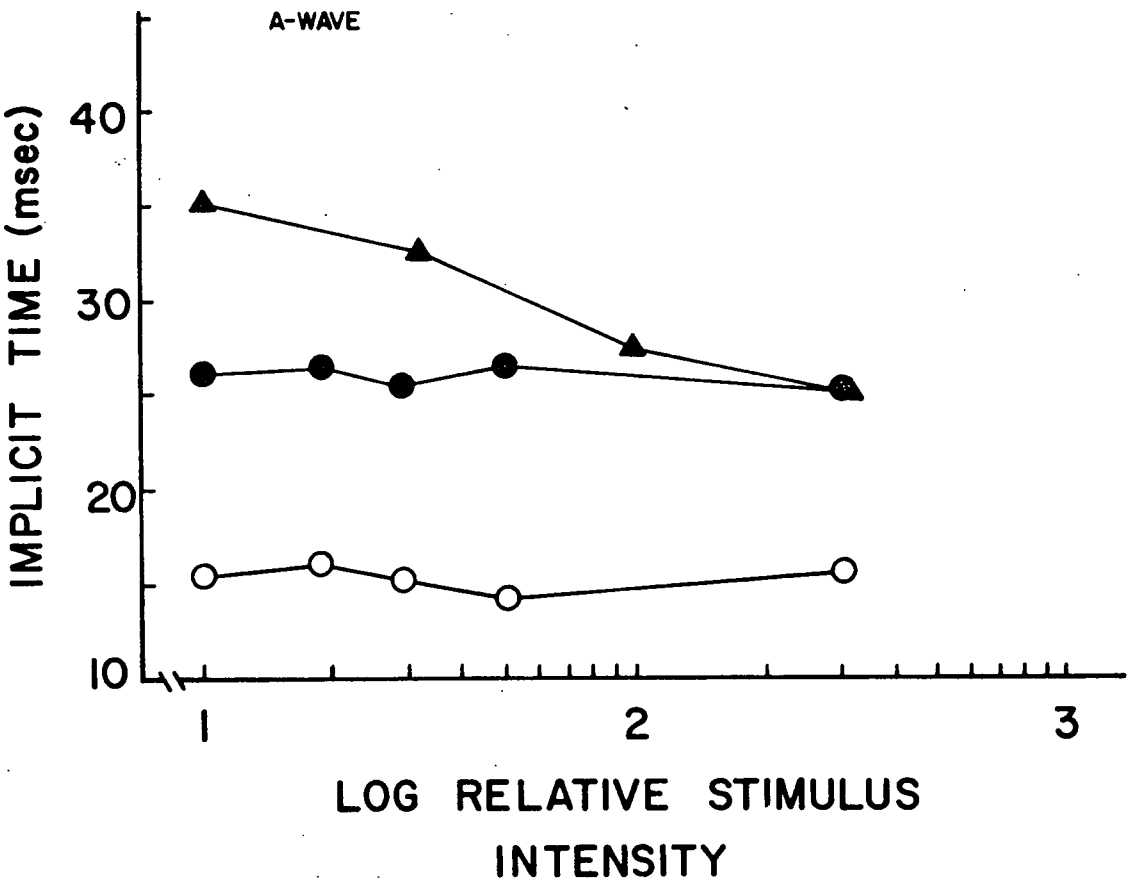


Figure 14. Mean a-wave implicit time plotted against log relative stimulus intensity. Sheep, light-adapted open circles; dark-adapted filled circles. For the human data (triangles) white stimulus, duration 30 sec. (From Krill and Lee, 1963).

same relative intensity range. It is possible that with the particular stimulus conditions employed the implicit times for the sheep are approaching some inherent minimum value. This could limit the range of response magnitudes resulting in little change with respect to changes in intensity.

In auxiliary studies higher stimulus intensities were used. Subsequently, it was observed that the light-adapted a-wave implicit times were shorter than those reported above (light-adapted a-wave 10.5 msec). This suggests that the implicit times are not approaching a minimum value. Instead, it indicates a slow changing implicit time versus amplitude function.

Normal Response Range

The criteria used to differentiate a normal from an abnormal ERG is usually based on the population mean and standard deviation. In general, a response value which falls within the range of the population mean $\pm$ 1 standard deviation is considered normal. Response values which fall between 1 and 2 standard deviations from the mean are considered borderline or suspicious, and auxiliary ERGs (e.g., high frequency stimulation) are needed to make the proper diagnosis. Amplitude and implicit time values which are greater than 2 standard deviations from the mean are considered abnormal. In this situation, auxiliary tests (e.g., ophthalmoscopy) should be performed in order to determine the cause of the abnormal response.

Use of different stimulus intensities has proved beneficial in differentiating normal from abnormal ERGs (Krill and Lee, 1963). However,

the standard deviations listed in Tables I and II are large with respect to the differences between the mean values for the G1 and G16 stimulus intensities (e.g., dark-adapted b-wave amplitude). Hence, by the criteria given above, the response values for the G1 and the G16 stimulus intensities should be considered to be from the same population. In other words, the advantage of using different stimulus intensities is lost due to the amount of response variability between individuals. But the changes produced by the different stimulus intensities are readily demonstrated in the responses from a single animal. A more sensitive baseline for the above criteria can be established if the response changes produced by the different stimulus intensities are measured relative to the response value for the lowest stimulus intensity. Notice the mean amplitude values for the dark-adapted b-wave (Table I). If the amplitude for the G1 intensity (352μ V) is considered the "zero" point, increasing the stimulus intensity to the G16 level should produce a 102 μV increase in the b-wave amplitude. By evaluating the changes produced by each intensity relative to the G1 intensity for the entire population of sheep, a mean and standard deviation for the changes produced by each stimulus intensity can be calculated. The mean and standard deviation of the changes produced by stimulus intensities G2, G4, G8 and G16 with respect to the G1 value are graphed in Figure 15. Data for the a-wave and b-wave amplitudes and implicit times for both the light-adapted and dark-adapted conditions are shown. For all a-wave and b-wave amplitude functions, the G1 value is outside the standard deviation for the G16 intensity. Despite the fact that the absolute values for G1 intensity

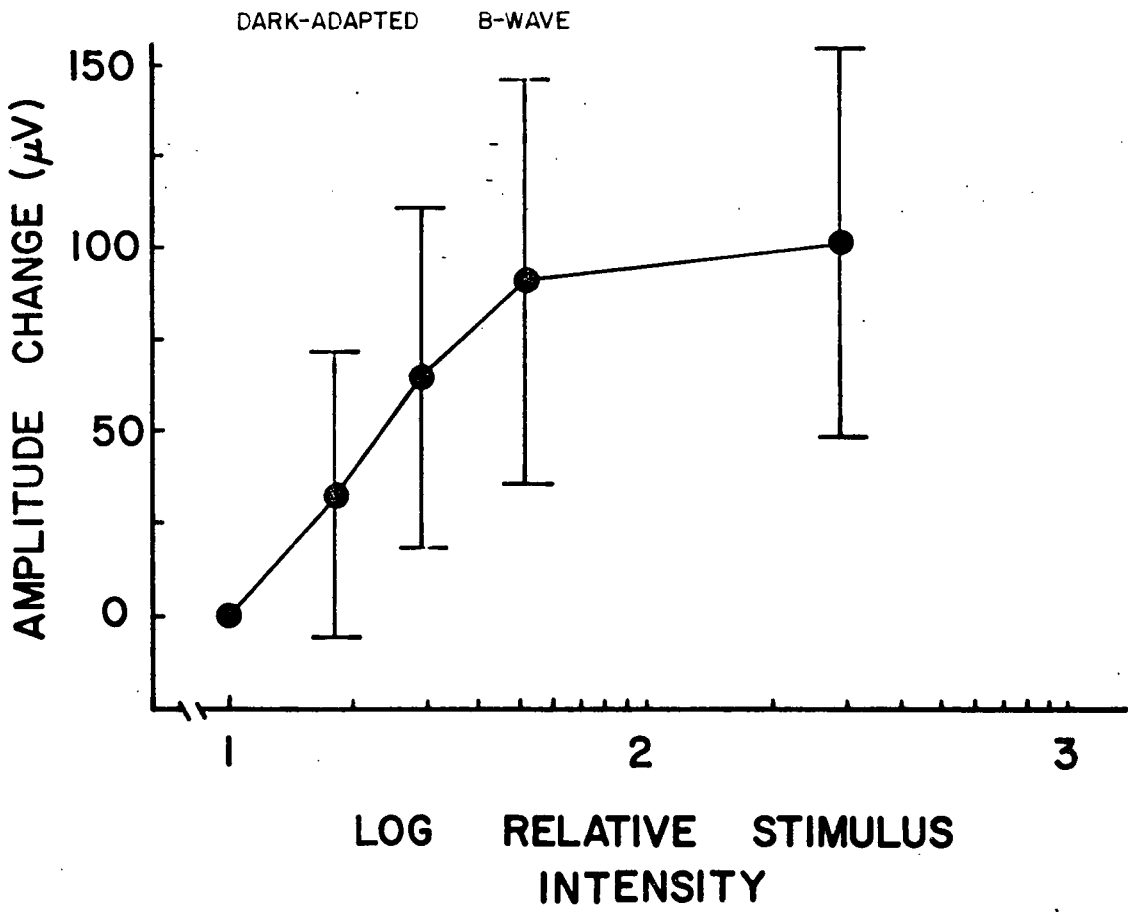


Figure 15A. The $\bar{X} \pm 1$ SD of the changes (ordinate) measured with respect to the GI intensity, produced by various white stimulus intensities (abscissa).

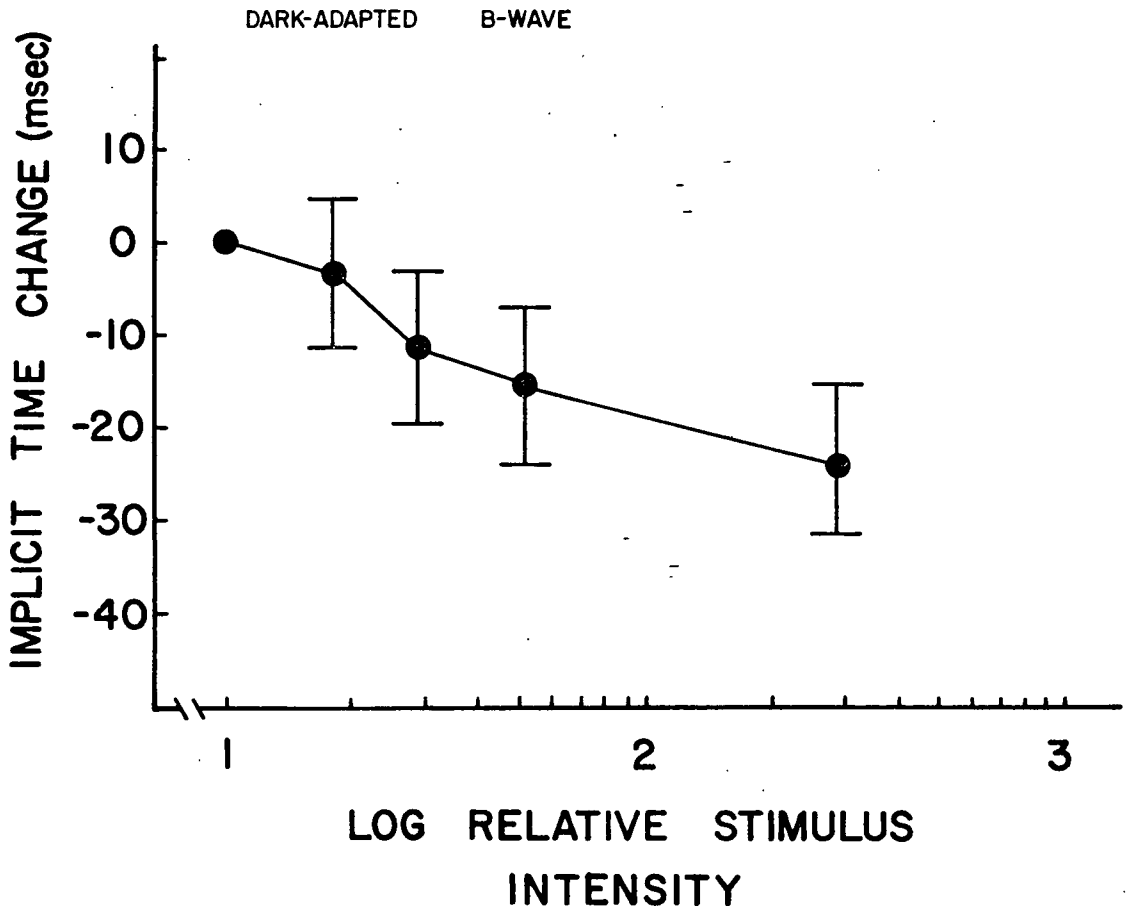


Figure 15B. $\bar{X} \pm 1$ SD of the changes measured with respect to the GI intensity.

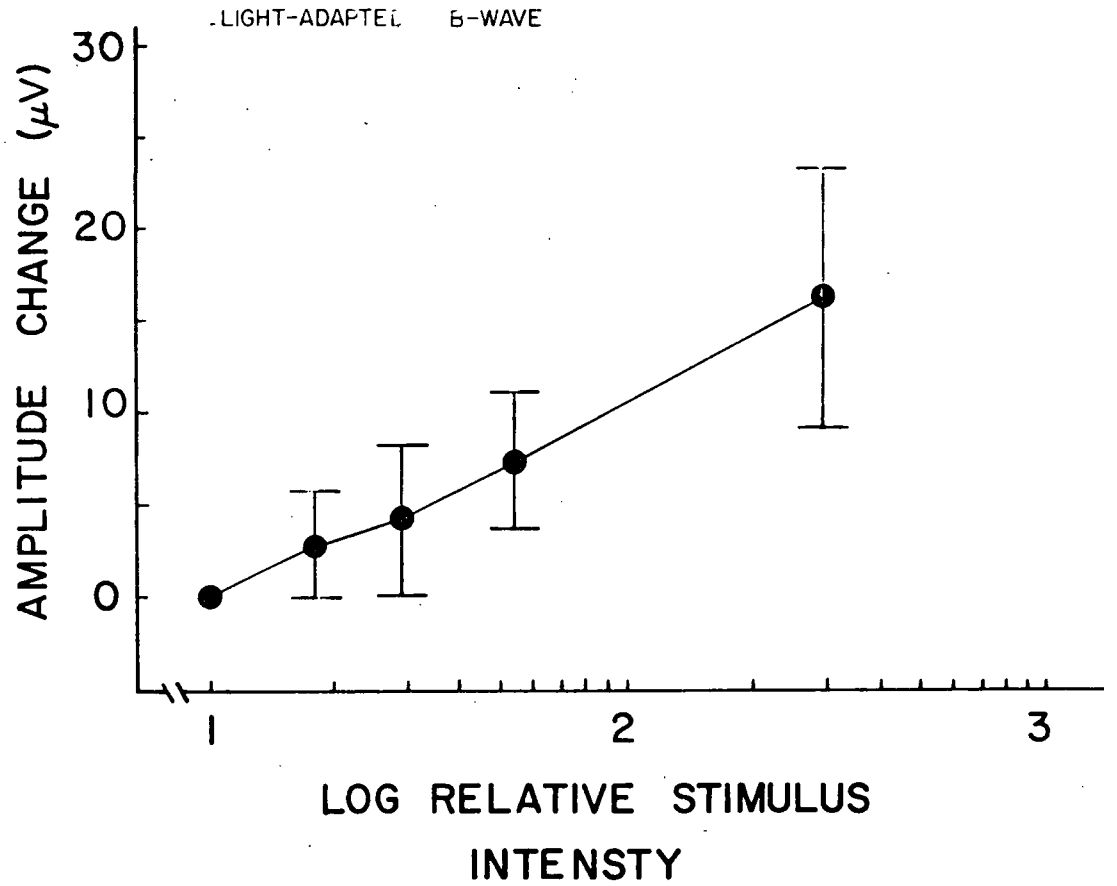


Figure 15C. $\bar{X} \pm 1$ SD of the changes measured with respect to the GI intensity.

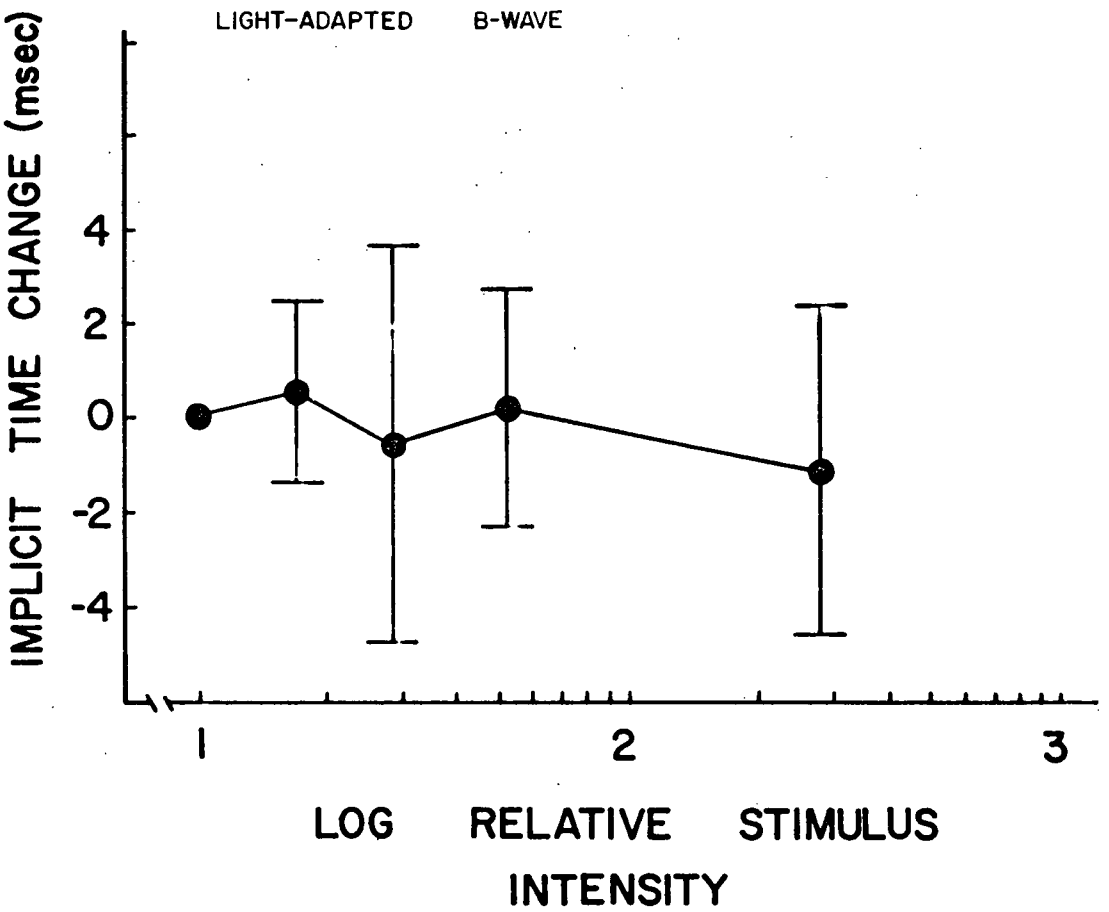


Figure 15D. $\bar{X} \pm 1$ SD of the changes measured with respect to the GI intensity.

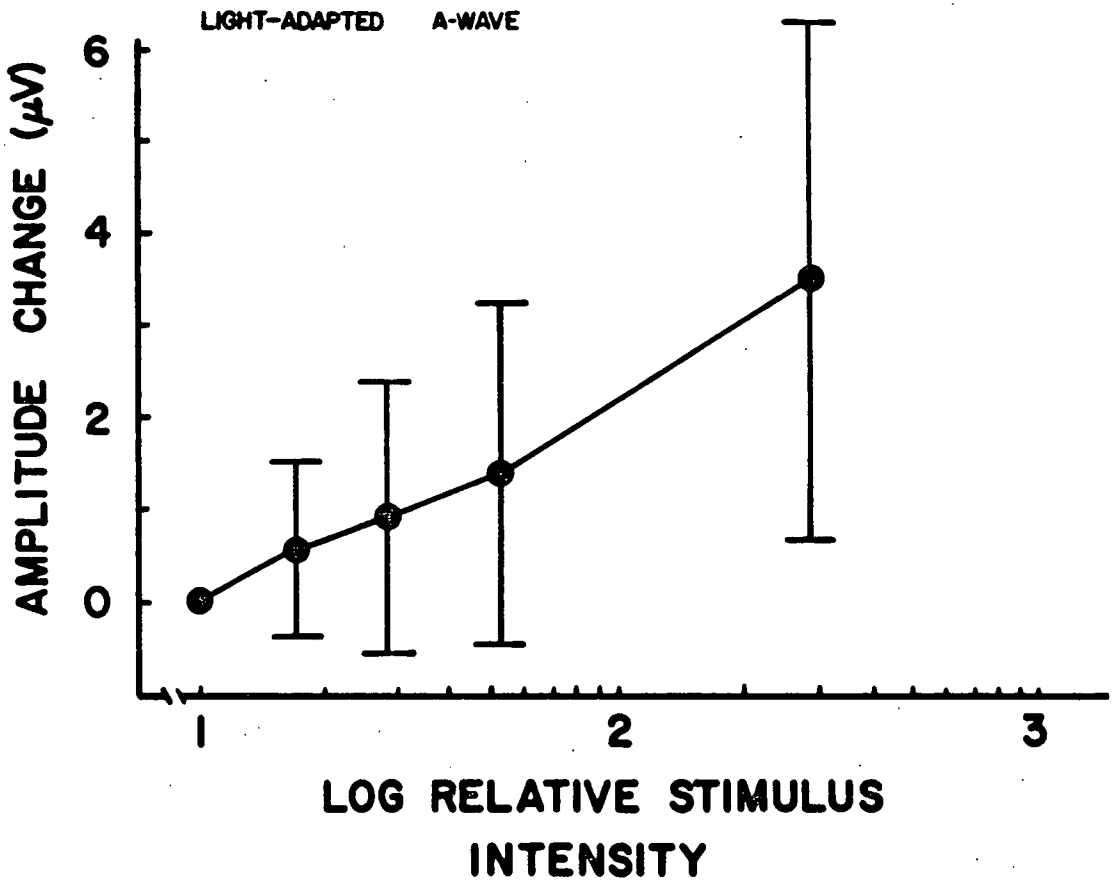


Figure 15E. $\bar{X} \pm 1$ SD of the changes measured with respect to the GI intensity.

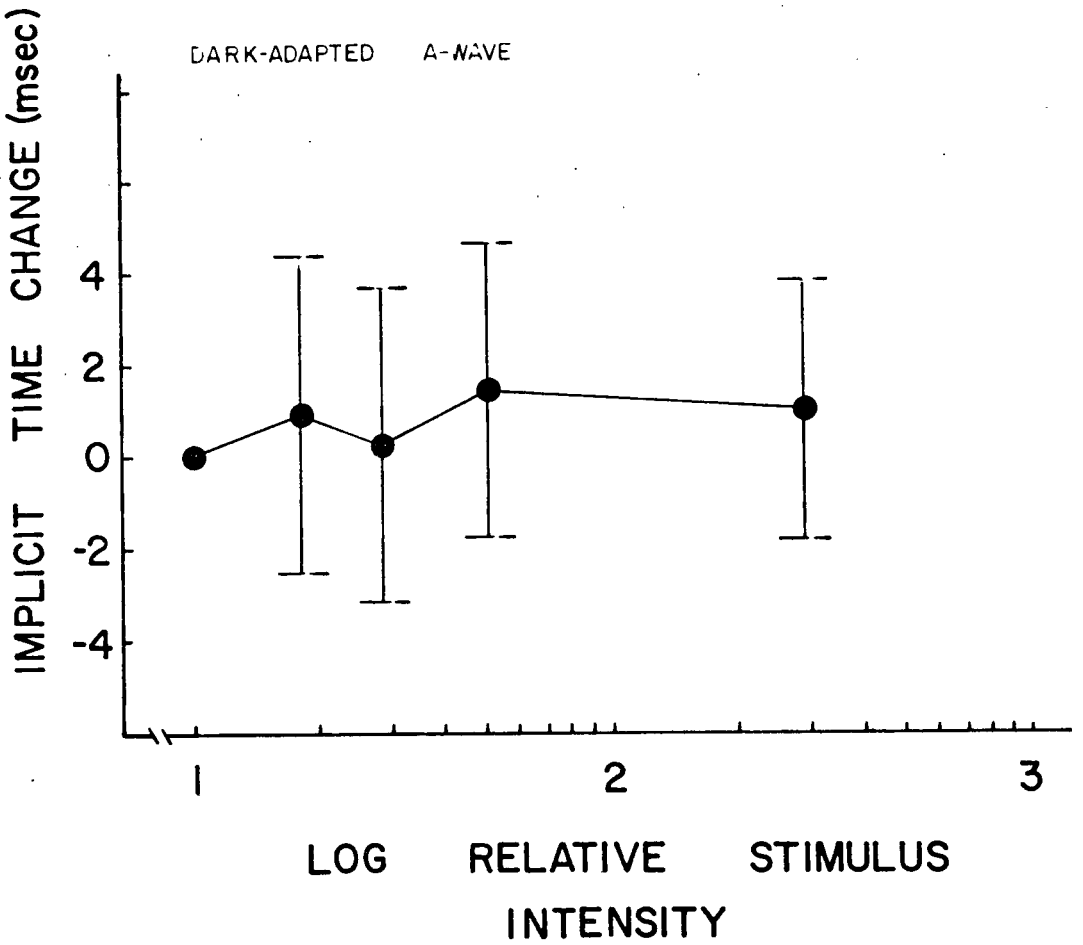


Figure 15F. $\bar{X} \pm 1$ SD of the changes measured with respect to the GI intensity.

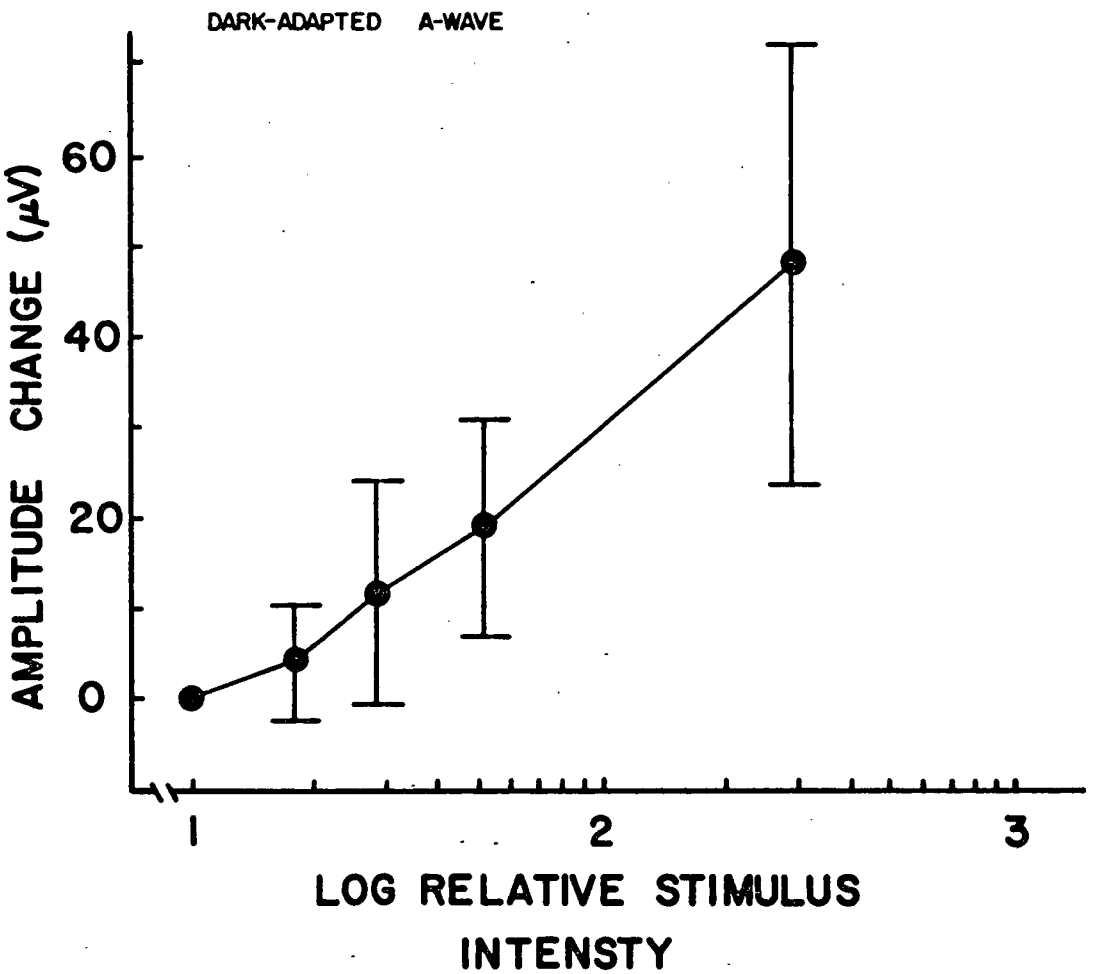


Figure 15G. $\bar{X} \pm 1$ SD of the changes measured with respect to the GI intensity.

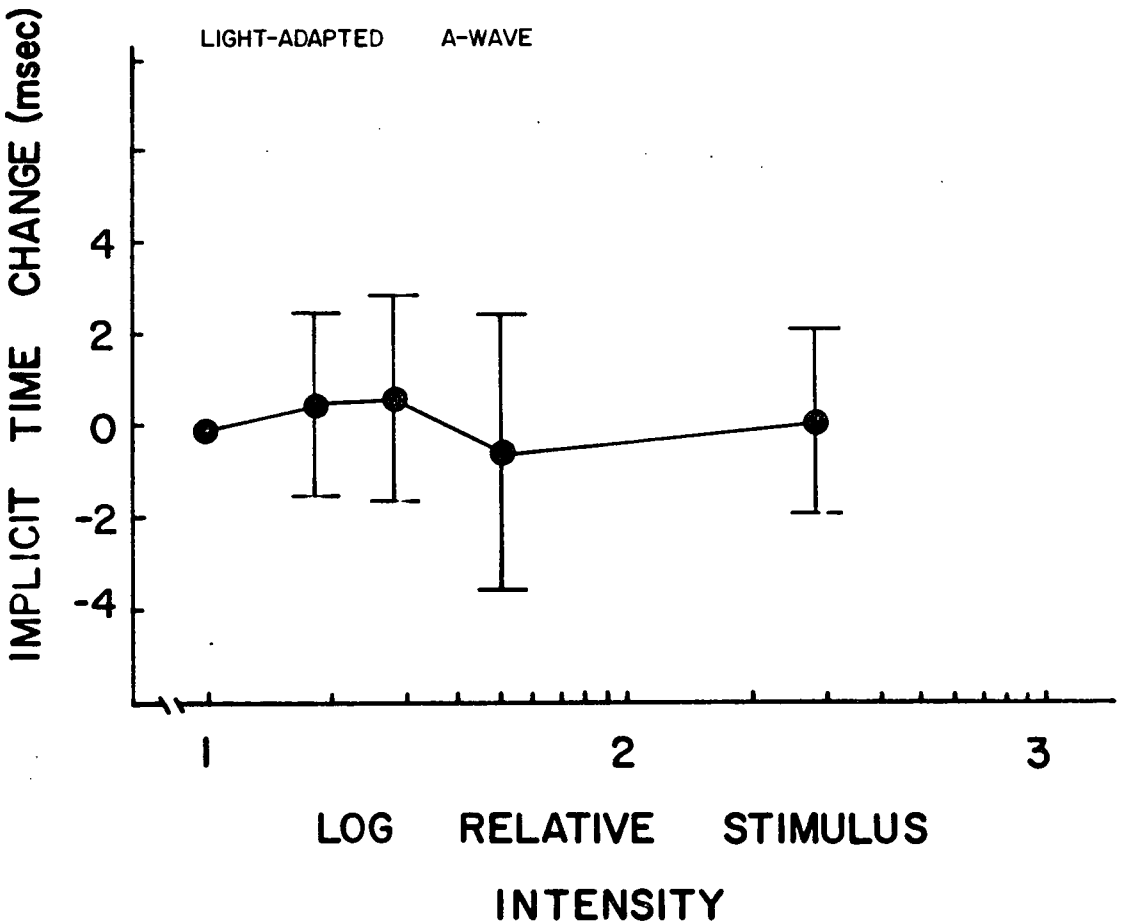


Figure 15H. $\overline{X} \pm 1$ SD of the changes measured with respect to the GI intensity.

vary between subjects, the criteria described earlier may now be used with respect to at least the G1 and G16 intensities. For the normal animal, a predictable increase in amplitude should accompany an increase from the G1 to the G16 stimulus intensities.

With the exception of the dark-adapted b-wave implicit times, virtually no change is seen with respect to changes in the stimulus intensity. This can be advantageous when evaluating the abnormal response. It allows the establishment of a single acceptance range for the normal response for all the stimulus intensities. In this situation, a normal implicit time would fall within one standard deviation of the mean for the G1 stimulus intensity. The dark-adapted b-wave implicit times may be evaluated in the same manner as the b-wave amplitudes.

B. Stimulus Wavelength

Typical ERGs recorded in response to blue, green and red stimuli are displayed in Figure 16. The light-adapted responses are shown in the left column; the right column of responses are dark-adapted ERGs. From the light-adapted ERGs it may be seen that the green stimulus is the most effective in producing a response. The blue stimulus is the least effective. Aside from the amplitude differences, the most noticeable changes are concerned with the relative contributions of the photopic and scotopic components to the form of the b-wave. Using the blue stimulus maximally stimulates the scotopic component while stimulating the photopic component minimally. The resulting b-wave is composed of approximately equal portions of photopic and scotopic components. With

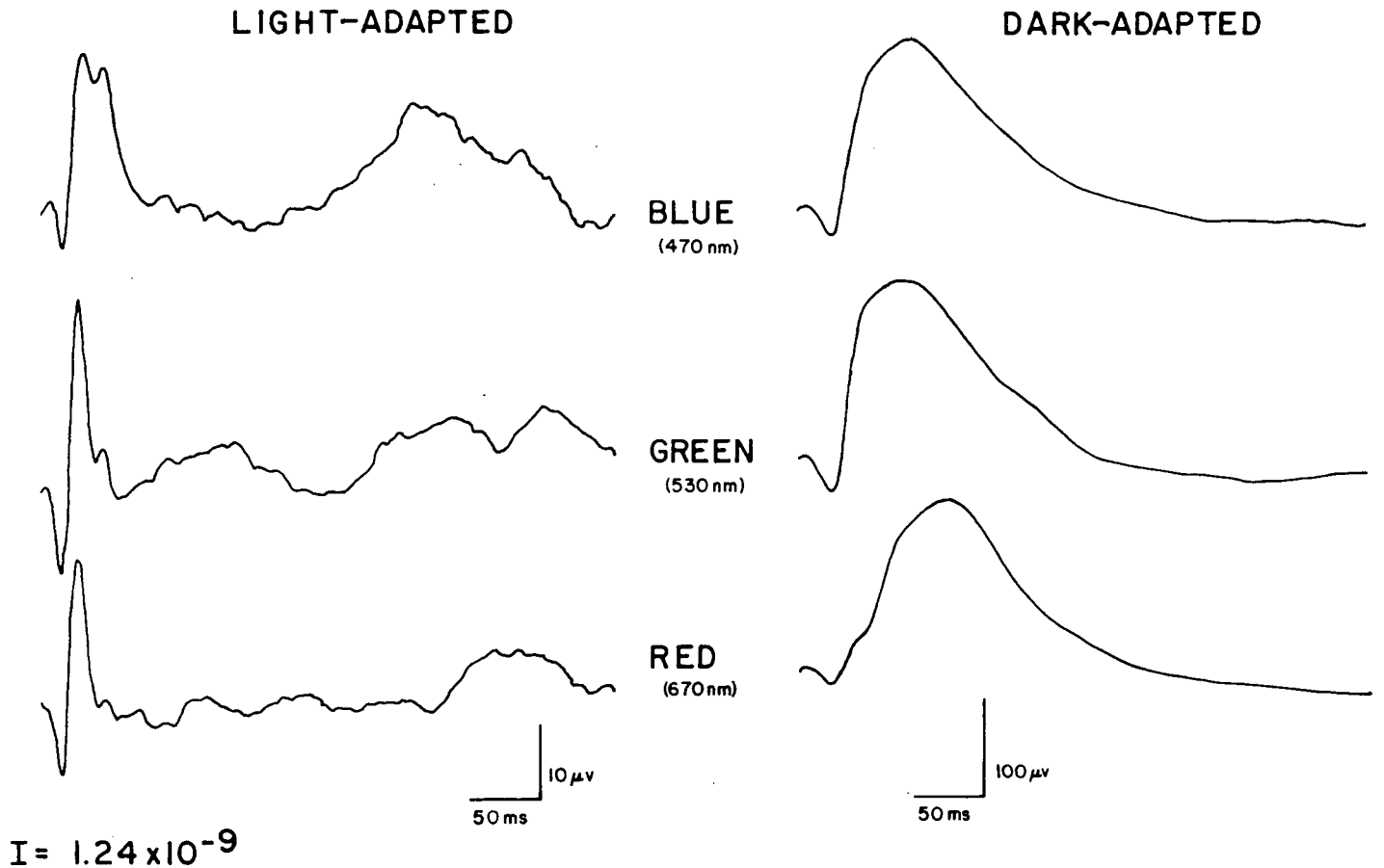


Figure 16. Light-adapted (left) and dark-adapted (right) ERGs. Stimulus intensity G16. Stimulus color, from top to bottom blue, green and red. Calibration, light-adapted 10 v, 50 msec; dark-adapted v, 50 msec.

the longer wavelength stimuli, the photopic component is increasingly stimulated. The inverse is true for the scotopic component. The green stimulus, as would be predicted, produces a response intermediate to those of the blue and red stimuli. The green response reveals a large photopic component along with a readily identifiable scotopic component.

However, during the process of dark-adaptation there is a shift in dominance from the photopic to the scotopic system. In the dark-adapted eye the shorter wavelength stimuli are more effective. As is shown in Figure 16, the green and blue flashes produce similar effects. The b-wave amplitudes and implicit times are nearly equal. The red stimulus is the least effective in evoking dark-adapted ERGs; the amplitude of the scotopic component is decreased and it shows a longer implicit time. Nevertheless, the red flash still readily stimulates the cone system. As a consequence, the photopic component of the b-wave is clearly isolated.

The mean amplitudes of the a-waves and b-waves for the entire group of animals (10 sheep; 20 eyes) confirm these single-animal findings. Table III contains the means, standard deviations and ranges of the amplitudes and implicit times for the a- and b-waves. Data for the red, green and blue stimuli under light-adapted and dark-adapted conditions are included. The mean amplitudes for the a- and b-waves under light-adapted conditions show that the green stimulus is the most effective. The blue stimulus shows the smallest amplitude while the red stimulus produces amplitudes slightly smaller than the green flash. The dark-adapted eye exhibits the smallest a- and b-wave amplitudes in response

TABLE III

Stimulus Condition and Color	A-WAVE						B-WAVE					
	AMP (μV)			LATENCY (msec)			AMP (V)			LATENCY (msec)		
	$\bar{X}$	S.D.	Range	$\bar{X}$	S.D.	Range	$\bar{X}$	S.D.	Range	$\bar{X}$	S.D.	Range
Light-Adapted												
Blue	3.7	1.8	0-7.5	14.8	1.8	12.0-18.4	18.9	6.9	9.1-36.5	33.1	4.6	25.4-45.6
Green	5.7	2.3	1.5-10.8	14.5	1.4	13.1-17.7	24.8	9.8	12.3-35.3	28.3	2.9	23.8-34.8
Red	4.6	2.9	.9-12.8	14.9	1.7	11.8-18.4	21.2	9.1	9.9-46.0	27.8	3.2	21.8-33.8
Dark-Adapted												
Blue	47.6	21.3	11.0- 96	25.0	2.3	21.5-29.7	445	132	246-680	87.9	7.7	72.2-100.1
Green	56.2	27.3	7.8-106	25.8	2.6	22.4-32.7	447	134	260-696	85.4	11.8	53.2-112.6
Red	24.7	13.6	7.1- 54	25.3	3.9	21.8-37.4	300	127	115-578	111.7	7.7	97 -124.9

to the red stimulus. In this condition, the blue and green stimuli produce equivalent b-wave amplitudes. The larger dark-adapted a-wave amplitudes with the green stimulus may be attributed to a larger contribution by the cone system. The a-wave implicit times are essentially constant for the three colored stimuli under both the light-adapted and dark-adapted conditions. The b-wave implicit times are relatively longer for the least effective stimuli in both adaptive states. The implicit time for the more efficient stimuli (green and red for the light-adapted eye; blue and green for the dark-adapted eye) are relatively equal.

ERGs produced with different wavelength stimuli are most often used in an auxiliary capacity when differentiating a normal from an abnormal response. The blue and red stimuli are most useful in differentiating pure rod or cone anomalies. The blue and red response are generally used in a qualitative manner although the mean and standard deviations found in Table III may be used to establish a normal range.

Repeatability of Quantitative Data

As a check on the repeatability of the recording procedures and systems a nonparametric paired variate t-test was applied to the quantitative data of five animals (Alder and Roessler, 1962). The quantitative findings recorded at two separate experimental sessions constituted the set of paired variates. For example, the left eye a-wave amplitude for a given stimulus condition at the first recording session was compared to its analogous value obtained from the left eye in the second recording session. The values from a given eye for the five sheep for a specific

stimulus arrangement constituted a population of 5 with 4 degrees of freedom. The t-test was applied to all of the quantitative data. This included the b-wave and a-wave amplitudes and implicit times for the five different white stimulus intensities and the three different wavelength stimuli. With the exception of the light-adapted left eye b-wave implicit times for the G16 ($t=3.759$, $p<.05$) and G8 ($t=3.433$, $p<.05$) intensities, no significant differences were found. On re-examining the comparison ERGs for the significant finding, it was noted that in two of the five animals the b_s-wave was dominant. A b_p-wave was not easily identifiable and hence, the implicit time measurements were made with respect to the b_s-wave. This resulted in significantly longer b-wave implicit times for the G16 and G8 conditions.

C. Stimulus Frequency

The effects of different frequencies of stimulus presentation were invesigated qualitatively. A single intensity (G16) of white light was used. Only responses from the light-adapted animals were considered. The typical ERGs recorded using stimulus frequencies of 10 fps, 20 fps, 30 fps, and 40 fps are displayed in Figure 17. As can be seen, the higher the stimulus frequency the smaller the amplitude of the recorded response. The scotopic component (b_s-wave) is reduced a greater amount by increases in the stimulus frequency than the photopic component (b_p-wave). However, the scotopic system of the sheep appears to respond to a higher range of frequencies than the scotopic system of the human. Krill (1972) reports that the fusion frequency for the human

Figure 17

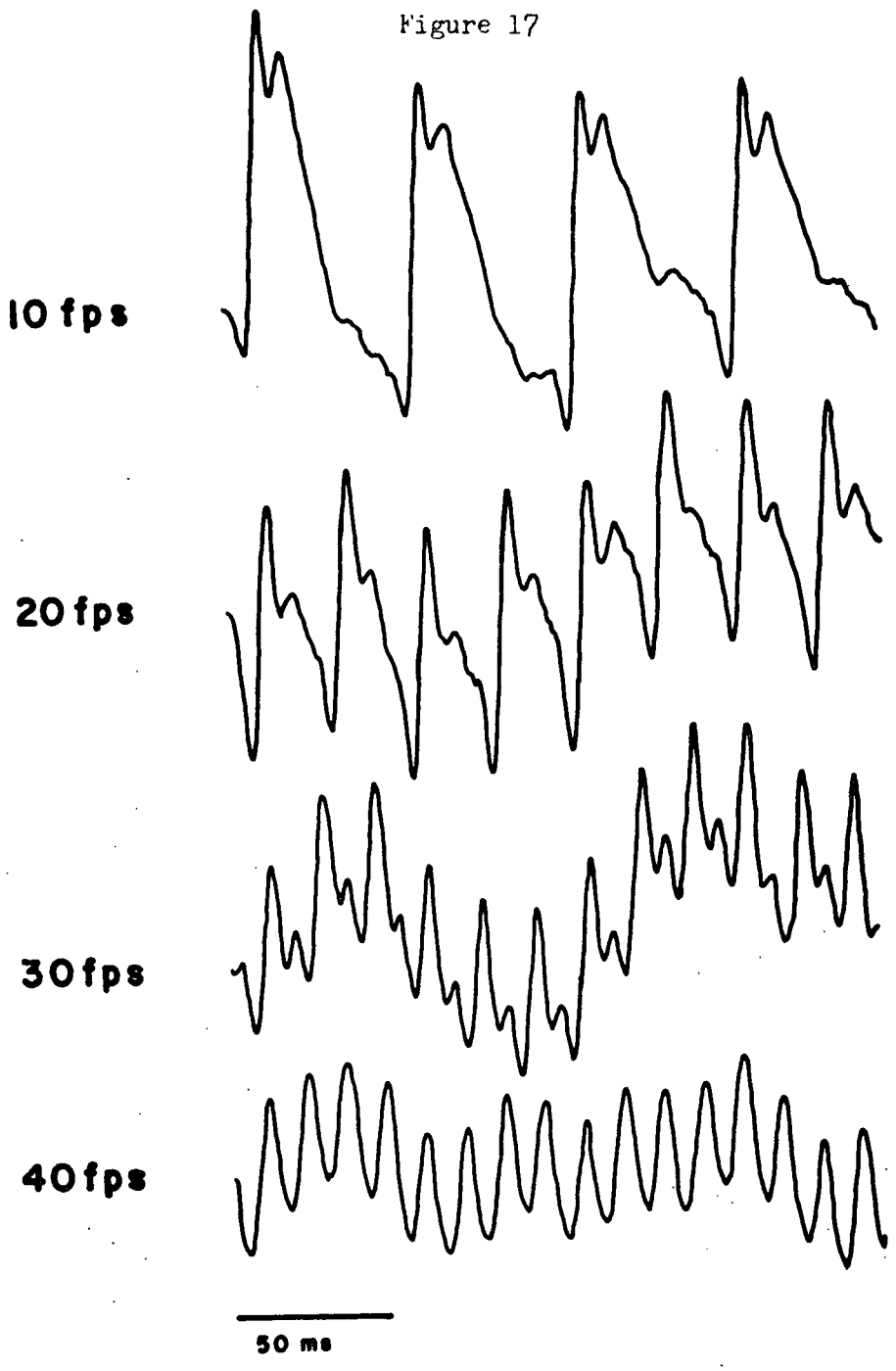


Figure 17. ERGs recorded using stimulus frequencies of 10, 20, 30 and 40 fps. Stimulus intensity G16. Calibration 10 v, 50 msec.

rod system is approximately 20 fps. The rod system of the sheep appears to be contributing to the ERG at frequencies of at least 30 fps. The 30 fps response reveals an easily identifiable secondary positive peak which is attributed to the rod system. To confirm the fact that the secondary positive peak was scotopic in nature, an auxiliary experiment was conducted on one animal. Blue and red color stimuli were used instead of the white stimulus. As before, stimulus frequencies of 10 fps, 20 fps, 30 fps and 40 fps were used. Since the blue flash enhanced the secondary positive peak and the red flash reduced its amplitude, it may be concluded that the secondary positive peak was generated by the rod system. The form of the 40 fps response was relatively unaffected by the different wavelength stimuli. This indicated that the fusion frequency for the scotopic response was between 30 and 40 fps. To determine the fusion frequency for the photopic and scotopic systems, the frequency of the stimulus (white light) was increased in 1 fps steps. The fusion frequency for the scotopic system was 34 fps. Due to limitations in the stimulus system, it was not possible to determine the fusion frequency for the photopic system. The photopic system was responding at the maximum stimulus frequency available, 60 fps.

High frequency stimulation is usually used to isolate cone activity. The normal response can be differentiated from the abnormal response on the basis of the waveform and the fusion frequency. For the sheep, where the scotopic component is easily identifiable, it may be more convenient to determine the fusion frequency for the scotopic system.

D. Stimulus Duration

The full ERG waveform (a-, b-, c- and d-wave) was also evaluated qualitatively. Light-adapted and dark-adapted ERGs produced with PSII (1 sec duration) are shown in Figure 18. Included in Figure 18 are the ERGs from the pure-cone retina of the grey squirrel (Arden and Tansley, 1955) and from the rod-dominated retina of the cat (Brown, 1968). Brown's (1968) schematic representation for a predominantly rod ERG and for a pure cone ERG are also displayed. The light-adapted sheep response, which is relatively cone dominated, shows a distinct a-wave followed by a rapidly rising b-wave. The falling phase of the b-wave is often interrupted by the peak of the scotopic component although it is not clearly visible in Figure 18. A positive c-wave comes after the b-wave. The amplitude of the c-wave varied greatly between subjects. Superimposed on the c-wave is what appears to be a biphasic d-wave. The d-wave is composed of an initial positive potential followed by a smaller trough which is negative with respect to the c-wave. The positive deflection of the d-wave occurs approximately at the time the light stimulus is turned off. The positive d-wave peak or the onset phase may be attributed to the decay of the cone late receptor potential (see Figure 6). The falling phase of the d-wave is considered to be generated by the decay of the positive d.c. component which has a slightly longer latency than the cone late receptor potential. In comparing the light-adapted sheep ERG with the squirrel ERG and Brown's schematic ERG for the pure cone retina, several differences are easily noted. First, the light-adapted sheep ERG possesses a c-wave which is not normally present in a cone dominated response. Second, the d-wave shows a negative trough which indicates a

Figure 18

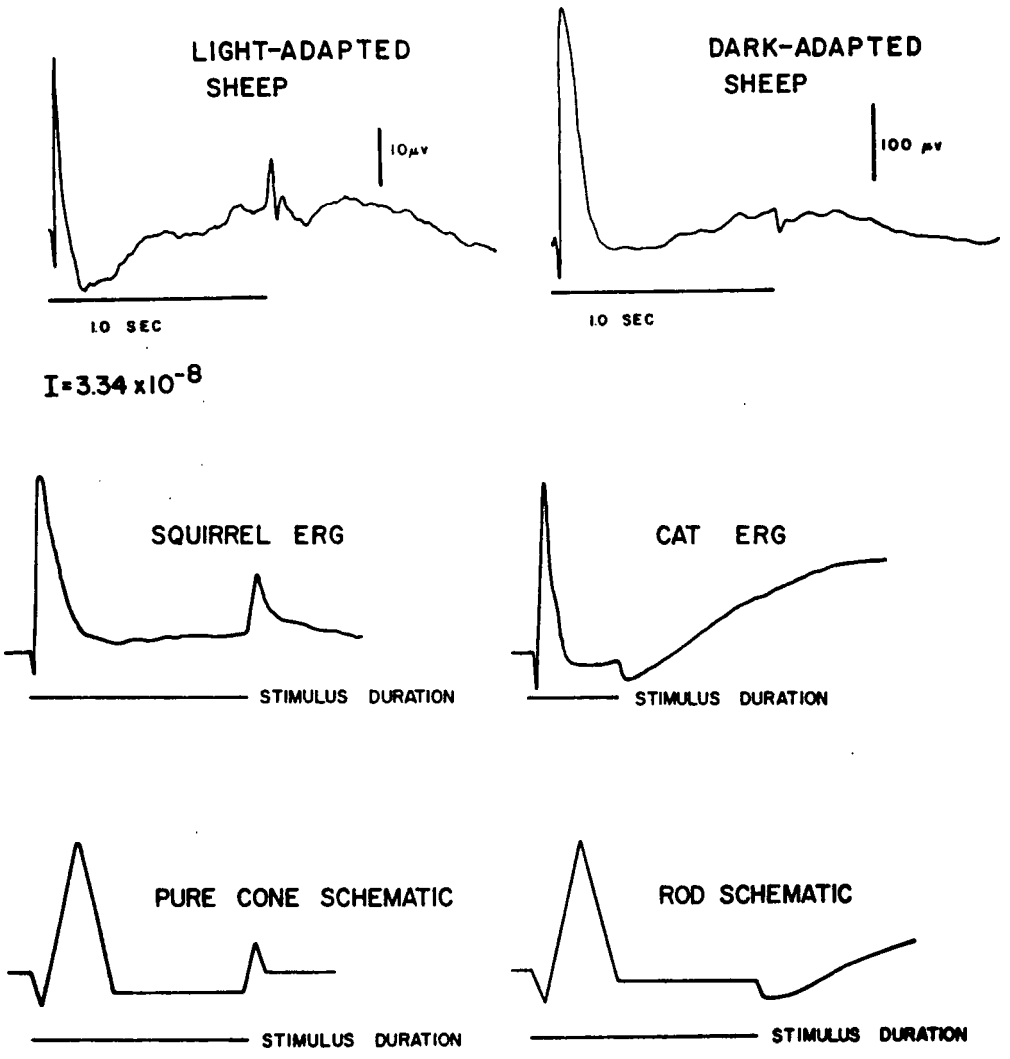


Figure 18. Light-adapted (left) and dark-adapted (right) ERGs. Stimulus intensity $3.34\ 10^{-8}$, duration 1 sec. Included: the ERGs from the grey squirrel (Arden and Tansley, 1955) and the cat (Brown, 1968). Brown's analysis of the rod dominated ERG and the pure cone ERG are included.

relatively large d.c. component. Again, this is not normally found in the cone dominated response.

The dark-adapted, rod-dominated sheep response exhibits a rapidly developing a-wave. It demonstrates a b-wave with a steep rising phase but the total duration is considerably lengthened with respect to the light-adapted response. A small c-wave is present. A negative d-wave is found after termination of the stimulus. The trough of the d-wave for the dark-adapted ERG has the same implicit time as the negative trough for the light-adapted d-wave. Decay of the positive d.c. component is responsible for the negative potential. The lack of a positive component in the d-wave is attributed to the slow decay rate of the rod receptor potential. The dark-adapted sheep ERG compares favorably with the cat ERG and Brown's schematic for predominantly rod retinae. Only the low amplitude of the c-wave is remarkable. However, it is known that the c-wave amplitude varies greatly among predominantly rod species.

The full ERG waveform is not universally used to differentiate the abnormal response from the normal response. The variability of the c-wave amplitude reduces its effectiveness as a diagnostic tool. Also, the quantitative values for the a- and b-waves may be more accurately evaluated with the expanded time base used with the short duration stimuli. However, the full ERG waveform is important when classifying the type of response being recorded (i.e. rod or cone dominated).

CHAPTER IV

DISCUSSION

The ERG of the sheep is basically similar to the ERGs recorded from other mammals with mixed receptor (rod and cone) retinae. The results obtained using different stimulus intensities generally agree with what is known about the effects of stimulus intensity on other mammalian ERGs. The sheep intensity/amplitude curve for the dark-adapted b-wave compares favorably with the human intensity/amplitude function (Riggs and Johnson, 1949). Its configuration is in line with those of the rat (Cone, 1963) and cat (Arden, Granit, and Ponte, 1960). It has been demonstrated (Knave, Moller and Persson, 1972) that the sheep dark-adapted b-wave intensity/amplitude function has two saturation levels. The amplitude at which the function levels off vary between subjects. The results of the present study indicate that the intensities employed are associated with the first or lower amplitude level. Burian and Pearlman (1964) state that the intensity region in which the amplitude of the b-wave first levels off may be considered to be in the mesopic range. The subsequent increases in amplitude with even higher stimulus intensities are attributed to the increasing involvement of the photopic system.

The intensity/amplitude function for the light-adapted b-wave (Figure 11) is linear over the range of stimulus intensities used. Crampton and Armington (1955) have shown that the intensity/amplitude curves for the light-adapted human b-wave are composed of two more or less linear

segments. The segment exhibiting the flatter slope is seen with the lower stimulus intensities. The results for the sheep (Figure 11) are consistent with the lower intensity findings of Crampton and Armington (1955).

The ratio of the dark-adapted to the light-adapted b-wave amplitudes for the sheep is greater than the ratio for the human ERG. This suggests that the sheep retina has relatively fewer cones than the human retina. The exact number of receptors in the sheep retina has not been determined. It is known that the receptors are mostly rods (Prince, Diesem, Eglitis and Ruskell, 1960).

Johnson and Bartlett (1956) have shown that the b-wave intensity/implicit time functions for both the light-adapted and dark-adapted human subject are linear. The intensity/implicit time functions for the sheep (Figure 12) appear to be in accord with findings in humans. However, the absolute values for the implicit times are different. This is particularly true for the light-adapted condition where the values for the human are longer by a magnitude of approximately 3. This may be due to differences in the stimulus intensities used, but there are some discrepancies in the literature concerning the implicit times of the light-adapted human b-wave. In a more recent study, Brunette (1973) was able to achieve a clear separation of the photopic and scotopic components of the b-wave. The ERGs from the study by Johnson and Bartlett did not demonstrate the degree of b_p-wave isolation as seen in the studies by Brunette. The implicit times Brunette reports for the human (14 msec a-wave; 30 msec b-wave) are equivalent to those of the sheep. Moreover,

Brunette reports essentially no change in the b_p-wave implicit time over a range of stimulus intensities equivalent to those used in the present study. Hence, it may tentatively be concluded that a clear isolation of the b_p-wave results in an implicit time/intensity function which demonstrates a relatively gradual slope.

The amplitude of the light-adapted and dark-adapted sheep a-wave varies predictably with the intensity (Figure 13). However, little change is seen with respect to the a-wave implicit time (Figure 14). Again, the apparent lack of change in the light-adapted a-wave implicit time with respect to changes in stimulus intensity is in agreement with the a-wave findings of Brunette (1973). Also, the values reported are in agreement with the implicit times indicated in the sheep ERGs recorded by Knave, Moller and Persson (1972). But the dark-adapted a-wave implicit time values are difficult to interpret with respect to the dark-adapted a-wave implicit time changes found in man.

The effects of the different wavelength stimuli on the sheep ERG are predictable from the results of studies on the human eye (Riggs, Berry and Wayner, 1949). The scotopic and photopic components appear to possess spectral response characteristics similar to those reported for man. In the light-adapted case (Figure 16) the relative contribution of the b_p-waves and b_s-waves to the total b-wave reflects the efficiency of the wavelength of the stimulus. For the dark-adapted condition, the amplitude of the b-wave most readily reflects the efficiency of the stimulus wavelength. Of interest is the fact that the dark-adapted amplitudes for the blue and green stimulus are approximately equal. In

view of the findings of Boynton (1952), the increased sensitivity to blue may be attributed to the increased scatter by the ocular media of the shorter wavelengths.

Dodt and Walther (1958) believe that flicker fusion frequencies for the ERG greater than 30 fps must be mediated by cones. Nonetheless, Figure 18 provided evidence indicating that the rod system of the sheep is responding at frequencies greater than 30 fps. Tansley (1961) suggests that flicker fusion frequencies vary as a function of the ratio of visual cells to inner nuclear and ganglion cells. The greater the convergence of receptors on to subsequent neurons, the lower the flicker fusion frequency for the electroretinogram and vice versa. This indicates that the rod system of the sheep demonstrates less convergence than most animals with rod dominated retinae. Tansley states that the higher flicker fusion frequencies seem to be associated with a "morphological elaboration" of the inner nuclear layer.

Prince et al. (1960) report that in the center of the sheep retina there are eight rows of receptor nuclei, ten to twelve rows of inner nuclei (bipolar), and one to three rows of ganglion cell nuclei. In the perimascular region of the human retina there are eight rows of receptor nuclei, five to six rows of bipolar nuclei, and up to eight rows of ganglion cell nuclei (Wolff, 1968). By comparing the number of rows of nuclei in the inner nuclear layer, it appears that the sheep retina possibly would demonstrate less convergence than the human retina. The greater number of rows of bipolar nuclei could be considered a "morphological elaboration". However, due to the extreme variations of cellular

architecture within a given retina, the lack of good anatomical knowledge of the sheep retina, and the increasing evidence for Müller cell involvement, any conclusion drawn from the above information must be considered highly speculative.

For the cone system, flicker fusion frequencies have been reported as high as 140 fps with responses for mixed receptor retinae starting from 30 fps (Tansley, 1961). The cone system of the sheep was shown to respond within at least the lower limit of the cone flicker fusion frequency.

Although the sheep ERG is altered predictably by various stimulus parameters, the configuration of its waveform is somewhat unusual. In the light-adapted condition a short (100 μsec) white stimulus produces an easily discernible bipartite b-wave. Both the b_p- and b_s-wave are easily recognizable. Many investigators (Finkelstein and Gouras, 1969; Auerbach and Burian, 1955; Brunette, 1973) have demonstrated the bipartite nature of the b-wave using a white stimulus. However, the separation of the b-wave subcomponents is somewhat more dramatic in sheep.

Using a longer (1 sec) white stimulus also induces some non-characteristic responses. In the light-adapted condition, the d-wave appears to be more complex than expected when compared to Brown's scheme for pure cone retinae. In particular, the negative component of the d-wave seems remarkable. Knave, Moller and Persson (1972) developed a modified component analysis for the light-adapted eye which proposes, in comparison to Brown's analysis, a much larger positive d.c. component. The presence of a large positive d.c. component could explain the negative

portion of the d-wave. In addition to the complex d-wave, an obvious c-wave is present in the light-adapted condition. The variability of the c-wave amplitude between subjects may be attributed to the cyclic fluctuations of an individual's c-wave amplitude (Calissendoriff,Knave, Persson, 1974). Nonetheless, according to Tansley a c-wave is not observed in a cone-dominated response. This indicates that even in the light-adapted state the sheep ERG reflects much activity of the rod system. The fact that the b_s-wave was occasionally larger than the b_p-wave in the light-adapted response suggests that the photopic system does not totally dominate the light-adapted ERG, at least with the stimulus intensities used in the present study. Nonetheless, factors such as the positive d-wave may be attributed to the photopic system.

In trying to fit the light-adapted sheep response to one of the existing component analyses (Brown, 1968 or Knave, Moller and Persson, 1972), one finds that no single scheme is adequate. The analyses put forth by Knave et al. (1972) for the light-adapted sheep ERG is inadequate because it predicts a simple negative d-wave. This fails to explain the positive d-wave found in the present study. In order to apply the model of Knave et al., the amplitude of the negative d.c. component must be increased. But the negative d.c. component, by virtue of counteracting a portion of the positive d.c. response would upset the balance between the positive d.c. response and the receptor response, which would in essence alter the b-wave. In determining the light-adapted scheme, Knave et al. (1972) used stimuli with a 0.1 sec duration while in the present study, stimulus durations of 1 sec were used to generate ERGs.

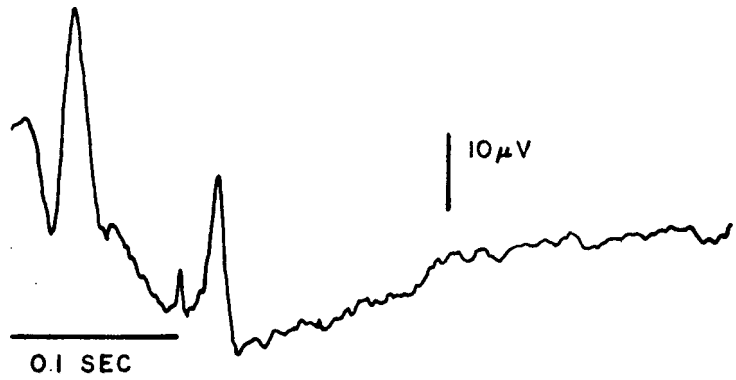
The lack af congruence between ERGs from the present study and the model of Knave, Moller and Persson may possibly have been explained by the different stimulus durations. However, by adjusting the electronic shutter of PSII, stimulus durations of 0.1 sec were possible. The ERG recorded from one animal along with the light-adapted model of Knave et al. are displayed in Figure 19. The obvious difference occurs at the termination of the stimulus. Knave et al. predict a negative off-effect; but as is shown, a positive off-effect is recorded. The discrepancies are not readily explainable. Since the recording conditions (e.g., stimulus intensity) were not identical, further research is needed to explain the differences.

Brown's pure cone ERG may be altered to fit the light-adapted sheep ERGs. The changes required involve increasing the amplitude of the positive d.c. component. This would allow for the negative component of the d-wave. A negative d-wave component could also be generated by combining Brown's pure cone schematic with his analysis for predominantly rod ERGs. This latter procedure would be the more justified since the sheep has a mixed receptor retina.

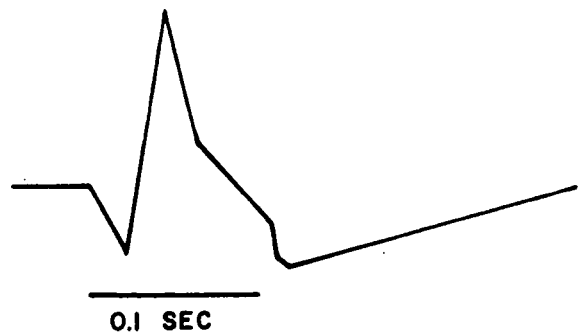
The sheep ERGs recorded under dark-adapted conditions are comparable to the component analysis for predominantly rod eyes proposed by Brown (1968). No alterations are necessary to explain the dark-adapted sheep ERG.

For subsequent toxicology studies, a normal response range may be established using the $\bar{X}$ and S.D. values given in Tables I, II and III. The normal response range may defined as the $\bar{X} \pm$ S.D. A suspicious or borderline range may be defined as being between 1 and 2 standard

Figure 19



LIGHT-ADAPTED SHEEP ERG



LIGHT-ADAPTED SCHEMATIC ERG

Figure 19. Light-adapted sheep ERG. Stimulus intensity 3.34×10^{-8}, duration 0.1 sec. Schematic of the light-adapted ERG from Knave, Moller and Persson (1972) is included.

deviations from the mean. A response can be considered abnormal if it is more than 2 standard deviations from the mean. The above criterion must be restricted to ERGs recorded under the experimental parameters described in Chapter II.

Summary and Conclusions

The ERG of the normal sheep was qualitatively and quantitatively investigated. The stimulus parameters studied were intensity, wavelength, frequency, duration and state of adaptation. The results of this study lead to the following conclusions:

1. The recording system and the procedures used were adequate to produce repeatable results.
2. With the possible exception of the dark-adapted a-wave implicit times, the ERG of the sheep responds predictably to changes in the stimulus parameters (i.e., wavelength, intensity and frequency).
3. The dark-adapted sheep ERG is rod dominated. The light-adapted sheep ERG is cone dominated although there is a significant contribution by the rod system.
4. The d-wave for the light-adapted ERG shows both rod and cone properties.
5. The bipartite nature of the sheep b-wave is readily demonstrated with both white and colored stimuli.
6. The rod system of the sheep demonstrates less convergence than the rod system found in most mammals.

7. Brown's component analysis may be applied to the sheep ERG.
8. There are differences in the form of the sheep ERG reported in the present study and those reported by Knave, Moller and Persson. The discrepancies are not readily explainable. Furthere research is needed to resolve the differences.
9. The anatomy of the sheep retina must be further investigated in order to possibly explain its unique characteristics.

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APPENDIX

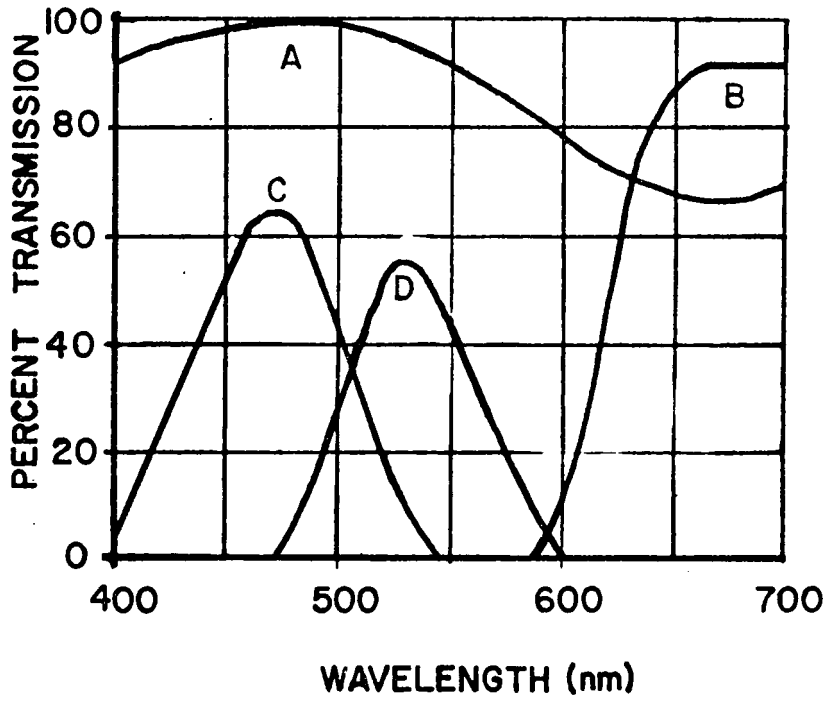
Figure 1. Spectral Characteristics for PSI and Color Filters.

Figure 2. Recording Electrodes.

Figure 3. A. Duration of PSI.

B. Duration of PSII.

Figure 1



Curve A – Flashlamp alone

B – Red

C – Blue

D – Green

Figure 1. Spectral characteristics for PSI and color filters.

Figure 2

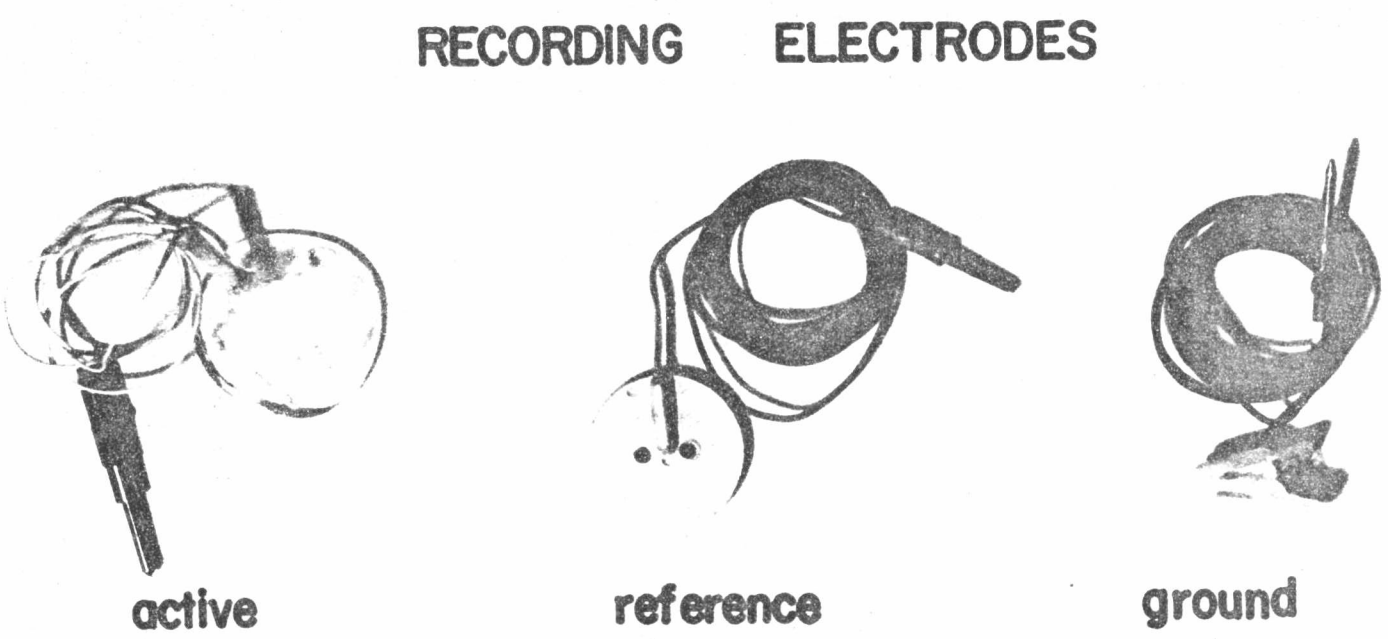


Figure 2. Recording electrodes.

Figure 3A

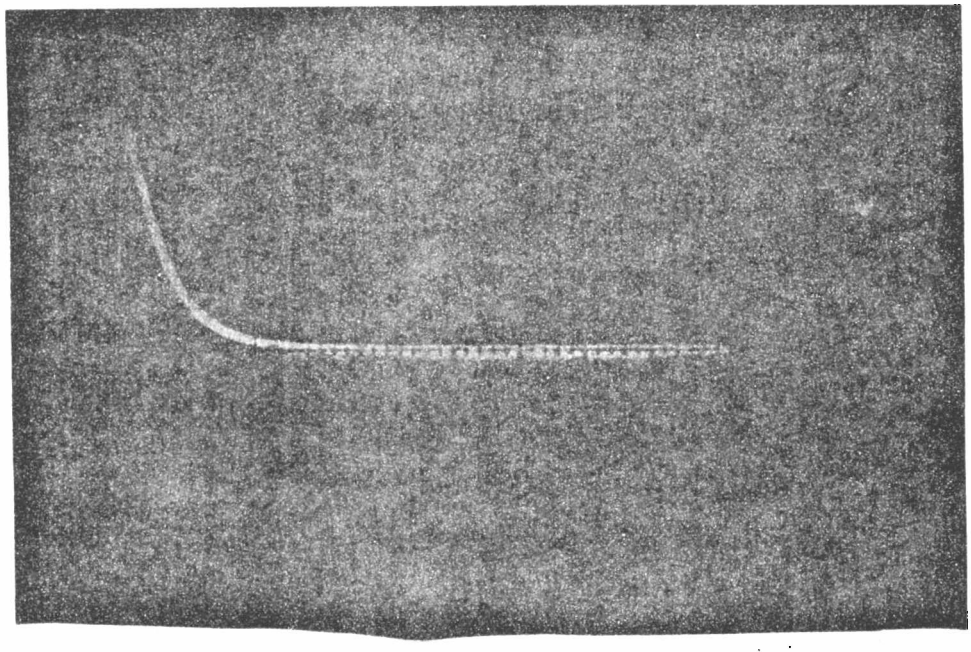


Figure 3A. Duration of PSI.

Figure 3B

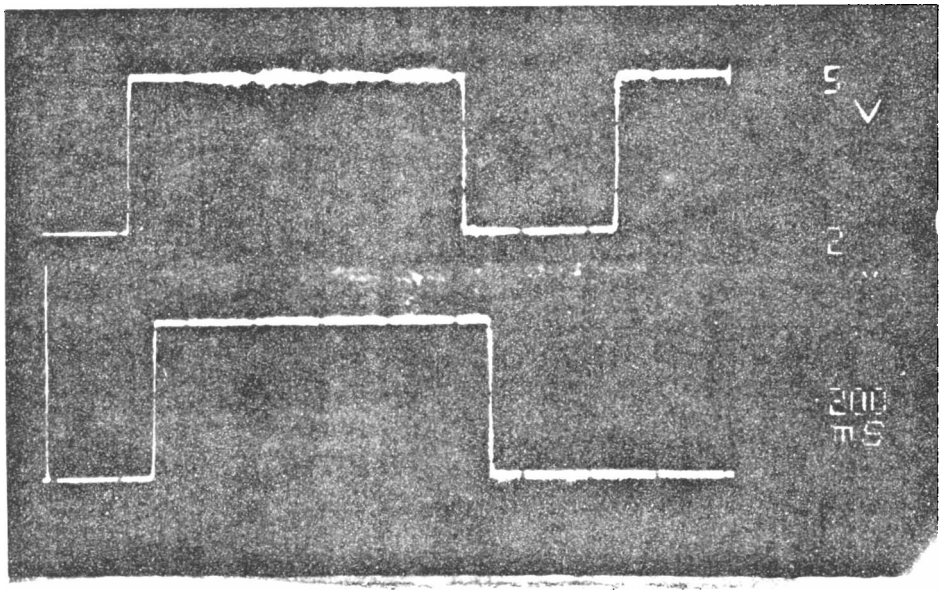


Figure 3B. Duration of PSII.