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THE SPECTRAL SENSITIVITY OF THE TURTLE
PSEUDEMYS SCRIPTA ELEGANS.

The Ohio State University, Ph.D., 1971
Biophysics, general

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THE SPECTRAL SENSITIVITY OF THE TURTLE

PSEUDEMYS SCRIPTA ELEGANS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Leathem Mehaffey, III, A.B., M.S.

* * * * *

The Ohio State University
1971

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Academic Faculty of Biophysics
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The following dissertation deals with the determination of the spectral sensitivity of the turtle *Pseudemys scripta elegans*. The approach used herein is behavioral rather than electrophysiological in nature, and thereby provides data which, when used in conjunction with the available electrophysiological data, allow some inferences to be drawn about the information processing in the visual pathway.

Although behavioral determinations of spectral sensitivity have been made in the past, a new approach, air reinforcement, employed for the first time herein, allows collection of a larger number of data points per unit time of experiment than previous approaches. This new method improves accuracy and provides information about the behavioral response to a new task.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>VITA</td>
<td>iii</td>
</tr>
<tr>
<td>FOREWORD</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vii</td>
</tr>
<tr>
<td>Chapter</td>
<td></td>
</tr>
<tr>
<td>I. SURVEY OF PREVIOUS WORK</td>
<td>1</td>
</tr>
<tr>
<td>II. AN AIR REINFORCEMENT TECHNIQUE</td>
<td>21</td>
</tr>
<tr>
<td>III. ANALYSIS OF THE DATA</td>
<td>27</td>
</tr>
<tr>
<td>IV. RESULTS</td>
<td>45</td>
</tr>
<tr>
<td>V. DISCUSSION</td>
<td>59</td>
</tr>
<tr>
<td>Appendix</td>
<td></td>
</tr>
<tr>
<td>A. DESIGN OF EQUIPMENT</td>
<td>67</td>
</tr>
<tr>
<td>B. Calibration of Optical Equipment</td>
<td>73</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>88</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Summary of spectral sensitivity data for the turtle</td>
<td>9</td>
</tr>
<tr>
<td>2.</td>
<td>Survey of behavioral performance for nine turtles</td>
<td>34</td>
</tr>
<tr>
<td>3.</td>
<td>Conversion of raw data to final form</td>
<td>36</td>
</tr>
<tr>
<td>4.</td>
<td>Sample of computer-analyzed data output</td>
<td>43</td>
</tr>
<tr>
<td>5.</td>
<td>Comparison of left and right optical channels</td>
<td>85</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summary of spectral sensitivity data for <em>Pseudemys scripta elegans</em></td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Muntz Vitamin A₂ photopigment absorption templates</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Difference spectrum for turtle photopigments</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>Diagram of the experimental apparatus</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>P(C) curves</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>FMR curves</td>
<td>31</td>
</tr>
<tr>
<td>7</td>
<td>P(RR) curves</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>Merit curves</td>
<td>33</td>
</tr>
<tr>
<td>9</td>
<td>Stimulus intensity as a function of time during a run</td>
<td>38</td>
</tr>
<tr>
<td>10</td>
<td>Stimulus intensity as a function of time during a run</td>
<td>39</td>
</tr>
<tr>
<td>11</td>
<td>Stimulus intensity as a function of time during a run</td>
<td>40</td>
</tr>
<tr>
<td>12</td>
<td>Probability-of-seeing curve</td>
<td>44</td>
</tr>
<tr>
<td>13</td>
<td>Spectral sensitivity curve for turtle #4 in the first interval</td>
<td>46</td>
</tr>
<tr>
<td>14</td>
<td>Spectral sensitivity curve for turtle #4, whole-run data</td>
<td>47</td>
</tr>
<tr>
<td>15</td>
<td>Spectral sensitivity curve for turtle #16 in the first interval</td>
<td>49</td>
</tr>
<tr>
<td>16</td>
<td>Spectral sensitivity curve for turtle #16, whole-run data</td>
<td>50</td>
</tr>
<tr>
<td>17</td>
<td>Spectral sensitivity curve for turtle #71 in the first interval</td>
<td>51</td>
</tr>
<tr>
<td>18</td>
<td>Spectral sensitivity curve for turtle #71, whole-run data</td>
<td>53</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.</td>
<td>Slope plot for turtle #4</td>
<td>55</td>
</tr>
<tr>
<td>20.</td>
<td>Slope plot for turtle #16</td>
<td>56</td>
</tr>
<tr>
<td>21.</td>
<td>Slope plot for turtle #71</td>
<td>57</td>
</tr>
<tr>
<td>22.</td>
<td>Block diagram of equipment operations</td>
<td>68</td>
</tr>
<tr>
<td>23.</td>
<td>Diagram of the optical pathway</td>
<td>70</td>
</tr>
<tr>
<td>24.</td>
<td>Normalized spectral sensitivity of barrier layer cell</td>
<td>74</td>
</tr>
<tr>
<td>25.</td>
<td>Normalized unfiltered light source power spectrum</td>
<td>76</td>
</tr>
<tr>
<td>26.</td>
<td>Normalized transmission spectrum of Wratten 29 stray-light filter</td>
<td>77</td>
</tr>
<tr>
<td>27.</td>
<td>Normalized transmission spectrum of Wratten 47 stray-light filter</td>
<td>78</td>
</tr>
<tr>
<td>28.</td>
<td>Calibration curves for fixed density filters</td>
<td>80</td>
</tr>
<tr>
<td>29.</td>
<td>Preliminary calibration curves for variable density wedge</td>
<td>82</td>
</tr>
<tr>
<td>30.</td>
<td>Calibration curve for variable density wedge</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>(400-600 nm)</td>
<td></td>
</tr>
<tr>
<td>31.</td>
<td>Calibration curve for variable density wedge</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>(600-700 nm)</td>
<td></td>
</tr>
<tr>
<td>32.</td>
<td>Normalized viewed power spectrum</td>
<td>86</td>
</tr>
<tr>
<td>33.</td>
<td>Log relative quantum flux spectrum</td>
<td>87</td>
</tr>
</tbody>
</table>
The spectral sensitivity of the turtle has been a subject of much interest in recent years. In the main, two approaches have been used, the electrophysiological and the behavioral. Some rather interesting differences have appeared in the results from these two approaches.

To begin with, the photo-receptors of *Pseudemys scripta elegans* appear to be of several types. Many are found to have a pigmented globule, usually called an oil droplet, contained within the inner segment of the receptor and located adjacent to the outer segment. Thus, light normally passes through the oil droplet before reaching the photopigment in the outer segment. This interposition of the globule into the light path has led to several investigations of the anatomy and optical properties of these globules.

Granda and Haden (1970) describe 3 basic oil droplets in *Pseudemys scripta elegans*; a colorless globule of 3.5 to 6.4 μm diameter; an orange-yellow globule of 5.4 to 6.4 μm diameter; and a red globule of 6.4 μm diameter. Some authors prefer to consider the orange-yellow oil droplets as two classes, one yellow, the other orange (Strother, 1963; Liebman, 1971); Granda and Haden measured the distribution of these droplet types. In the visual streak, red comprises 38% of the droplets, orange-yellow 47%, and clear 15%.
Droplets of all types became much scarcer farther from the visual streak.

Strother (1963) has measured the transmission spectra for the red, "light orange," yellow, and clear oil droplets in Pseudemys. He found that the droplets acted as sharp-cutoff, high-pass optical filters. The red droplet cuts off maximally below 570 nm. Longest wavelength cutoff for the "light orange" droplets is below 570-540 nm. For the yellow, the longest wavelength cutoff is below 510 nm. The clear droplets have a maximum absorption of 60% at about 380 nm. Lipetz (1971) has measured the λ_i of yellow, orange, red, and clear oil droplets for Emys blandings. The λ_i values is the wavelength at which the line tangent to the linear portion of the rising absorption curve intersects the level of 100% absorption. For the yellow droplets, λ_i = 510 nm; λ_i for the orange is 535-555 nm; for the red, λ_i = 570-595 nm; and for the clear, λ_i = 395 nm. While these cutoff values agree with those of Strother, Strother claims only about 90% absorbance as a maximum, while Lipetz has estimated optical densities greater than 4 at peak cut-off for the red oil droplets in Emys blandings.

The next topic of interest is the receptor pigments themselves. The most recent work is that of Liebman and Granda (1971). By microspectrophotometric techniques, these authors have examined the absorption spectra of the pigments which they found in the outer segments of the photoreceptors of Pseudemys scripta elegans. These outer segments they grouped into rods and cones by their geometry, rods being cylindrical, 3 μm diameters by 40 μm long; cones being conical, 30-40 μm long,
tapering from a 2 μm diameter base to a 1 μm tip. The rod pigment has an absorption λ\text{max} of 518 ± 2 nm and was considered to be porphyropsin. Cones were seen to have several pigments and differed morphologically. Most commonly cones were seen associated with oil droplets. Of this type, the majority sampled had a pigment with an absorption λ\text{max} of 620 ± 5 nm. Others of the same morphologic type had a pigment whose absorption λ\text{max} appeared at 450 ± 5 nm. Another type of cone was a cone in that it showed the characteristic tapering morphology, but was not seen to be associated with an oil droplet. Pigment in these cones was measured to be spectrally the same as the rod pigment. The authors surmise these receptors to be the accessory cones, found paired with oil droplet-containing cones in other species.

Once one moves beyond the physically measurable oil droplet and receptor pigment characteristics, one must depend upon inferential techniques such as electrophysiology and psychophysics. To gather knowledge about the information available at different processing stages in the nervous system from levels directly proximal to the receptors up into higher control nervous system levels, electrophysiological techniques must be employed.

The electrophysiological techniques most often employed are those of direct recording from within cells or cell layers of the retina, or from the optic nerve fibers, and those of indirect recording of mass discharges, such as E.R.G.'s and cortical-evoked potentials. The E.R.G. is usually employed by taking the voltage difference between
the trough of the negative-going a-wave (predominantly receptor response) and the peak of the b-wave (response of cells proximal to the receptors). The most common procedure is to measure the stimulus intensity required to cause this voltage difference to reach some criterion value. Thus an action spectrum is obtained. A similar method, measuring the stimulus intensity needed for some criterion voltage amplitude, is used in the tectal-evoked-potential methods.

Another method of study involves the behavioral response of the animal to colored light. This behavioral methodology cannot directly point out information processing within various levels of the visual nervous system, but is nonetheless useful in determining the overall processing of the information available at the retina, to the extent that that information is utilized for the particular behavioral task. The most common task is a discrimination, or choice between two targets. When the illumination of the targets is varied to produce some criterion of percent choices correct, an action spectrum results. If a single target is used and response rate measured, a response spectrum results. Let us investigate, then, these tests as applied to the turtle, and examine the results.

Granit (1941) measured the spectral sensitivity of the ganglion cells of the retina of Testudo, and found a primary sensitivity maximum at 600-640 nm, with a secondary peak at 515-530 nm. Orlov and Maksimova (1964), using the null indicator of no change in the mass optic nerve discharge when substituting one spectral distribution
for another, derived color matching functions for the turtle *Emys orbicularis*. They found a Purkinje shift, with a scotopic maximum of 520 nm and a photopic maximum of 630 nm. Interestingly enough, they found that mixtures of four primaries were needed to match colors, indicating a tetrachromatic mechanism of color vision in these turtles.

Using the overall amplitude of the ERG (trough of a-wave to peak of b-wave) as a measure Armington (1954) studied the spectral sensitivity of *Pseudemys scripta elegans*. He found sensitivity to be maximal in the 630-650 nm range, with a secondary peak at 500 nm. Granda (1962), using the same technique and the same species, found the same maximum, 640-650 nm, and uncovered a secondary peak at 575 nm. Both of these studies were perforce carried out on light adapted turtles because of the intensity of stimulus necessary at that time to evoke an ERG sufficiently far above the noise level for reliability. As equipment became better, and electronic signal averaging was developed, Granda and Stirling (1966) were enabled to study the ERG's of more completely dark adapted turtles. Using the a-wave trough to b-wave peak amplitude of *Pseudemys scripta elegans* as a measure, and by recording the stimulus intensity at each wavelength required to evoke a criterion response, they determined spectral sensitivity curves for several criteria of response amplitude. Larger criterion amplitudes require larger stimulus intensities. They found the same peaks in the red (640-660 nm) and yellow (560-580 nm) for larger
criterion amplitudes, as had previous workers, but they found that for small criterion amplitudes a peak at 500 nm became visible, corroborating Armington's finding (1954). Granda and Stirling (1965) had previously observed this 500 nm peak when measuring spectral sensitivity by use of the overall height of the evoked potential of the tectum of *P. scripta elegans*.

Prior to this work, Deane, Enroth-Cugell, Congaware, Neyland, and Forbes (1958), also recording the tectal evoked potential amplitude in *P. scripta elegans*, had found the peak sensitivity to high intensity stimuli to be at 645 nm, while the peak sensitivity to low intensity stimuli was found to be between 465 and 545 nm.

Armington (1954) used a behavioral paradigm to measure the spectral sensitivity curve on one *P. scripta elegans*. The turtle moved down a runway toward the illuminated one of two panels in order to obtain a food reward. The illumination was decreased until no preference was exhibited. By this means, he found a sensitivity maximum at 525 nm, and a secondary shoulder at 650 nm, the opposite of what he found electrophysiologically.

Bitterman (1964) designed an apparatus used by most researchers to date. The test chamber is roughly boat-shaped, with a starting box at the flat end. The far walls thus form an angle in the middle, facing the starting box. Centered on each of these angled walls is a 2" hole, through which may be seen a target which may be transilluminated with light of controlled spectral composition. To complete a response, the
turtle moves down the chamber, puts its head through one of the two holes, and presses on the target. Reinforcement in the form of food, usually horsemeat, is then given the animal.

Sokol and Muntz (1966) used a one-target Bitterman box (as we shall call it) to measure the spectral sensitivity of *Chrysemys picta picta*. Turtles were trained to respond to an illuminated target and not to a dark one. Response rate as a function of intensity was then used as a measure of spectral sensitivity. Turtles were dark-adapted before testing. When high intensity stimuli were used, the sensitivity was found to be maximal between 650 and 660 nm, while for low intensity stimuli the maximum was found to be in the blue, between 420 and 500 nm.

Later, Muntz and Sokol (1967), performed a similar experiment, but now using a two-choice Bitterman box. Stimulus intensity was lowered until the turtle no longer chose the illuminated target at greater than chance level. As before, maximum sensitivity to low-intensity stimuli was found to be in the 430 to 500 nm range, even after prolonged pre-adaptation to white (tungsten) light. The shift in maximum sensitivity from blue to red with increasing stimulus intensity would thus not appear to be a classical light-adaptation phenomenon, since the pre-adaptation should cause the shift, as well as the increased stimulus intensity. In the same experiment, Muntz and Sokol also found an inflection in the sensitivity curve at 560 nm.

Graf (1967) also used a two-choice Bitterman box to measure
spectral sensitivity in *Chrysemys picta picta*. But Graf used the powerful technique of flicker photometry. He trained the turtles to respond to flickering stimuli only. The animal was then presented with two targets, one of which was monochromatic and flickering well above the animal's critical fusion frequency, the other of which was illuminated with the test wavelength, flickering at a frequency below CFF, and whose modulation was controlled. The modulation level at which the animal was unable to distinguish the flickering from the non-flickering target was then measured. By this means, Graf determined the spectral sensitivity function for wavelengths between 575 nm and 715 nm, and found a peak at 625-640 nm. Graf's data do not go below 575 nm, so correlation to Armington's and Sokol and Muntz's (1966) dark-adapted peak in the blue is not possible. He states, however, that his data do indicate the existence of a second mechanism below 575 nm, based on an apparently different response to flicker which begins to show up as 575 nm is approached.

Table 1 and Figure 1 summarize the results of the foregoing studies of turtles. Table 1 includes the data for all species, while Figure 1 is only for *Pseudenvs scripta elegans*. Let us confine our attention, for the moment, to this latter data.

In Figure 1, the solid lines represent maxima obtained under photopic conditions, while the dotted lines represent maxima obtained under scotopic. The combined tectal and ERG maxima, scotopic and photopic, show a striking grouping into three (or possibly four) main regions. All the ranges in the region 620 to 660 nm appear to
### TABLE 1

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>SPECIES</th>
<th>MEASURED VARIABLE</th>
<th>PEAKS DETECTED (in order of size)</th>
<th>SCOTOPIC (S)</th>
<th>PROTOPIC (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grantt (1941)</td>
<td>Testudo</td>
<td>Ganglion cell output</td>
<td>600-640; 515-530</td>
<td></td>
<td>(P)</td>
</tr>
<tr>
<td>Orlov and Maksimova (1964)</td>
<td>E. orbicularis</td>
<td>Mass optic nerve discharge</td>
<td>520</td>
<td>(S)</td>
<td></td>
</tr>
<tr>
<td>Armington (1954)</td>
<td>P. scripta elephas</td>
<td>ERG</td>
<td>630-650; 500</td>
<td>(P)</td>
<td></td>
</tr>
<tr>
<td>Granda (1962)</td>
<td></td>
<td>&quot;</td>
<td>640-650; 575</td>
<td>(P)</td>
<td></td>
</tr>
<tr>
<td>Granda and Stirling (1966)</td>
<td></td>
<td>&quot;</td>
<td>630-650; 540-580; 460-480</td>
<td>(P)</td>
<td></td>
</tr>
<tr>
<td>Granda and Stirling (1965)</td>
<td></td>
<td>Tectal evoked potential</td>
<td>650-660; 620-630; 560</td>
<td>(P)</td>
<td>(S)</td>
</tr>
<tr>
<td>Deane, et. al. (1958)</td>
<td></td>
<td>&quot;</td>
<td>665</td>
<td>(P)</td>
<td>(S)</td>
</tr>
<tr>
<td>Armington (1956)</td>
<td></td>
<td>Behavioral discrimination</td>
<td>525; 650</td>
<td>(S)</td>
<td></td>
</tr>
<tr>
<td>Sokol and Muntz (1966) Chrysonyx pica</td>
<td>Behavioral: single</td>
<td>650-660; 420-500</td>
<td>(P)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muntz and Sokol (1967)</td>
<td></td>
<td>target response rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graf (1967)</td>
<td></td>
<td>Behavioral discrimination</td>
<td>430-500</td>
<td>(1)</td>
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</tr>
</tbody>
</table>
FIGURE 1

RECORDED LOCATIONS OF SENSITIVITY MAXIMA

(PSEUDENYS SCRIPTA ELEGANS)

BEHAVIORAL

TECTUM

E.R.G.

PIGMENTS

WAVELENGTH (nm)
overlap, indicating that it is possible that all the different researchers were dealing with the same peak. Indeed, a peak at 640 nm would fall within the ranges of certainty of all of the experiments with the exception of Granda and Stirling's (1965) tectal data, where two peaks are shown equidistant on either side of the hypothetical 640 nm peak and yet not overlapping. Deferring discussion of this dual peak for the moment, let us go on to consider the remainder of the spectrum.

There is also clear evidence of a peak in the 560 to 580 nm region. The separation between this hypothetical peak and that occurring around 640 nm is quite clear, there being no evidence of a peak anywhere between 580 and 620 nm.

There are two reports of a peak in the 500 nm region and one of a peak in the 460 to 480 nm region. The separation in the ranges within which these peaks might lie is only 10 nm, but one would not be justified in concluding that only one peak existed.

Thus, there appear to be these main peaks: 460-480 nm, 490-510 nm, 540-580 nm, and 630-650 nm. A remarkable feature of this series of peaks is the discrepancy between their spectrum loci and the pigment absorption maxima, given by Liebman and Granda (1971) as occurring at 450 nm, 518 nm, and 620 nm. How might such a discrepancy be resolved?

A widely-held view of color vision in oil droplet-bearing animals, such as the turtle, is that these oil droplets are used to
shift the output maxima of different receptors each containing pigment with the same absorption spectra. By this reasoning, an oil droplet whose cutoff is at some wavelength $\lambda_c$ is associated with a receptor containing a photopigment whose absorption maximum occurs at $\lambda_a$. Assuming that the oil droplet is a high-pass filter, then as $\lambda_c$ approaches $\lambda_a$ from the short wavelength side, the output maximum of the receptor will begin shifting toward the longer wavelengths as the filter begins to cut down incoming light at $\lambda_a$. The output maximum continues to move to longer wavelengths as $\lambda_c$ reaches and passes $\lambda_a$. But this shift can be accomplished only at a cost in the sensitivity to light, since the filter is absorbing incoming light which would normally affect the photopigment. This drop in output amplitude occurs at a rate proportional to the slope of the cutoff of the filter as $\lambda_c$ reaches and passes $\lambda_a$. Since oil droplets have sharp cutoffs, it is therefore obvious that output maxima cannot be moved very far (only 10 to 20 nm) by use of oil droplet filters. In addition, the oil droplet can only drive the peak toward the wavelengths in which the oil droplet is less absorptive, thus the yellow, orange, and red oil droplets, all of which have peak absorption at 475 nm (Lipetz, 1971), can only drive output peaks of receptors containing the 518 and 620 nm pigments farther into the longer wavelengths from the absorption maxima of these photopigments, and drive the output peaks of the 450 nm pigmented receptors toward shorter wavelengths.
In terms of accounting for the observed ERG and tectal peaks, then, the oil droplet hypothesis is a poor one. All the droplets have absorption maxima at 475 nm, so no droplet could cause a photoreceptor with a 450 nm pigment to have an output maximum of a higher wavelength. Thus, the 450 nm pigment cannot be used in this model to account for the 400-480 nm and the 490-510 nm peaks. As previously shown, no oil droplet can shift an output peak toward lower wavelengths for a pigment with an absorption maximum greater than 475 nm. Thus, the 518 nm pigment cannot be used in this model to account for the 460-480 nm, or the 490-510 nm peak. Clearly, these peaks cannot be accounted for by the model.

Oil droplet filtering might shift the 518 nm pigmented receptor's absorption peak up towards the observed 540 to 580 nm sensitivity maximum. But only the yellow oil droplet has a $\lambda_c$ less than 518 nm (about 510 nm). And with a 510 nm $\lambda_c$, the 518 peak could not be shifted very far, certainly not far enough to account for observations of peaks at, or near, 580 nm. By similar reasoning, the cutoffs of the orange droplet (535-555 nm) and the red oil droplet (570-595 nm) are too far removed from the 620 nm absorption maximum to shift it very far. Certainly not as far as 660 nm, where some observers have reported peaks. Nor could a 620 nm pigment and oil droplet account for the dual peak observed in the tectum by Granda and Stirling.

There is another model which might be employed with more
success. Naka and Rushton (1966) have described mathematically a process by which two maxima may give rise to a number of others. This process is differencing. That is, the output of one unit containing one photopigment is subtracted from the output of another unit with a different pigment. The position of the maximum of the resultant, or the difference peak, depends on the broadness of the absorption spectra of the two primary pigment processes, their spectral separation, and the weights given to the inputs from each at the differencing point.

Figure 2 shows the Muntz templates for vitamin A₂ pigments with absorption maxima at 450, 518, and 620 nm. Note that there is a good deal of overlap between the 450 and 518 nm pigments, and between the 518 and 620 nm pigments. Thus, a significant differencing can occur over a wide range of wavelengths.

Figure 3 shows a rather simplistic approach to the differencing operation. Here, the differences between the 450 and 518 nm pigments and the 518 and 620 nm pigments are shown. The approach is simplistic in that equal weights are given to both inputs. Ignoring polarity, which is, after all, only a function of the direction of the differencing, four possible peaks result. There are peaks at 430 nm, 500 nm, 530 nm, and 630 nm. Note that by this simplistic, equally-weighted, differencing operation, the 450 nm pigment maximum shows up as a 430 nm output maximum in the difference channel; similarly, the 620 nm pigment gives rise to a 630 nm output maximum.
MUNTZ PIGMENT TEMPLATES
(A2 SYSTEM)

FIGURE 2
And the 518 nm pigment has given rise to two output maxima, at 500 and at 530 nm. Thus, differencing can not only shift output maxima either way from the pigment maxima, but can produce additional peaks.

Applying the differencing model to the available data then becomes a matter of choosing which pigments to difference and what weights to assign each to obtain the observed sensitivity maximum. It is worth noting that even the simple operation employed in deriving Figure 3 from Figure 2 produced a 500 nm and a 630 nm peak, both of which have been observed, and neither of which can be accounted for by the oil-droplet-and-photopigment model. Also, the dual peaks at 620 and 650 nm can be explained in terms of a second differencing operation, or possibly a difference peak at 650 nm and a direct pigment peak at 620 nm, if the latter information were to be carried through to the tectum.

The ERG data is helpful in terms of locating the differencing mechanism. The ERG data show only difference peaks, no primary peaks. The ERG data result from measuring the voltage difference between the trough of the a-wave and the peak of the b-wave. The b-wave is considered to arise proximal to the receptor's synaptic region, somewhere in or near the outer plexiform layer. The dominant component of the a-wave is associated with activity in the outer plexiform layer. Since both a- and the b-wave show difference peaks rather than primary peaks, it is evident that the differencing occurs in the outer plexiform layer. In this layer, interactions are possible between receptors
and other receptors; between horizontal cells, receptors, and bipolar cells; and between horizontal cells.

The behavioral data of Sokol and Muntz (1966), and Muntz and Sokol (1967), taken together with the electrophysiological data of Granda and Stirling (1965), show that the spectral sensitivity maximum shifted with change in stimulus intensity. The peak at 630-650 nm, which is dominant in the ERG, tectum, and behavior for high intensity stimuli, shifts to 460-500 nm as the stimulus intensity drops. Thus, there occurs a shift in the spectrum locus of the sensitivity maximum with changing stimulus intensity.

To explain the foregoing, Muntz and Sokol (1967) suggested the following hypothesis. It is assumed that the blue receptors do exist at the retina, but their number is small compared to the red receptor population. Also, it is assumed that both receptor types are equally sensitive at all supra-threshold intensities, but that the red receptors have a higher threshold than the blue. Thus, for intensities below the threshold of the red receptors the blue response dominates, while at higher intensities the more numerous red receptors come to dominate the response. As a physiological basis for the threshold difference, Muntz and Sokol suggest that the less numerous blue receptors have a higher degree of convergence onto higher neural elements, which would cause these elements to be excited even when relatively few blue receptors are active. At higher intensities, red receptor driven higher elements, being more numerous, would
dominate the response. The scarcity of blue receptors would explain why the blue peak is often not seen in electrophysiological recordings at the retina. This hypothesis is also used by the authors to explain the failure of white adapting light to cause a shift in the sensitivity maximum.

This model has several drawbacks. It assumes a fixed anatomy and therefore, cannot produce changes in spectral sensitivity as a function of anything other than stimulus light intensity. Yet, James Maxwell (1971), a student of Granda, recently has demonstrated that there are indeed spectral sensitivity changes occurring during dark adaptation. It is considerably simpler to explain the shift in terms of the differencing model. In terms of this model, the shift in the maximum reflects a change in the weighting functions assigned to the primaries. This change might occur over time at the retinal differencing site and could be correlated with stimulus intensity; or the change in weighting might occur over space, in that one set of weights could be used at the retina and another at the tectum for differencing. This last supposes that primary pigment absorption information gets to the tectum, a fact pointed to by Grandi and Stirling's (1955) 620 nm tectal peak, but somewhat doubtful for lack of corroborative evidence at other neural levels.

Thus, it is seen that a differencing model can much more readily account for the observed data than can a pigment-oil droplet model. With the possible exception of one observation, nowhere in
visual system is the primary pigment absorption information to be found beyond the receptors. The present study of the spectral sensitivity of *Pseudemys scripta elegans*, tests whether or not the processing of information involved in the behavioral task utilized primary pigment information or differencing information, or a combination of both.
The concept of using breathable air as a reinforcement in behavioral studies with turtles was first introduced by van Sommers (1963) and later verified by Morlock et al. (1968). Van Sommers demonstrated that turtles could be conditioned to press a lever in order to obtain breathable air when submerged. He studied the effects on the animals' behavior of varying such parameters as duration of access to air, extinction in the presence of a (red) discriminative light, presence of carbon dioxide in the air, and water temperature.

For this dissertation, the choice of the gas mixture was based on the more recent work of Jackson (1968), and Frankel et al. (1969), who studied the metabolism of diving turtles. These studies led to the use of a 5-second duration access to a mixture of 5% oxygen, 95% nitrogen, as the reinforcement. Experiment runs were kept to less than one and one-half hour so as not to unduly stress the animal. Water temperature was maintained at 27°C.

Spigel (1963, 1964, 1965a,b) studied the effects of preconfinement on turtles and found that such preconfinement drastically interfered with performance. The idea of dark-adapting the turtles prior to each run was therefore abandoned.

Since the study involved the determination of stimulus intensities at the visual threshold, it was decided to employ a procedure
which would allow a higher density of data to be collected at near-threshold stimulus values. Such a procedure is the "tracking" procedure of Muntz and Northmore (1968). According to this procedure, the values of the stimulus on any given trial depends on the response of the animal during the preceding trial. As employed in this experiment, the procedure was to have the stimulus decrease in intensity if the preceding response was incorrect. A feature which was added here was that of variable intensity steps; in the beginning of a run, when the stimulus was well above threshold, the step changes in intensity were large; as the run progressed the increments became smaller, thus enabling a rapid descent to threshold without subsequent loss of accuracy in determining the actual threshold intensity value.

In its final form, the air reinforcement technique employed in this experiment was as follows. A light stimulus of known spectral composition appeared from behind one of two diffusing targets (each 1-1/2" x 1-1/2", and 2-1/2" apart) which were visible to the turtle, who was submerged. Each target formed one wall of a chamber which was slightly above the level of the underwater ceiling, but plainly visible because of a slanting back wall (see Figure 4). The turtle then swam up to and placed his head near one of the targets. This action interrupted an infrared beam and caused a response to be registered. If the response was correct, i.e., the turtle approached the illuminated target, then the gas mixture was vented into the chamber from the top under 1-1/2 psi, driving the water level down to the
FIGURE 4
level of the air vents (Figure 4) and providing breathing space for the turtle. Inflow continued for five seconds, during which time the target remained illuminated. At the end of the five seconds, the inflow stopped, the target light went out, and the gas inlet orifice was vented to the open air, allowing the water level to rise. Within two seconds or less, the air space was occluded. Had the response been incorrect, no gas would have been administered, and the target illumination would have been immediately terminated. In either case, a fifteen-second cycle time next followed the response. During this time, the stimulus intensity was automatically adjusted (see above mentioned tracking procedure) and a randomizer decided which target would be illuminated at the next presentation. At the end of this time, the next presentation was made by illuminating one of the panels, unless the animal remained in the reinforcement chamber, in which case, no presentation was made until the animal withdrew his head.

Normally then, this cycle was repeated until the animal had caused the stimulus to reach threshold intensity, at which time, the number of incorrect responses came to equal the number of correct responses, causing the intensity to oscillate about the threshold value. It became evident, however, that for some wavelengths of light to which the animals were relatively insensitive, several animals would adopt the strategy of responding to one target only, "position preference"), whether or not illumination was present.
Such a strategy resulted in 50% reinforcement, since the target to which the animal was responding was the correct one 50% of the time, on the average. This was not in itself serious, since Kirk and Bitterman (1965) demonstrated probability-learning in the turtle, leading to the conclusion that under normal circumstances, the turtles would prefer the strategy which produced 100% reinforcement. But such a position preference, adopted near but not at threshold, could throw off results by creating a false threshold in the record. To diminish the position preference, the experimental paradigm was modified so that the stimulus intensity was allowed to decrease by tracking for, at most, two log units, after which time it would be reset to a known suprathreshold value and held constant, regardless of the response pattern, for five minutes. During this period, the additional modification was made that while the stimulus had a 50:50 chance of changing sides following a correct response, it would never change sides following an incorrect response, thus forcing the turtle to change sides. After this five-minute reshaping period, the stimulus was reset to the previous low intensity level, and tracking was reinstated for the next two log units decrease in intensity, or until threshold. In some cases, severe position preference had to be treated by fixing the stimulus on the non-preferred side for five or ten correct responses. In the majority of cases, this procedure eliminated position preference, at least temporarily.

As time and response rate permitted, the stimulus intensity was allowed to track to threshold several times in the course of an
experiment, thus providing for several responses to be made at each intensity. This allowed computation of the probability of a correct response at each tested intensity interval (see Chapter III). The exhibition of position preference, and the time necessary for the reshaping periods, of course, interfered with this procedure. Records were also kept of the total number of responses, the number of responses made at each target, and the number of times each target was illuminated. Thus, any net position bias inherent in either turtle or equipment could be detected.
CHAPTER III
ANALYSIS OF THE DATA

As the experiment progressed, a strip-chart recorder kept a record of the position of the neutral density wedge as a function of time. This record is referred to as ND(t). In addition, the wavelength, turtle number, date, and time of starting, as well as presence of any stray-light filters were recorded on the strip chart at the outset of the experiment. Each response caused a change in ND(t); a correct response caused an increase, and an incorrect response a decrease. As each response occurred, it was analyzed and recorded on the cumulative behavioral recorder, a device consisting of four cumulative digital counters and associated logic circuitry. One counter accumulated CR, or response correct-stimulus right, responses; another CL, or response correct-stimulus left, responses; a third IR, or response incorrect-stimulus right, responses; and the last IL, or response incorrect-stimulus left, responses.

The cumulated CR, CL, IR, and IL data were used in two ways: firstly, in the pretraining and training evaluation of performance, and secondly, as an aid in the evaluation of performance during a run. During pretraining selection of turtles and subsequent training of selected turtles, these data were further analyzed into 

P(C) (Probability of a correct response), P(RR) (probability of response right), P(SR) (probability of stimulus right), P'(C) (corrected
probability of a correct response), FMR (fraction of maximum rate), and M (figure of merit). These derived data were obtained from the raw CR, CL, IR, IL data in the following way. First, the total number of responses, \( N = CR + CL + IR + IL \) was calculated. Then \( P(CR) = CR/N \), \( P(CL) = CL/N \), \( P(IR) = IR/N \), and \( P(IL) = IL/N \) were calculated. Then \( P(C) = P(CL) + P(CR) \), \( P(RR) = P(CR) + P(IL) \), \( P(SR) = P(CR) + P(IR) \). The datum \( P(RR) \) was taken to be an index of position preference on the part of the turtle. \( P(SR) \) similarly measured a position bias on the part of the automatic machinery.

The corrected \( P(C) \) or \( P'(C) \) was so designed as to compensate for coincidental biases on the part of the turtle and the machine. \( P'(C) \) was derived in the following manner. Note that \( P(SR) \cdot P(RR) \) gives the probability of a correct response due solely to similar right-hand biases on the part of the animal and the machine. Then \( P(SR) \cdot P(RR) \cdot P(SL) \cdot P(IL) = P(SR) \cdot (1-P(SR)) \cdot (1-P(RR)) \) gives the total probability of a correct response based on any coincidental machine-animal side bias. Thus, for a first approximation to a corrected \( P(C) \), one may use

\[
P''(C) = P(C) - P(RR)P(SR) - (1-P(RR))(1-P(SR))
\]

\[
= P(C) - 1 + P(RR) \cdot P(SR) - 2P(RR)P(SR)
\]

\[
= P(CR) + P(CL) - 1 + P(CR) \cdot P(IL) + P(CR) \cdot P(IR) - 2P(RR)
\]

\[
= P(SR)
\]

and thus,

\[
P''(C) = 2P(CR) - 2P(RR)P(SR),
\]

since \( P(CR) \cdot P(CL) \cdot P(IR) \cdot P(IL) = 1 \). But it may be shown that
-1/2 ≤ P''(C) ≤ +1/2, and therefore we may choose P'(C) = 2P'(C) = 4(P(CR) - P(RP(SR))) so that -1 < P'(C) < 1. Thus, P'(C) = 0 implies a purely random response situation, P'(C) < 0, implies position preference, and P'(C) > 0, implies stimulus-driven performance.

The FMR figure was computed by dividing the elapsed time of the run by the number of seconds taken by each stimulus-response-stimulus cycle so as to get the number of possible responses. This number is then divided into the actual number of responses, so as to produce the fraction of maximum rate, or FMR.

The figure of merit (M) is a rather highly arbitrary measure of overall performance during a run, arrived at by setting M = 10 P'(C) \cdot FMR for P'(C) > 0, or M = FMR/10P'(c) < 0. Thus, -10 < M < 10.

Records of the parameters P'(C), FMR, P(RR) and M were kept on each potentially useful turtle. Such records are shown in Figures 5, 6, 7, and 8. Such records were used to select those turtles showing the best performance. And there does, indeed, appear to be a dichotomy in performance. Table 2 shows the results of a survey of 15 naive turtles. Note the markedly higher performance indices for Numbers 4, 16, and 71, showing why these turtles were selected.

The parameters P(RR), P(SR), and P(C) continued to be monitored during data-collecting runs, so as to continually evaluate these aspects of turtle and machine performance. By these means one breakdown in the randomizer was diagnosed, and incipient position preferences on the part of the turtles could be detected and corrected, even during a run.
FIGURE 5
TURTLE No. 4 P(C) CURVE (TRAINING)

TURTLE No. 16 P(C) CURVE (TRAINING)
FMR FIGURES

- • N° 4
- • N° 16

FIGURE 6
**FIGURE 7**
FIGURE 8

MERIT FIGURES

- [Line with dots at 5.0, labeled "No. 4"]
- [Line with dots at 4.0, labeled "No. 10"]

FIGURE OF MERIT (M)

TRIALS
After a run was completed, the first step in analysis was to convert the raw ND(t) into an intensity, I(t). This procedure made use of calibrations outlined in Appendix II. As described therein, the relative spectral output power $E(\lambda)$ of the unfiltered monochromator light source was determined. Since, however, it is widely accepted that visual receptor systems respond only to number of quanta caught by the receptor and not to the wavelength or energy of the quanta, this $E(\lambda)$ was converted to RQN, or relative quantum number. This was done by use of the relation $E(\lambda) = \frac{Nhc}{\lambda}$, where $E(\lambda)$ is the absolute power as a function of wavelength, $N$ is the absolute number of photons per unit time, $h$ is Planck's constant, and $\lambda$ is the wavelength of the light. Then, in relative terms, $E(\lambda) = \frac{Nhc}{\lambda}$. Or, multiplying by $\lambda$, $\lambda E(\lambda) = Nhc = KN$, where $K$ is a constant, so that we can restate the relationship as $\lambda E(\lambda)$ is proportional to the relative quantum number. For convenience in calculating the intensity output with neutral density filters in place, the RQN was converted to logarithmic (base 10) form, as shown in Figure 33.

Having log RQN for each wavelength, conversion of ND(t) was easily accomplished. The sum of the neutral wedge's optical density (as determined by calibration outlined in Appendix II) and the optical density of the fixed neutral filter value (also from Appendix II) was subtracted from the log RQN to get the final I(t). Table 3 shows such a conversion.
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<th>Raw Wedge</th>
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**TABLE 3**
The plot of raw I(t) was useful in evaluating the turtle's performance during a run. Some examples of I(t) plots are shown in Figures 9 through 11. In the ideal case, I(t) starts out at some level well above threshold, and moves steadily downward toward threshold. As threshold is neared, more mistakes are made, and the rate of decline of I(t) falls off to asymptote to the threshold value. In actual practice, this form of I(t) was not usually seen. First of all, the wedge moved through two optical density units before it was reset to zero, and another fixed filter added (see solid arrows, Figures 9 through 11). Since the fixed filter increased the total optical density by 1 unit, I(t) showed an increase at this changeover point before descending toward threshold again. Behavioral aberrations were reflected in the I(t) plot. Often turtles would make frequent mistakes at the outset of a run, so that I(t) remained at a high level before commencing to decrease. Position preference on the part of the turtle would cause I(t) to level out at some value above threshold (see Figure 11, dotted arrow). This is particularly obvious when several determinations of threshold were made in the course of one run.

A computer program was constructed to analyze the data from each run. For each response, the computer was provided data giving the elapsed time from the start of the run to that response, the final intensity at which the response was made, and whether the response was correct or incorrect. The program first rearranged the responses by intensity. The intensities were then grouped into intervals of 0.2
log units. At each interval, the number of correct and incorrect responses was counted. \( P(C) \) was then calculated, and a subroutine called into play to correct the raw \( P(C) \) data for the fact that in the two-alternative forced-choice situation, \( P(C) \) at threshold is 50%, not zero. Correction was made by employing Abbott's correction: \( \text{SMP} = \frac{P(C) - C}{1 - C} \) where \( C \) is the expected lower probability limit, or 50% in this case, and SMP the corrected probability. Thus, here, Abbott's Correction reduces to \( \text{SMP} = 2(P(C) - 0.5) \).

In order to fit a line to the probability data, the SMP values were transformed into probits. Probits (\( Y \)) are related to probability (\( P \)) by the following nonlinear formula:

\[
P = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\sqrt{Y-5}} e^{-1/2 u^2} du
\]

This conversion to probits has the effect of linearizing the probability axis, so that points which fell along an ogive now fall along a straight line. In practice it was necessary to calculate by use of an approximation to the inverse of the normal distribution, namely,

\[
Y = (P - 0.5)(1.5865 + 11.338)(P - 0.5)^2.
\]

A two-iteration, least-squares analysis was then employed to fit a straight line to the probit values. The routine also calculated the mean and variance of the probits; the chi-square fit of the line to the raw data; and any correction necessary to the assumed minimum-probability value of 0.4,
50%. A sample of output is given in Table 4. A sample $P(C)$ versus intensity curve is given in Figure 12.

The computer next calculated the spectral sensitivity curves. For a given turtle, the $P(C)$ versus intensity curves at each wavelength were used to determine the intensity required to reach criterion $P(C)$'s of 50, 60, 70, and 80 percent. A plot of the criterion intensities versus wavelength then gave the inverse spectral sensitivity curve. It remained only to replot the intensity values multiplied by $-1$, since these values were $\log_{10}$ values, to convert the plot to a spectral sensitivity plot.
WAVELENGTH 520 NM

<table>
<thead>
<tr>
<th>INTENSITY</th>
<th>P(C)</th>
<th>PROBIT</th>
<th>PERCENT</th>
<th>INTENSITY</th>
</tr>
</thead>
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<tr>
<td>2.4000</td>
<td>1.000</td>
<td>5.1700</td>
<td>50</td>
<td>-0.0870</td>
</tr>
<tr>
<td>2.2000</td>
<td>1.000</td>
<td>5.0000</td>
<td>60</td>
<td>0.1061</td>
</tr>
<tr>
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<td>1.000</td>
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<td>70</td>
<td>0.3146</td>
</tr>
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<td>1.000</td>
<td>7.2105</td>
<td>80</td>
<td>0.5618</td>
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<td>1.000</td>
<td>2.7895</td>
<td>90</td>
<td>0.9016</td>
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<tr>
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</tr>
<tr>
<td>0.8000</td>
<td>0.851</td>
<td>5.4139</td>
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<td>**********</td>
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<tr>
<td>0.6000</td>
<td>0.831</td>
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<td>4.6848</td>
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<tr>
<td>0.2000</td>
<td>0.667</td>
<td>4.6848</td>
<td>**</td>
<td>**********</td>
</tr>
</tbody>
</table>

PROBIT LINE EQUATION IS Y = 1.2947X + 5.1127
SLOPE VARIANCE IS 1.8455
PROBIT VARIANCE IS 0.1451
CHI SQUARE FIT OF LINE IS 0.794E 00 WITH 5. DEGREES OF FREEDOM
MEAN OF THE P.O.S. CURVE IS -0.0870
VARIANCE OF THE P.O.S. CURVE IS 0.7724
CORRECTION TO ESTIMATED C IS -0.3128
470 nm

PROBIT LINE: \( y = (1.9 + 0.044)x + 4.34 \)

\( \mu = 0.3457 \)
\( \sigma = 0.5261 \)
\( p < 0.1 \)

FIGURE 12
CHAPTER IV

RESULTS

Figure 13 shows the spectral sensitivity curve of turtle No. 4, taken during the first 45-minute period of each run. Three peaks are clearly delineated. There is a peak at about 470 nm, one at around 570-580 nm, and a third at about 640 nm. At the 50% criterion level, the peaks are all of about the same amplitude. With increase in criterion, however, there is a change in relative amplitude, such that the red (640 nm) peak comes to be the dominant peak, with the blue (470 nm) peak, secondary, and the yellow (570-580 nm) peak, tertiary. There is some evidence of an increase in sensitivity between 440 nm and 400 nm at all criterion levels. At the other end of the spectrum, the dropoff between 660 nm and 690 nm becomes more marked with increased criterion, as if a sensitivity shoulder or peak near 700 nm were dropping out as the criterion is increased.

Figure 14 shows the spectral sensitivity curve of the same animal, but here, derived from the total data for each run. The same three major peaks are present, and their relative amplitudes change as before with increase in criterion. Some new changes appear in this figure, however, with criterion change. The shoulder at 600 nm drops out and becomes a minimum with criterion increase. The same is true for the shoulder at 670 nm. This change at 670 nm may explain the long-wavelength change noted for the first interval.
FIGURE 13

WAVELENGTH (nm)

LOG SENSITIVITY

80% ---
70% ----
60% -----
50% ---

NO 4, INTERVAL 1
FIGURE 14

LOG SENSITIVITY

WAVELENGTH (nm)

400 500 600 700

NO 4, TOTAL
Figure 15 shows the spectral sensitivity curve for Turtle No. 16, derived from data taken during the first 45-minute interval of each run. The main peaks here are at 470 nm and 670 nm. There is, for the 50% criterion, a slight peak at 560 nm. This peak shifts toward 600 nm with criterion increase. Also, with criterion increase, a shoulder at 450 nm becomes more prominent, while a shoulder at 530 nm drops out. The relative amplitude of the three peaks remains fairly constant for the different criterion levels.

Figure 16 shows the curve derived from the data taken over the entirety of each run for Turtle No. 16. This family of curves shows some very interesting changes with change in criterion. At the 50% level, the main peaks are at 470 nm, 570 nm, and 670 nm, as they were for the first interval. The same shoulders at 450 nm and 530 nm are visible. There are additional possible shoulders here, however, at 400 nm, 620 nm, and 650 nm. The 400 nm, 620 nm, and 650 nm shoulders drop out with increase in criterion, the 620 and 650 nm shoulders actually becoming minima. The 450 nm shoulder increases with increase in criterion to produce a shift in the local maximum from 470 nm to 450 nm. Similarly, the 530 nm shoulder increases and causes a shift from 570 nm to 530 nm. There is also a marked downward shift from 670 to 660 nm with increase in criterion. It is worthy of note that all three shifts were toward the short-wavelength end of the spectrum.

Figure 17 shows the spectral sensitivity curve for Turtle No. 71, derived from data taken during the first 45 minutes of each run.
FIGURE 15

LOG SENSITIVITY

WAVELENGTH (nm)

80% ——
70% ——
60% ——
50% ——

NA 16, INTERVAL 1

400 500 600 700
FIGURE 17

LOG SENSITIVITY

WAVELENGTH (nm)

N 71, INTERVAL I

80%  70%  60%  50%
Here again, interesting changes occur with changing criterion. At the 50% criterion, the main peaks are at 490 nm, 570 nm, 610 nm, and 690 nm. Note that the dual peak in the red (610 nm and 690 nm), is seen for the first time in this turtle. A shoulder may be seen at 440 nm with criterion increase; this shoulder develops into a peak, causing a shift in the local maximum from 490 nm to 440 nm. The shift is even more dramatic than shown here, since the 490 nm peak and the 440 nm peak are not shown at the 50% level because of size; but at this level the 490 nm peak is considerably higher than the 440 nm shoulder. There is the development of a shoulder at 530 nm, causing an upward shift in the sensitivity local minimum from 530 nm to 540 nm. Another development takes place at 590 nm, where a local peak appears to develop. The peak at 610 nm shifts upward to 620 nm. The peak at 690 drops out entirely.

Figure 18 shows the spectral sensitivity curve for Turtle No. 71, based on data taken from the entirety of each run. At the 50% criterion level, the major peaks are at 450 nm, 570 nm, 620 nm, and 690 nm. Shoulders are visible at 470 nm, 530 nm, and 610 nm. With increase in criterion level, the 450 nm peak drops and the 470 shoulder increases, causing a shift in the local maximum from 450 to 470 nm. Similarly, the 530 nm shoulder increases and the 590 nm peak drops to cause a shift in the local maximum from 570 nm down to 530 nm. A peak develops at 610 nm, as in the first interval. The shoulder at 620 nm drops out. While the 690 nm peak does not entirely drop out,
FIGURE 18

LOG SENSITIVITY

WAVELENGTH (nm)

N4 71, TOTAL
as in the first interval, it declines from a value higher than that for the 620 nm peak at the 50% level to one lower than that for the 620 nm peak at the 80% level.

As mentioned in Chapter III, the data processing includes a linearization of P(C) by conversion to probits, and a line-fit to the probits. The slope of this probit line, then, is related to the rate of decline of the POS curve for the given wavelength. The rate of decline is then, in turn, related to the difficulty of perception experienced by the animal in detecting and responding to a target of the given spectral composition. It is, therefore, worthwhile to plot the probit line slope for each wavelength vs. wavelength. Such a plot will be termed the slope plot.

Figure 19 is a slope plot for Turtle No. 4, based on data for the entire course of each run. Note that there are three minima. These minima indicate those wavelengths which were easiest for the turtle to detect and respond to. They occur at 470 nm, 570 nm, and 640 nm, the points where the maxima for spectral sensitivity occurred for that turtle (Figure 14).

Figure 20 is a slope plot, again based on data from the entire time course of each run, for Turtle No. 16. Minima occur at 470 nm, 580 nm, and 670 nm. With the exception of the 580 nm minimum, which does not exactly match the 570 sensitivity peak, these minima match the spectral sensitivity maxima for this turtle (Figure 16).

Figure 21 is a slope plot of whole-run data for Turtle No. 71. Minima occur at 450 nm, 570 nm, 620 nm, and 670 nm. The 450 nm
FIGURE 19

No. 4, SLOPE PLOT
FIGURE 20

WAVELENGTH (nm)

SLOPE

No. 16, SLOPE PLOT
FIGURE 21

NA 71, SLOPE PLOT

SLOPE

WAVELENGTH (nm)

400 500 600 700
minimum matches that turtle's 450 nm sensitivity peak (Figure 18). The 570 nm slope minimum corresponds to the 570 sensitivity maximum, and the 620 nm slope minimum corresponds to the sensitivity maximum at 620 nm. The 670 nm slope minimum is close to, but does not quite, match the sensitivity maximum at 690 nm.
Chapter V
Discussion

Interval 1

Blue Peaks.—At the 50% criterion level in the first time interval, Turtle No. 4 has a peak in the blue at 470 nm. The blue peak at this criterion level for Turtle No. 16 is in the same spectral location, while for Turtle No. 71, it is at 490 nm. The uncertainty for the peak for Turtle No. 4 places it between about 460 and 480 nm. For Turtle No. 16, the blue peak could range between about 460 and 500 nm. The peak for Turtle No. 71 could be located anywhere between 460 and 510 nm. All three ranges overlap, so that it is not possible to positively state that these peaks are different even though they appear so. For Turtles 4 and 6, however, this 470 nm peak remains in the same spectrum locus despite changes in criterion, while for the same criterion increase the 490 nm peak of Turtle 71 shifts toward the blue end of the spectrum. Such a result might be expected if this peak was actually not a pigment absorption peak but was, instead, a difference peak, and if Turtle 71 was at a different state of adaptation from that of Turtles 4 and 16, so that as the intensity was increased, there was a change in the weighting of the receptor contributions to the difference channel, which change did not appear for Turtles 4 and 16.
Yellow Peaks.—In the first interval, Turtle 4 shows a sensitivity peak located at approximately 590 to 580 nm. Turtle 16 shows a peak located at, or around, 560 nm, while Turtle 71 shows a peak near 570 nm. The peak for Turtle 16 is broad and therefore the exact spectrum locus of the peak is ill-defined. The peaks for Turtles 4 and 71 are rather sharply delineated. Thus, it is not possible to say definitely that the yellow peak for Turtle 16 is not derived from the same channel as those for Turtles 4 and 71, as it could well be located at 570 nm. With an increase in criterion, these peaks for Turtles 4 and 71 show no appreciable shift in location, but the peak for No. 16 shifts toward the red. As before, this movement of spectrum locus with increased intensity implies a difference channel rather than information directly related to photopigment absorption.

Red Peaks.—Turtle 4 shows, for the first interval, a peak located at about 640 nm for the 50% criterion. Turtle 16, for the same interval and criterion, shows a peak sensitivity near 670 nm, while Turtle 71 shows two peaks, one located near 610 nm and one close to 690 nm.

The peak for Turtle 4 could range between perhaps 625 and 650 nm. The peak for Turtle 16 could range between 660 and 680 nm. Thus, it would appear that these are two distinct maxima. The 610 nm peak for Turtle 71 could be located anywhere between 595 and 615 nm. The 690 nm peak could be anywhere between 675 and 695 nm. It is therefore
possible that the 670 nm peak for Turtle 16 and the 690 nm peak for Turtle 71 are the same, but we are still left with three separate ranges in which peaks are located: 595-615 nm, 625-650 nm, and 660-695 nm. The only photopigment available in this range is Liebman and Granda's 620 nm pigment. No oil droplet with a cutoff of 595 nm or less could drive the maximum output of a 620 nm pigment receptor up to the 660-695 nm range, or even very far into the 625-650 nm range. Nor could any oil droplet with an absorption maximum at 475 nm drive the output maximum of a 620 nm pigment receptor downward to the 595-615 nm range. The only reasonable way to account for the 595-615 nm and 660-695 nm ranges is through the use of difference channels.

By a similar argument, one can show that no oil droplet can be used in conjunction with the 518 nm pigment to produce the yellow (560-580 nm) peak seen in all three animals. Thus, here too, is evidence of a difference channel.

In the blue, all three animals show peaks at around 470 nm. Once again, no oil droplet with a maximum absorption at 475 nm could move the 450 nm pigment maximum up to 470 nm. A difference channel is again indicated. It is interesting to note, however, that there is the shift from the 470 maximum at the 50% level for Turtle 71 toward a 450 maximum which would coincide with a pigment maximum. This shift would perhaps indicate greater weight being given to the 450 nm pigment information at higher intensities.
We deal, in this section, with data derived from the entirety of each run at each wavelength for each animal. The time taken for any given run could vary from 45 minutes to 1-1/2 hours, but usually averaged about 1-1/2 hours. Differences between these results and the results seen for the first 45-minute interval alone can be attributed to several factors. These factors are physiological (example: dark adaptation), behavioral (example: fatigue, stress, and other factors leading to behavioral alterations such as position preference or to a drop in response rate) and mathematical, since, if there is no significant change in the animal's physiology and/or behavior after the first 45-minute interval, then the statistics derived from the whole-run data will be more accurate than those derived only from the data of the first 45 minutes of each run.

Blue Peaks.--Turtle No. 4 shows a distinct peak in the blue located near 470 nm. This peak does not shift with change in criterion, although a small rise is seen at 430 nm over the same criterion range. Turtle 16 shows the same peak near 470 nm, but in this case, there is a shift toward 450 nm with criterion increase, a process hinted at in the first interval data. Turtle 71 shows a very marked peak in this region, located at about 450 nm. This peak appears to shift in the opposite direction from that of Turtle 16, with increase in criterion being displaced from 450 nm toward 470 nm. Such a result is not incompatible with a single difference channel in the 450-500 nm area.
with variable weights being applied to the differencing operation.

Yellow Peaks.—Turtle 4 shows only one peak in the yellow, located at, or near, 580 nm for the 50% criterion. With increase in criterion, this peak broadens out toward the shorter wavelengths, suggesting perhaps, an increased weighting being given to a shorter wavelength mechanism. Turtle 16 shows the same 570-580 nm peak and the same broadening or even partial shift toward shorter wavelengths with increase in criterion. The same result is seen for Turtle 71. Thus, a consistent trend is shown in all three animals: a sensitivity maximum located at about 570-580 nm broadens or shifts toward shorter wavelengths with increasing stimulus intensity. A difference model with variable differencing weights could explain this shift.

Red Peaks.—Turtle 4 shows a local maximum in the red, located at about 640 nm for all criteria. A possible shoulder located near 670 nm drops out with increase in criterion. Also, the relative sensitivities at 600 nm and 630 nm shows a reversal with changing criterion. Turtle 16 shows a peak located at, or near, 660 to 670 nm at the 50% level. This peak could correspond to the 670 nm shoulder seen in the sensitivity curve for Turtle 4. The same change with change in criterion occurs in both cases, in that the sensitivity near 670 nm decreases with increase in criterion. In the case of Turtle 16, this change results in a shift of the 670 nm peak towards 660 nm. An increase in sensitivity is seen at about 640 nm with increasing criterion. This might correspond to the broadening toward 630 nm of the
640 nm maximum seen in the sensitivity curve for Turtle 4, with increase in criterion. For Turtle 16, it is the relative sensitivities at 610 nm and 640 nm which interchange with changing criterion, not the sensitivities at 600 nm and 620 nm, as was the case for Turtle 4. The data points in this region are too widely separated, however, for any conclusions to be drawn from this difference. The spectral sensitivity curves for Turtle 71 show the very interesting property in the red of having two distinct peaks for the 50% criterion, one located at, or near, 630 nm, the other at about 690 nm. With increase in criterion, however, the 690 nm maximum declines rapidly, while the 630 nm maximum remains. Thus a shift occurs, producing a single maximum at 630 nm. For Turtle 71, it is the relative sensitivities at 590 nm and 610 nm which interchange with increase in criterion, but again, there are not enough data points for the accuracy needed for conclusions.

Conclusions

The spectral sensitivity data from this study show several maxima and numerous shoulders. The maxima occur in roughly four groups. One group consists of maxima located between 460 and 510 nm. Another group consists of maxima located between 560 and 580 nm. A third, of maxima located between 625 and 650 nm, and a fourth, of maxima located between 660 and 695 nm. These data are plotted in Figure 1, along with the data from the literature. The figure shows that, with the exception of the highest values, the ranges are
compatible with the ranges found in the literature. The main difference is in the spectral spread covered by the set of peaks. Of all studies to date done with *Pseudemys scripta elegans*, only the present study shows a range of possible maxima from 460 nm to close to 700 nm. The only other behavioral study (Armington, 1954), shows a range from 500 nm to 650 nm. Close examination of Armington's data indicates some possible inflection in the sensitivity curve in the 440-460 nm region, but no clear sensitivity peak. Armington's data also show a possible shoulder far in the red, at about 660-680 nm. Thus, the behavioral data are not incompatible between this and Armington's study. Yet, there are distinct differences, especially in the clarity of the local maxima shown by this study. These differences are most likely attributable to the different nature of the task. Speaking teleologically, it makes sense that the submerged turtle would use all the spectral information available to him in order to detect the location of the brightest area, which would correspond to an unobstructed surface to the water. Also, differences might be the result of the use of the upper part of the retina (lower visual field) in Armington's paradigm versus the use of the lower part of the retina (upper visual field) in this study.

Of much greater significance is the relation of the data from this study to the alternative models for color perception in the turtle. Figure 1 shows that no sensitivity maximum corresponds to any of the three photopigment maxima. Thus, no information directly related to quantum absorption by a photopigment is used by
the turtle in performing this task. Even using oil droplet filters, only the lowest portion of the 620 to 640 nm sensitivity maximum can be accounted for. Should more evidence in favor of the difference model be needed, the shifting of the maxima with change in criterion provides it. Photon absorption at the maxima can be increased or decreased with changing intensity, but only a differencing operation (or other interaction of the pigment systems) can account for shifts in maxima along the spectrum axis.

In conclusion, then, air reinforcement as a technique for behavioral determination of spectral sensitivity in the turtle has been proven to be a workable technique. The results obtained are of considerable interest in considering the alternative models of color vision, namely, the oil droplet-pigment model versus the difference channel model.
At the outset of the experiment, it was decided that the experimental paradigm should be automated as fully as possible, so as to avoid experimenter bias and variability. To this end, a special purpose hybrid solid-state and relay controller was designed and constructed, as were the electromechanical input-output devices, which coupled the controller to the test apparatus and the recorders. The experimental paradigm is described in Chapter II. The operation of this paradigm included the following steps: (1) presentation of the stimulus; (2) recording of stimulus position; (3) detection of response; (4) evaluation of response correctness; (5) delivery of reinforcement; (6) removal of stimulus; (7) alteration of stimulus intensity; (8) decision as to position of next stimulus presentation; (9) timing to end of 15-second cycle; and, if necessary (10) delaying of next presentation until turtle's head is withdrawn. In addition, manual control of the various processes was decided to be essential, and was included, since such control allowed modification of the paradigm as circumstances dictated. The operations are summarized in Figure 22.

The above steps were implemented in various ways. The stimulus originated in a Bausch and Lomb Hi-Intensity Grating Monochromator, Model 33-86-25-02, with a tungsten light source. A shutter
FIGURE 22

left turtle pickup

right turtle pickup

stimulus left

stimulus right

pickup inhibit

evaluate response and time cycle

direct wedge out

direct wedge in

reinforce

advance tracking program

record wedge position

change wedge position

record wedge position

direct wedge out

change wedge position

record wedge position

record behavior

change pulse generator

wedge clutch timer

shutter

randomizer

mirror motor

FIGURE 22
was installed between the light source and the grating housing of the monochromator. Light from the monochromator's output slit passed through a series of lenses and mirrors (see Figure 24) to finally reach the translucent diffusing Plexiglas target located at the front of the response chamber. The stimulus position was detected by means of limit switches which were activated by the stimulus interchange mirror (Figure 23).

The response was detected by the interruption of the beam passing from an RCA 40736R GeAs photoemissive diode (emission $\lambda_{\text{max}} = 930$ nm, 40 nm half maximum bandwidth, to a GE L14A502 phototransistor on the opposite side of the response chamber. The signal which occurred upon the breaking of the beam by the turtle closed a relay. The output behavioral recorder then recorded, for each response, the stimulus position and the response position. A relay logic circuit compared position of stimulus and response, and evaluated the response. For either a correct or an incorrect response, a 15-second solid-state cycle timer was started, and the shutter was commanded to occlude the stimulus. For a correct response, a solenoid valve system switched the reinforcement chamber roof inlet from the atmosphere to the 1.5 psi gas line. This forced breathing gas into the chamber and lowered the water level in the chamber. Simultaneously, the signal to the solenoid valve system overrode the occlusion command to the shutter, so that the stimulus remained on throughout the reinforcement period. Immediately, upon the occasion of a response, the direction of
OPTICAL SYSTEM

FIGURE 23
rotation of a Hurst PC-DA 10 RPM synchronous motor, which drove the variable density wedge, was or was not altered, depending upon the evaluation of the response. After a fixed delay of 1.0 second to allow inertia to be overcome if the rotation was to be reversed, the clutch was engaged and the wedge was moved. The clutch remained engaged for a period, controlled by a solid-state timer whose own period was controlled by the tracking program. Thus, for the first few responses the wedge moved for a longer period than for subsequent responses.

Another Hurst PC-DA synchronous motor was used to control the stimulus position at the next presentation by controlling the position of the rotating mirror. At the end of the 15-second cycle, a 0.05 second pulse was generated, and sent through the contacts of a relay. This relay was independently cycling at 1 Hz, and was carefully set to a 50% duty cycle (0.5 sec. closed, 0.5 sec. open). Thus, the pulse had a 50% chance of passing the relay. If the pulse passed, a circuit was closed and the motor driven through one cycle, causing the mirror to rotate to the alternate position. The rotation cycle controlled by a limit switch, was fairly exact, but for absolute accuracy in positioning the mirror, a 90° Geneva gear was employed.

The signal occasioned by the breaking of the infrared beam passed through and then triggered a delay-on-operate relay, so that the initial response was shaped into a pulse. This relay was held open by either the 15-second cycle timers, or the mirror motor while
it was cycling, or the response pickup itself, or any combination of
these sources. Thus the response switches were inactivated through-
out the normal 15-second cycle, and beyond if the animal did not
withdraw before the end of the 15 seconds. The shutter was also
driven by the same signal which drove the response switch cutoff
relay, thus preventing premature presentation of the stimulus.

The system could run in either the automatic mode as described
above, or in the manual mode. In the manual mode, the automatic se-
quencing was not present. Front panel controls allowed manual con-
trol over shutter position, stimulus position, reinforcement, and
wedge (stimulus intensity). In addition, parts of the tracking pro-
gram could be manually bypassed. The tracking program was set man-
ually by means of a 20 x 10 crossbar switch, which allowed selection
of any of 10 intensity increment sizes for each one of the first 20
sequential responses; the 21st and following responses kept to the
same increment as the 20th.

In addition to pure manual, or pure automatic operation, the
system provided for some manual modification of the automatic opera-
tion. For example, in the reshaping procedure, the system might be
run in the automatic mode, but with the stimulus intensity control
decoupled, so as to keep the stimulus at a constant intensity, while
the stimulus changeover pulse was set to occur only if the response
preceding was correct (see Chapter II).
The optical equipment and light pathways are shown in Figure 23. The monochromator was a Bausch and Lomb Hi-Intensity Grating Monochromator, Model 33-86-25-02, with tungsten light source. The continuously variable wedge (OD 0-2) was a Kodak photographic emulsion on acetate type of Wratten filter. The fixed density filters were Wratten Type 96 2-inch squares in glass. Kodak Wratten 29 and Wratten 47 3-in. squares in acetate were used to eliminate scattered light from the monochromator.

For calibration purposes the monochromator's output was defined as the measured output with the neutral wedge in place and set to the minimum density (highest transmission value) which was to be used in the experiments to follow. Calibration was carried out by use of a Weston barrier layer cell whose current output was transduced to voltage by a solid state, zero impedance, linear operational amplifier circuit. The voltage was then read by a Fluke 845A B High Impedance Voltmeter. A Keithley digital voltmeter was often used to facilitate reading of the Fluke meter's output. The barrier layer cell was calibrated in Dr. Ingling's standards laboratory, and its spectral sensitivity curve is given in Figure 24.

The barrier layer cell was first placed inside the aquarium with no target screen in place so that the light beam passed through
NORMALIZED SPECTRAL SENSITIVITY OF BARRIER LAYER CELL
the optical system and emerged via the right hand channel, then passed through the glass of the aquarium to strike the cell. The cell was held normal to the beam and positioned so that the maximum sensitive area (2" diameter) was maximally covered by the light beam (this maximum coverage was about 95%). The cell was positioned along the axis of the beam so that the maximum reading did not exceed 1 volt. Readings of output voltage were made every ten nanometers from 400 to 700 nm, both ascending and descending the spectrum. Eight such sets of readings were made. The averages are shown in Figure 25. Note the curious bumps on the output curve, whose envelope otherwise corresponds to the manufacturer's curve. The notching is due to some spectrally nonlinear optical element, probably the polished aluminum mirrors used.

Using this unfiltered spectral output curve, the transmission of the Wratten scattered-light filters could be calculated. The reading with one of these filters in place was divided by the product of the barrier cell's normalized sensitivity and the normalized unfiltered spectral output for each wavelength measured. Results were then normalized, and are shown in Figures 26 and 27.

Fixed optical density filters were calibrated at each wavelength between 400 and 700 nanometers by taking a reading without the filter in place, and then with the filter in place. The ratio of these two readings was then calculated. Ratios at each wavelength were averaged over four spectral scans (alternating ascending and descending), and then converted to logarithms. The results may be
Figure 25

Normalized Power Spectrum (unfiltered)
NORMALIZED TRANSMISSION SPECTRUM

WRATTEN 29

FIGURE 26
NORMALIZED TRANSMISSION SPECTRUM

WRATTEN 47

FIGURE 27
seen in Figure 28. Also shown in this figure are the means and standard deviations for the readings. Note that there is more variability for the OD2 than for the OD1 filter. Also, both filters showed an increase in density at the blue end of the spectrum. Much of this variability could be attributed to the fact that for the OD2 filter across the spectrum, and for both filters in the blue, the signal out of the barrier layer cell was weak (see Figure 24), and thus variability due to a lowered signal to noise ratio became more apparent. It was decided to use the mean density values of 2.1441 O.D. for the nominal ND2 filter, and 1.0880 O.D. for the nominal ND1 filter. A spot check of O.D. across the spectrum, performed later on a Cary spectrophotometer revealed that the nominal ND2 filter had a maximum O.D. of 2.30 at 400 nm, while the nominal ND1 filter had a maximum OD of 1.32, also at 400 nm. Thus a maximum error of 0.16 OD units and 0.22 OD units were introduced for the nominal ND2 and ND1 filters, respectively. Since intensities were grouped in intervals of 0.2 log units for data processing, this error is not excessive. The approximations employed in fitting a curve to the data points also lessened the effects of this error.

Calibration of the variable density wedge was done by a procedure similar to that employed for the fixed density filters. For the variable wedge, however, spectral scans of optical density were made at fixed intervals along the wedge's travel range. The wedge's position was detected by a potentiometer mechanically coupled to it so that the wedge's position could be read out in millivolts. The lower end (maximum transmission) was set to 0 mV. Spectral readings were taken at each 10 mV interval up
NEUTRAL DENSITY FILTER CALIBRATION CURVES

FIGURE 28
to the maximum of 70 mV and then the log ratio, or increase in optical density for each increment was calculated. The results appear in Figure 29. Mean values were used, since this introduced an error of less than 0.05 OD., which was smaller than the positioning error. This allowed using a single curve of mean density value versus position for 400-600 nm as the calibration curve for the wedge. Because of the pronounced increase in optical density at higher wavelengths, a second calibration curve for wedge position versus optical density was calculated for those wavelengths. These calibration curves appear in Figures 30 and 31.

A check was made of the equality of the two light channels by repeating the nonfiltered output calibration described above, now on the left-hand channel, and comparing the normalized results for the two channels. The ratios appear in Table 5. The ratios are all very close to 1.0 and show no consistent shift with spectrum locus.

As a final calibration, four scans were made using the Wratten scattered-light filters as indicated (i.e., in their regions of maximum transmission) and placing the barrier layer cell behind the diffusing plexiglas target. Thus, this calibration curve represents the power spectrum as viewed by the turtle. This spectrum is shown normalized in Figure 32, and converted to log relative quantum flux in Figure 33. To obtain this latter figure, the normalized values of the power spectrum of Figure 32 were multiplied by the wavelength at which that figure occurred and the product, or relative quantal flux, was converted to log $\log_{10}$ form.
FIGURE 29

OPTICAL DENSITY

NEUTRAL DENSITY WEDGE CALIBRATION

WAVELENGTH (nm.)

0.480

0.400

0.300

0.200

0.100

0-10 mV

10-20 mV

20-30 mV

30-40 mV

40-50 mV

50-60 mV

WAVELENGTH (nm.)
OPTICAL DENSITY - NEUTRAL DENSITY WEDGE CALIBRATION CURVE
(for 400 to 600 nm.)

FIGURE 30
NEUTRAL DENSITY WEDGE CALIBRATION CURVE

(for 600 to 700 nm.)

FIGURE 31
<table>
<thead>
<tr>
<th>WAVELENGTH</th>
<th>CHANNEL RIGHT</th>
<th>CHANNEL LEFT</th>
<th>RATIO</th>
<th>WAVELENGTH</th>
<th>CHANNEL RIGHT</th>
<th>CHANNEL LEFT</th>
<th>RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>0.0406</td>
<td>0.0444</td>
<td>1.0935</td>
<td>560</td>
<td>0.5789</td>
<td>0.5908</td>
<td>1.0205</td>
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<tr>
<td>410</td>
<td>0.0681</td>
<td>0.0743</td>
<td>1.0910</td>
<td>570</td>
<td>0.5286</td>
<td>0.6614</td>
<td>1.2512</td>
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<tr>
<td>420</td>
<td>0.0645</td>
<td>0.0871</td>
<td>1.0307</td>
<td>580</td>
<td>0.6764</td>
<td>0.7521</td>
<td>1.1119</td>
</tr>
<tr>
<td>430</td>
<td>0.1190</td>
<td>0.1322</td>
<td>1.1109</td>
<td>590</td>
<td>0.8258</td>
<td>0.7822</td>
<td>0.9472</td>
</tr>
<tr>
<td>440</td>
<td>0.1654</td>
<td>0.1716</td>
<td>1.0374</td>
<td>600</td>
<td>0.8215</td>
<td>0.7545</td>
<td>0.9164</td>
</tr>
<tr>
<td>450</td>
<td>0.1702</td>
<td>0.1708</td>
<td>0.9962</td>
<td>610</td>
<td>0.7294</td>
<td>0.7870</td>
<td>1.0789</td>
</tr>
<tr>
<td>460</td>
<td>0.2133</td>
<td>0.2536</td>
<td>1.1861</td>
<td>620</td>
<td>0.7140</td>
<td>0.7989</td>
<td>1.1189</td>
</tr>
<tr>
<td>470</td>
<td>0.2901</td>
<td>0.2904</td>
<td>1.0010</td>
<td>630</td>
<td>0.7295</td>
<td>0.8094</td>
<td>1.1095</td>
</tr>
<tr>
<td>480</td>
<td>0.2698</td>
<td>0.2573</td>
<td>0.9536</td>
<td>640</td>
<td>0.6256</td>
<td>0.8363</td>
<td>1.0129</td>
</tr>
<tr>
<td>490</td>
<td>0.2532</td>
<td>0.3273</td>
<td>1.2435</td>
<td>650</td>
<td>0.9038</td>
<td>0.8230</td>
<td>0.9105</td>
</tr>
<tr>
<td>500</td>
<td>0.3581</td>
<td>0.4181</td>
<td>1.0772</td>
<td>660</td>
<td>0.6586</td>
<td>0.8599</td>
<td>1.0015</td>
</tr>
<tr>
<td>510</td>
<td>0.4230</td>
<td>0.3859</td>
<td>1.0961</td>
<td>670</td>
<td>0.8182</td>
<td>0.9283</td>
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</tr>
<tr>
<td>520</td>
<td>0.3705</td>
<td>0.4059</td>
<td>1.0955</td>
<td>680</td>
<td>0.9322</td>
<td>1.0000</td>
<td>1.0727</td>
</tr>
<tr>
<td>530</td>
<td>0.4229</td>
<td>0.5438</td>
<td>1.2858</td>
<td>690</td>
<td>1.0000</td>
<td>0.9651</td>
<td>0.9651</td>
</tr>
<tr>
<td>540</td>
<td>0.6447</td>
<td>0.6607</td>
<td>1.0248</td>
<td>700</td>
<td>0.9699</td>
<td>0.9397</td>
<td>0.9688</td>
</tr>
<tr>
<td>550</td>
<td>0.6808</td>
<td>0.6058</td>
<td>0.8898</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 5**

AVERAGE RATIO 1.0532 ± 0.0997
NORMALIZED POWER SPECTRUM
(viewed through Wrattens and diffuser)

FIGURE 32
LOG RELATIVE QUANTUM FLUX

(viewed spectrum)

FIGURE 33
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