Correlation Between the Physiologic and Morphologic Changes in Experimental Autoimmune Uveitis Induced by Peptide G of S-Antigen

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Purpose. The authors followed and correlated the physiologic and morphologic changes occurring in experimental autoimmune uveitis (EAU) induced by the peptide G of S-antigen.

Methods. EAU was induced in Lewis rats by footpad inoculation of a 13-amino acid synthetic peptide (peptide G) in complete Freund's adjuvant. Electrotetrographtography (ERG) was used to follow the physiologic changes, and light and electron microscopy were used to examine the morphologic changes.

Results. Serial ERG recordings showed a progressive decrease in the b-wave amplitude and a depression of retinal sensitivity beginning on day 18–21 postinoculation (PI). By day 35 PI, the b-wave was decreased by 91%, and the sensitivity was depressed by 4.68 log units. Negative ERG were recorded during the intermediate and late stage. Light and electron microscopy of the retina showed better correlation of the pathologic changes with b-wave depression than with PI day.

Conclusions. ERG recordings were a good method to detect, follow, and quantify the severity of EAU. Their good correlation with the morphologic changes showed that this method can be used to assess the condition of the retina noninvasively. Invest Ophthalmol Vis Sci. 1993;34:1861–1871.

Footpad inoculation of microgram amounts of a soluble retinal protein (S-antigen) in complete Freund's adjuvant (CFA) will induce a severe inflammatory eye disease called experimental autoimmune uveitis (EAU) in several mammalian species including primates.‡§ EAU has been studied for at least four decades, and it is considered to be an animal model for human inflammatory eye diseases, eg, birdshot retinochoroidopathy.¶ It is characterized by the infiltration of inflammatory cells into the anterior segment, vitreous, retina, and choroid, which is accompanied by a loss of the photoreceptors and nuclei of the outer nuclear layer (ONL), retinal detachment, and cells and exudate in the subretinal space. In its most severe form, there is, in addition, hemorrhage and gliosis of the retinal tissue.¶ The severity of the retinal changes is dependent on the source of S-antigen, the dose, the species and strain of animal, and the type of adjuvant.¶ In addition to the cell-mediated inflammation, antibodies also play a role in the process of the disease induction.¶

S-antigen is a 45-kD protein extractable from the retina of many species of animals. It is a highly conserved protein and occurs in species from Drosophila melanogaster‡§ to humans.¶ It is expressed at high levels in the rod photoreceptors and the pinealocytes in the pineal gland¶ and at low levels in the brain and lens.¶ It has been identified as arrestin, which termi-
nates the visual cascade initiated by light stimulation. The amino acid sequence of S-antigen has been determined in various species and found to be made up of 403–406 residues. Initially, it was reported that a 24-kD peptide isolated by alpha-chymotrypsin proteolysis of S-antigen retained its uveitogenic capacity. Further studies with synthesized peptides showed that there are several immunogenic determinants in the S-antigen.

The severity of the EAU induced by a given experimental protocol has been graded by slit-lamp examination of the anterior segment and from histologic studies of the eye. The scoring is qualitative, and the severity of EAU is classified as mild, intermediate, or severe. Although electroretinography (ERG) has been used to follow the physiologic alterations induced by a retinal homogenate and S-antigen, currently, ERG has not been used to grade the severity of EAU. Because ERG is a noninvasive procedure and the alterations can be quantified, this method offers a means to detect, grade, and follow the different degrees of alteration in the retinal physiology. In addition, ERG can be used to study the disease induced by a single immunogenic determinant in S-antigen, which might induce damage to a restricted groups of cells.

In our study, we followed the physiologic and morphologic changes induced by inoculating a 13-amino acid synthetic peptide of S-antigen (peptide G). We used ERG to follow the physiologic changes and correlated these changes with the morphologic alterations determined by light and electron microscopy (EM). We shall show that inoculation of peptide G in CFA will induce a severe reduction in the ERG and the loss of the photoreceptor cells. The changes induced were similar in some respects to those induced by the complete S-antigen but also differed in several important ways.

MATERIALS AND METHODS

The experimental procedures used throughout this study conformed to the ARVO Resolution on the use of Animals in Research.

Animals

All experiments were conducted on 6–8-week-old female Lewis rats purchased from Charles Rivers Laboratories (Wilmington, MA). The animals were housed in clear plastic boxes and were fed Lab Chow (Purina, St. Louis, MO). The illumination in the animal room ranged from 4–8 ft/cd, and the light was kept on a 12 hr:12 hr light/dark cycle.

Inoculation

Under ketamine–xylazine–urethane (15 mg, 15 mg, and 600 mg/kg, respectively) anesthesia, 200 μg of peptide G was inoculated into the left hind footpad of the animal. Peptide G (50 μl) was emulsified in an equal volume of CFA. Bordetella pertussis vaccine (2 × 10⁹ dead cells in 1 ml) was injected intraperitoneally at the same time.

ERG

ERG were detected by a wick electrode placed on the cornea of the anesthetized rats. The indifferent electrode was placed subcutaneously on the head, and the animal was grounded by an electrode placed subcutaneously in the neck region. The pupil was dilated with 0.2% tropicamide, and the cornea was anesthetized with 0.5% topical proparacaine HCl.

The ERG were amplified and displayed on an oscilloscope with the half-amplitude band width set at 0.1–10 KHz. The amplified signals were fed to a Texas Instruments (Houston, TX) 960A minicomputer to average 16 responses with a bin size of 10 msec. The ERG were stored on digital tapes for permanent records. The amplitude and implicit times of the averaged ERG were measured from the printout of the digital values of the ERG.

Stimulus

The light for the stimulus was obtained from a 150-W quartz–halogen lamp bulb. The filament of the light bulb was focused in the plane of a shutter and by another lens onto a 3-mm diameter fiberoptic bundle. The fiberoptic bundle was brought into a Faraday cage and positioned so the tip was 2–3 mm from the corneal surface of the animal.

The luminance of the unattenuated stimulus was 5.431 log cd/m² (Salford Electrical Instruments, Ilford, England), and neutral-density filters (Eastman Kodak, Rochester, NY) were used to reduce the stimulus intensity. A Uniblitz shutter (Eastman Kodak), controlled by a Grass S4 stimulator (Quincy, MA), was used to deliver 250-msec duration flashes at an inter-stimulus interval of 5 sec.

Procedure

The anesthetized animal was strapped down with tapes to a platform with its head resting in a U-shaped holder. The upper and lower lids were retracted with masking tape to hold open and propose the eye. After the electrodes were in place and the fiberoptic bundle was adjusted to give uniform illumination of the eye, the animal was dark adapted for 30 min. The ERG recordings were begun with a 8.0-log unit neutral-density filter; in preliminary experiments, this was found to be lower than the stimulus intensity necessary.
Alteration of the ERG During EAU

to elicit a 100-μV b-wave. The stimulus intensity was increased in 0.5-log unit steps, and the responses were recorded from 8.0 to 3.5 log units (ten intensities). A recovery time was allowed between each increase in the stimulus intensity: 30 sec between filters 8.0 to 7.5 and increasing in 30-sec steps with each increase in intensity. The recovery time between the 4.0 and 3.5 filters was 4.5 min.

All recordings were made from the right eye except on the day the animals were killed for histologic studies; at that time, the ERG were recorded from both eyes.

Histologic Analysis

After the ERG were recorded from both eyes, the animal was deeply anesthetized and perfused through the heart with 4% glutaraldehyde. The eyes were enucleated and placed in cold glutaraldehyde for 1–4 hr. After this, a section of the cornea was sliced off with a razor blade. The eyes were kept in the refrigerator for 1–4 days, and then the crystalline lens was removed and discarded. The eye was placed cornea down, and the long posterior ciliary arteries were identified in the sclera. Then the eye was divided by cutting it perpendicular to the vessels with the cut made next to the optic nerve. The portion of the eye containing the optic nerve was embedded in paraffin, and the sections were stained with hematoxylin and eosin. Because of the orientation of the eye, these sections demonstrated the changes induced in the superior to the inferior hemispheres of the retina. These sections also included the optic nerve head, ciliary body, iris, the angle, and part of the cornea. The other half was trimmed further and embedded in plastic. The plastic-embedded tissue was cut at 1–1.5 μm and stained with toluidine blue for light microscopy. For EM, 0.6–0.7-μm sections were cut and examined on a JEOL EM (Tokyo, Japan).

RESULTS

ERG

The ERG recorded from the same eye on days 18, 23, 28, and 37 after footpad inoculation of peptide G in CFA are shown in Figure 1. The number to the left of each ERG is the nominal value of the neutral-density filter used to reduce the full intensity stimulus. Full intensity stimulus = 5.431 log cd/m². Calibration: 25 μV for intensity (I) = 8.0 and 7.0 on all days PI and for I = 6.0 on days 28 and 37 PI; 50 μV for I = 6.0 on days 23 PI and for I = 5.0 and 4.0 on day 37 PI; 100 μV for I = 6.0 on day 18 PI for I = 5.0 and 4.0 for days 23 and 28 PI; 200 μV for I = 5.0 and 4.0 on day 18 PI and 100 msec.

Examples of ERG recordings from a single eye on days 18, 23, 28, and 37 after footpad inoculation of peptide G are shown in Figure 1. The number to the left of each ERG is the nominal value of the neutral-density filter used to reduce the full intensity stimulus. Full intensity stimulus = 5.431 log cd/m². Calibration: 25 μV for intensity (I) = 8.0 and 7.0 on all days PI and for I = 6.0 on days 28 and 37 PI; 50 μV for I = 6.0 on days 23 PI and for I = 5.0 and 4.0 on day 37 PI; 100 μV for I = 6.0 on day 18 PI for I = 5.0 and 4.0 for days 23 and 28 PI; 200 μV for I = 5.0 and 4.0 on day 18 PI and 100 msec.

FIGURE 1. ERG recorded from the same eye on days 18, 23, 28, and 37 after footpad inoculation of peptide G. The number to the left of each ERG is the nominal value of the neutral-density filter used to reduce the full intensity stimulus. Full intensity stimulus = 5.431 log cd/m². Calibration: 25 μV for intensity (I) = 8.0 and 7.0 on all days PI and for I = 6.0 on days 28 and 37 PI; 50 μV for I = 6.0 on days 23 PI and for I = 5.0 and 4.0 on day 37 PI; 100 μV for I = 6.0 on day 18 PI for I = 5.0 and 4.0 for days 23 and 28 PI; 200 μV for I = 5.0 and 4.0 on day 18 PI and 100 msec.

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The ERG recorded 5 days later on day 23 PI showed the early ERG changes. The b-wave amplitude elicited with the 8.0 filter was reduced to 15 μV and to 62 μV, with the 7.0 filter. The V max was reduced by 30% to 812 μV. There were further decreases in the ERG recorded on day 28 PI. Only a small response was detected with the 8.0 filter, and the V max was 474 μV.

On day 37 PI, the responses elicited with filters 8.0 and 7.0 consisted of a negative-going wave that resembled the PIII component of the ERG. With fur-
ther increases in the stimulus intensity, the b-wave appeared, but the $V_{\text{max}}$ was reduced by 74.3% to 302 μV.

**Intensity–Response Curves (V–log I Curves)**

The b-wave amplitudes of the ERG shown in Figure 1 were measured and plotted against the stimulus intensity (Fig. 2). ERGs were also recorded from this eye on days 8, 49, and 56 PI, and the V–log I curves for these days are also plotted. The V–log I curves for days 8 and 18 PI did not differ significantly from those of normal eyes. As shown by the ERG in Figure 1, there was a depression of the b-wave amplitude and a shift of the curve to higher stimulus intensities on day 23 PI. With increasing days PI, there was a progressive decrease in $V_{\text{max}}$ and a shift of the curve to higher stimulus intensities. The ERG were last recorded on day 56 PI when the $V_{\text{max}}$ was 140 μV.

To follow the change in retinal sensitivity, the amount of light necessary to elicit a 100-μV b-wave was determined from each of the V–log I curves. The retinal sensitivity for this eye before inoculation was 7.35 log units, which was comparable to the mean sensitivity of 16 control eyes (7.44 ± 0.04 log units). The retinal sensitivity on days 8 and 18 PI was 7.25 log units. On day 23 PI, when $V_{\text{max}}$ was reduced by 30%, the retinal sensitivity was depressed by 0.60 log units to 6.75 log units. With additional time, there was a progressive decrease in retinal sensitivity, and on day 56 PI, the retinal sensitivity was depressed by 2.24 log units.

**Changes of $V_{\text{max}}$ and Retinal Sensitivity With PI Times**

The $V_{\text{max}}$ and retinal sensitivity determined from each of the V–log I curves for 15 right eyes were tabulated and the means and standard errors of the means were plotted in Figures 3 ($V_{\text{max}}$) and 4 (retinal sensitivity). Also shown are the means determined from the five control animals that received CFA and *B. pertussis*. The numbers in parentheses represent the number of eyes measured for that period. The value plotted on day 7 PI is the mean (and standard error of the mean) of the ERG recorded on days 6–9, that plotted on day 12 PI is the mean for the ERG recorded on days 11–14, and that plotted on day 17 PI is the mean for ERG recorded on days 16–19. Because the eyes were removed for histologic studies at various times, the number of eyes tested decreased with increasing time.

For days 7 and 12 PI, the mean $V_{\text{max}}$ of the experimental animals were not significantly different from the mean of the controls, and the variance of $V_{\text{max}}$ was not large (less than the size of the symbol). On day 17 PI, there was a large variation in the $V_{\text{max}}$ (788–1465 μV) with three $V_{\text{max}}$ significantly lower than the means for the control eyes. However, the mean $V_{\text{max}}$ (1068 ± 56 μV) was not statistically different from the $V_{\text{max}}$ of
Alteration of the ERG During EAU 1865

the 16 normal eyes. The first significant decrease in the mean $V_{\text{max}}$ was recorded on day 21 PI when the mean $V_{\text{max}}$ was 683 ± 100 $\mu$V ($t = 5.210; P < 0.001$). Thereafter, there was a rapid decrease in $V_{\text{max}}$ until on day 35 PI; the mean $V_{\text{max}}$ was 127 ± 40 $\mu$V. For the CFA-treated animals, the mean $V_{\text{max}}$ on day 35 PI was 997 ± 138 $\mu$V, which was not significantly different from the $V_{\text{max}}$ recorded from normal eyes ($t = 2.040; P > 0.05$).

The mean retinal sensitivity (Fig. 4) was not significantly altered on days 7 and 17 PI. The first significant depression in retinal sensitivity was measured on day 21 PI; the sensitivity was depressed by 0.14 log units ($t = 2.686; 0.01 < P < 0.02$). By day 35 PI, the mean retinal sensitivity was depressed by 4.68 log units. In the CFA-treated animals, the mean retinal sensitivities on days 21, 28, and 35 PI were also lower than those of the normal eyes.

Negative-Type ERG

A consistent and unexpected finding was the recording of a negative-type ERG during the course of EAU, ie, an ERG in which the b-wave was abolished or markedly reduced so that the b-wave/a-wave ratio was less than 1.0. The responses recorded from an eye on day 22 PI are an example in which the b-wave was extinguished at all stimulus intensities (Fig. 5). At this early stage, the retinal sensitivity was not depressed significantly because a small response was elicited at a stimulus intensity of 8.0. The amplitude of the negative wave was small, and the maximum response was ~80 $\mu$V at a stimulus intensity of 5.0. At later stages, the retinal sensitivity was also reduced.

Retinal Morphology Before ERG Changes

Because the examination of 12 pairs of eyes showed that the degree of morphologic alterations was better correlated with the ERG depression than with the PI day, the morphologic changes will be presented in terms of ERG depression.

One pair of eyes was obtained on day 7 PI and another pair on day 13 PI before any ERG changes. In both cases, the morphologic appearance of the retinas did not differ from that of normal eyes. The anterior segment of the eyes taken on day 7 PI did not show any changes, but in both eyes obtained on day 13 PI, there was dilation of the iris vessels. However, there were no inflammatory cells in the anterior chamber or around the ciliary body.

Early Morphologic Changes When First Depressed ERG Were Recorded

Two pairs of eyes were taken for histologic study when the first reduced ERG were recorded. One pair of eyes was obtained when $V_{\text{max}}$ was reduced by 30% (day 22 PI) and another when the $V_{\text{max}}$ was depressed by 38% (day 18 PI). The $V$-log $I$ curves and photomicrographs of the eye obtained on day 18 PI are shown in Figure 6. Up to day 13 PI, the b-wave amplitude and retinal sensitivity did not differ significantly from the preinoculation recordings (Fig. 6A). On day 18 PI, the retinal sensitivity was not altered, but the $V_{\text{max}}$ was reduced by 38% to 788 $\mu$V.

Histologic examination of this eye showed that the anterior segment was normal, except for the dilation of the iris vessels (not shown). There were no inflammatory cells present in the anterior chamber or around the ciliary body. At low power, the retina appeared normal with the preservation of the architecture of the retina (Fig. 6B). Inflammatory cells were not found in the vitreous, retina, and choroid. At higher power (Fig. 6C), the retina appeared grossly normal. In the posterior pole, the ONL was 10–12 rows thick. The outer segments (OS) and inner segments (IS) of the photoreceptors were long and aligned. The inner nuclear layer and ganglion cell layers did not appear different from normal retina. However, in localized regions, the tips of the photoreceptors were separated from the retinal pigment epithelium (RPE). Similar observations of early changes have been reported after inoculation with rhodopsin.25

EM examination of this retina found that the overall structure of the photoreceptors and RPE was

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933398/) Responses recorded from an eye on day 22 PI. The b-wave was extinguished leaving only a negative-type ERG. Calibration, 25 $\mu$V and 100 msec.
normal (Fig. 6D). However, there were vacuoles in both the OS and IS of the photoreceptors, which were not seen in the control retinas. The inner layers of the retina appeared normal. The other pair of eyes in which $V_{\text{max}}$ was reduced by 30% showed similar morphologic changes.

**Morphologic Appearance When ERG Depressed 50–80%**

One pair of eyes was taken on day 56 PI when $V_{\text{max}}$ was reduced by 63%, and another pair was taken on day 22 PI when the ERG was reduced by 77%. Examination of the $V$–log $I$ curves for the eyes taken on day 56 PI showed the progressive reduction of the b-wave with increasing PI days (Fig. 7A). On day 47 PI, the b-wave

was reduced by 63%, and on day 56 PI, the reduction was essentially unchanged at 66%.

The eye was removed for histologic examination after the ERG recordings on day 56 PI, and a low-power view of the posterior pole showed that, although the overall architecture of the retina was preserved, the thickness of the photoreceptor and ONL were noticeably reduced (Fig. 7B). There were no inflammatory cells in the vitreous or retina, and the retina was not detached. A higher-power view demonstrated that the ONL was reduced to 4–5 rows of nuclei (10–12 in normal eyes), and the OS and IS of the photoreceptors were markedly shortened and distorted (Fig. 7C). There were mononuclear cells in the subretinal space. The inner retina appeared normal.

EM examination of this retina showed that the OS
of the photoreceptors were short and fragmented (Fig. 7D). The IS were also shortened but maintained their alignment. The distance between the RPE and external limiting membrane was considerably reduced. The other pair of eyes with V<sub>max</sub> reduced by 77% showed inflammatory cells in the anterior and posterior segment and will be described subsequently (Fig. 8).

**Morphologic Picture When the ERG Is Reduced by Greater Than 80%**

Six pairs of eyes were obtained after the ERG were depressed by more than 80%. The PI times ranged from 20–93 days. The results from a representative eye taken on day 35 PI are shown in Figure 8. The V–log I curves show that the initial ERG reduction was detected on day 17 PI when V<sub>max</sub> was reduced by 18% and retinal sensitivity was also slightly depressed (Fig. 8A). Four days later (day 21 PI), the V<sub>max</sub> was reduced by 66%, and on day 28 PI, the V<sub>max</sub> was reduced by 98%. There was no further reduction in the ERG when tested on day 35 PI, and the eye was removed for histologic study.

A low-power view of the posterior pole of this eye showed that the thickness of the photoreceptor and ONL was markedly reduced (Fig. 8B). Examination of the peripheral region of the retina demonstrated that the ONL was composed of two to three rows of nuclei, but the photoreceptors did not appear normal. Despite the marked loss of photoreceptors, there were no signs of invasion of inflammatory cells into the vitreous or retina. A high-power view showed two to three
ERG Changes in an Eye Showing Invasion of Inflammatory Cells

One of the hallmarks of EAU after the inoculation of the complete S-antigen or peptide M and G is the presence of inflammatory cells in the anterior segment, vitreous, retina, and choroid. Under our experimental conditions, there was a noticeable lack of inflammatory cells when peptide G was used to induce EAU. Only 2 of the 14 retinas showed any sign of the invasion of inflammatory cells into the eye. In one eye taken on day 22 PI, there were cells around the ciliary body and a few cells in the vitreous, but inflammatory cells were not found in the retina. Thus, the changes would be considered mild. The $V_{\text{max}}$ in this eye was reduced by 78%.

The second eye, obtained on day 20 PI, showed greater numbers of inflammatory cells. The $V$–log $I$ curves for this eye showed normal amplitude responses on days 7 and 12 PI (Fig. 9A). On day 17 PI, there was a reduction of $V_{\text{max}}$ by 28% and a loss of...
FIGURE 9. Data obtained from an eye after inoculation of peptide G. The eye was removed for histologic study after the ERG recordings on day 20 PI. (A) V-log I curves recorded on days 7, 12, 17, and 20 PI. (B) Low-power photomicrograph of the anterior segment showing the infiltration of inflammatory cells around the iris and ciliary body (CB, hematoxylin and eosin, X40). (C) Intermediate-power photomicrograph of retina showing the invasion of inflammatory cells into the vitreous and retina. The choroid is free of inflammatory cells (hematoxylin and eosin, X100). RPE, retinal pigment epithelium; Ph, photoreceptors; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. (D) High-power view of retina showing inflammatory cells in the different retinal layers (hematoxylin and eosin, X160).

sensitivity of 0.45 log units. Three days later, the $V_{\text{max}}$ was markedly reduced by 84%, and the sensitivity was decreased by 1.95 log units. ERG were also recorded from the left eye, and they were normal.

A photomicrograph of the anterior segment of the right eye showed dilation of the iris vessels as seen in other eyes but which differed from the other eyes by the presence of inflammatory cells (mainly mononuclear cells) around the iris and ciliary body (Fig. 9B). Inflammatory cells were also found in the vitreous and the retina (Fig. 9C). The overall layering of the retina was preserved, except in the small focal areas where there was a loss of ONL. The choroid was surprisingly lacking in inflammatory cells. Even in areas where the ONL was preserved, there were many inflammatory cells in all layers of the retina. A high-power view of the retina showed that there was a marked infiltration of inflammatory cells into all layers of the retina, including the subretinal space (Fig. 9D).

DISCUSSION

Our results showed that the inoculation of a synthetic, 13-amino acid peptide of S-antigen will induce significant alterations in the physiology and morphology of the retina. Physiologically, the changes consisted of the reduction of all components of the ERG and the depression of retinal sensitivity. The changes were seen as early as day 18 PI, and by day 35 PI, the mean reduction of the b-wave was 91%. The changes were permanent; no recovery was detected in eyes tested up to 93 days PI.

Morphologically, the changes consisted mainly in the loss of the photoreceptors and the ONL, with the
inner retinal layers appearing grossly normal. Except in two eyes, these changes were found without the invasion of inflammatory cells into the anterior segment, vitreous, retina, or choroid. Even in the two eyes with inflammatory cells, the degree of inflammation was mild. In addition, peptide G also did not induce retinal detachment and gliosis of the retina as has been reported with S-antigen.

The mild inflammatory changes was unexpected because the EAU induced by S-antigen is characterized by a massive invasion of T-cells. Several possibilities might be considered to explain our observations. First, we used a 13-amino acid peptide; the complete S-antigen has 404 amino acids with at least five uveitogenic epitopes. In addition, the peptide G site has been shown to be uveitogenic but only weakly proliferative to lymphocytes in culture. An adjacent site (353–373) induced a strong proliferative response but not EAU. A similar dissociation of pathogenic and proliferative sites has been found in experimental autoimmune encephalomyelitis. Second, the preparation and injection of peptide G was different from that used in earlier studies so that an effectively lower dose was given. Because the severity of EAU is dose dependent, this lower dose would explain the minor inflammatory changes. The lower dose could have resulted from incomplete emulsification or the site used for inoculation. Third, the ERG testing procedure may have altered the experimental conditions, and in this respect, others (who also used the ERG to follow the EAU induced by S-antigen) reported that the inflammation in their animals was minimal and the pathologic changes were limited to vasculitis and photoreceptor degeneration. We cannot state which is the correct explanation without further experimentation.

The absence of cellular invasion of the retina highlights the question of the mechanism for the photoreceptor degeneration. Although it was known from the early studies that there is a rise in antibody titers accompanying the disease process, the ability to transfer EAU adoptively using sensitized T-cells has demonstrated the importance of cell-mediated immunopathogenicity. However, one group induced choroidal lesions similar to EAU by subconjunctival injections of hyperimmune antiovine rod OS serum prepared in enucleated guinea pigs. More than one half of the treated guinea pigs had clinical and histologic uveitis. Additional experiments must be done to determine what role humoral immunity plays in the development of EAU.

We found a progressive depression of all components of the ERG as reported in the earlier ERG studies, but our observations differed in at least four important ways from those made when the complete S-antigen or retinal homogenate was inoculated. First, we did not detect any sign of enhancement of the ERG as reported in these earlier studies. This was not caused by the absence of inflammation because the two eyes that had invasion of inflammatory cells into the retina did not show any enhancement of the ERG.

Whether a different dose of peptide G or another epitope of S-antigen is required to induce the enhancement will need to be tested. Second, both earlier ERG studies reported that there was partial recovery of the ERG with increasing PI time. None of the animals inoculated with peptide G followed for 51–93 days PI showed any degree of recovery. The morphologic studies detected the loss of the photoreceptors and their nuclei, and it is difficult to conceive of a "regeneration" of the postmitotic photoreceptor cells. Third, the recording of negative-type ERG had not been reported by the earlier studies. Because this type of ERG is associated with an alteration in the physiology of the inner retina, further examination of the physiology and morphology of the inner retina is in progress. Fourth, the earlier ERG studies did not describe any change in retinal sensitivity. We found this to be one of the early physiologic changes. The significance of this observation is that the loss of sensitivity has been correlated with an alteration of the physiology of the inner retina. Although the morphology of the inner retina was grossly normal in routine hematoxylin and eosin-stained sections, our histochemical study demonstrated glial fibrillary acid protein expression in the Mueller cells. Others have reported a breakdown of the blood–retinal barrier during the course of EAU. The recording of negative-type ERG during the later stages of EAU (Figs. 1, 5) supports the idea of inner retinal alterations. The late appearance of the negative responses would suggest that the inner retinal changes did not result directly from peptide G treatment but were probably secondary to the damage to the photoreceptors.

In one study, negative-type ERG were recorded in more than one half of the patients with birdshot retinochoroidopathy. This is of considerable interest because EAU in rats has been considered to be an animal model of birdshot retinochoroidopathy. An important finding made in this study was that the severity of EAU (as measured by the morphologic changes) was better correlated with the ERG reduction than with the PI days. This is illustrated in the data presented in Figures 7 and 8. In the eye taken on day 35 PI (Fig. 8), the ONL in the posterior pole was reduced to two to three rows of nuclei, and the ERG was decreased by 98%. The eye taken 14 days later on day 56 PI (Fig. 7) showed six to seven rows of nuclei in the ONL, and the Vmax was reduced by 66%. Thus, the eye taken at an earlier PI time showed significantly greater physiologic and morphologic alterations. In future studies in which finer differences may be induced by other epitopes of S-antigen, examination of the retinas obtained on a given PI time might give a distorted picture of the changes induced. We suggest that the...
ERG be used to follow the physiologic alterations and the eyes be taken for histologic studies when comparable changes in the physiology have occurred.

**Key Words**

experimental autoimmune uveitis, electroretinogram, peptide G, histopathologic changes in EAU, correlation of EAU and ERG

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**References**