Retinal disease preferentially affects the sensitivity of the "blue"-cone pathways. This vulnerability to disease may be due, in part, to a more limited response range. A psychophysical technique, the probe-flash paradigm, was used to test this hypothesis. The data suggest that the S-cone pathways have a more limited response range than the L-cone pathways. Explanations for blue-cone vulnerability are discussed in the context of this finding. Invest Ophthalmol Vis Sci 25:864-867, 1984

One of the features of the short-wavelength sensitive (S) or "blue"-cone pathways is their apparent vulnerability to retinal disease. For example, during the early stages of retinitis pigmentosa or diabetic retinopathy, there is a preferential loss of blue-cone sensitivity.\(^1\)\(^{-3}\) We propose that this apparent vulnerability measured psychophysically can be attributed, in part, to the limited response range of the blue-cone pathways.

In this paper, we use a psychophysical technique, the probe-flash paradigm, to assess the relative response ranges of the S- and L-cone (long-wavelength sensitive) pathways. The data support the hypothesis of a more limited response range, and in the discussion we relate this explanation for blue-cone vulnerability to other explanations found in the literature.

**Materials and Methods.** The probe-flash paradigm requires the observer to detect the presence of a small, brief light—the probe—upon flashed backgrounds. Threshold intensity for probe detection is determined for a range of flash intensities. A steady adapting field, continuously present, controls the state of adaptation. Figure 1 displays the parameters in this experiment. The circular probe, 1 degree in diameter and 40 msec in duration, was presented simultaneously with the onset of the flash, which was larger (2 degrees in diameter) and longer (500 msec). Special precautions were taken in order to isolate the S-cone system. The probe and the flash were blue (430 nm), and the adapting field was orange (600 nm). A complete tvi curve was measured for the 430-nm probe on the 600-nm adapting field. The conditions were similar to those used by Stiles, and the tvi curve showed characteristic pi-1 and pi-3 branches. The L-cone system is easier to isolate. Here we used a probe and a flash that were red (640 nm) and an adapting field that was "white" (unfiltered tungsten). Isolation of the S- and L-cone systems at these adapting levels was confirmed with spectral sensitivities. A method of limits procedure was used and a three-channel Maxwellian view system provided the light stimulation (for details see Finkelstein and Hood\(^4\)).

**Results.** Probe-flash data for two observers are shown in Figure 2. The open symbols represent the 640-nm (L-cone pathway) condition, and the closed symbols the 430-nm (S-cone pathway) condition. This figure includes data collected at different adapting intensities. First consider the circles, which represent data collected at relatively low-adapting intensities. Here, the white-adapting field used in the 640-nm condition was set at 1.7 log td, and the orange-adapting field used in the 430-nm condition was set at 2.7 log td. The probe-flash curves are all positioned with respect to threshold; that is, the value 0 along the abscissa and ordinate indicates flash and probe threshold when each is presented alone. The important aspect of the data is the effectiveness of the flashes at raising probe threshold. For the 640-nm condition, probe threshold remains unchanged until the flash threshold is exceeded by at least 0.4 log unit. The lower range of flashes is ineffective in raising probe threshold. On the other hand, for the 430-nm condition, probe threshold is increased by flash intensities near threshold.

Conditions in the present experiment were chosen so that the S- and L-cone pathways were approximately equally adapted. By this we mean that the two adapting levels raised the threshold for the L- and S-cone pathways (pi-5 and pi-1) an approximately equal amount above absolute threshold. The shape of the probe-flash curve is known to be affected by the intensity of the adapting field.\(^5\)\(^6\) We know that the data for the L-cone pathway condition at high-adapting intensities will ap-
peared more like the 430-nm data. Although this effect of adapting intensity is too small to affect our conclusion, we sought to ensure that the difference in results for the two conditions was not due to the particular adapting levels used. We collected data for the 640-nm condition at a higher adapting intensity (4.0 log td). Data for the 430-nm condition also were collected at a higher adapting intensity (4.3 log td). The probe-flash data for the new conditions are shown as the triangles in Figure 2. At both adapting levels, low-flash values raise probe threshold for the S-cone pathways, while they fail to raise it for the L-cone pathways. Large changes in the level of adaptation appear to have little influence on the difference in S- and L-cone pathway measurements.*

Discussion. To relate the probe-flash data to the response range of a pathway requires several assumptions. These assumptions and the general approach of relating psychophysical data to response-intensity functions of the visual system are discussed in great detail in the literature. A key assumption is that the probe is detected when the response to the probe plus flash exceeds the response to the flash by some constant or criterion amount. We further assume that this constant amount is the same for both pathways. The question of interest here is, given these assumptions, what do the data suggest about the relative response ranges of the S- and L-cone pathways?

If the S- and L-cone pathways have identical response functions, then identical probe-flash curves should result; the probe-flash curves shown in Figure 2 should be superimposed. Clearly they are not. In fact, the 640-nm data fall over 0.4 log unit to the right of the 430-nm data. The degree to which the two sets of data fail to coincide is a measure of the difference in response range between the S- and L-cone pathways. The data suggest that the response range of the S-cone pathways is more limited than that of the L-cone pathways.*

* Virtually identical results were obtained with a 5-degree flash.
† An indistinguishable explanation can be based on a difference in detection criteria. The model relating the probe-flash data to the response functions in Figure 3A does not distinguish between changes in \( R_{\text{max}} \) and changes in detection criteria \( \delta \). (Notice that equation 2 in Hood et al. has the parameter \( \delta = b/R_{\text{max}} \).) We could assume that the response range of the S- and L-cone pathways are identical but that a larger response is needed for detection in the normal S-cone pathways. The conclusions are the same. An equivalent decrease in the responsiveness of all cone pathways to retinal disease, as shown in Figure 3B, will lead to larger disease-related changes in threshold in the S-cone pathways. We focus on the response range in this paper because it is easy to conceptualize. It makes little difference whether we assume a difference in detection criteria or response range. What is important is that there are differences in the normal system that can produce the apparent vulnerability of S-cones to retinal disease.

Fig. 1. Spatial and temporal paradigm (see text for details).

Fig. 2. Log threshold intensity of a probe presented upon a series of flashes (closed symbols: 430-nm probe/430-nm flash condition; open symbols: 640-nm probe/640-nm flash condition). Two levels of steady adaptation were used for the 430-nm condition: 2.7 (closed circles) and 4.3 (closed triangles) log td. Similarly, two levels of steady "white" (unfiltered tungsten) adaptation were used for the 640-nm condition: 1.7 (open circles) and 4.0 (open triangles) log td. All intensities are expressed relative to threshold; that is, the value 0 along the abscissa and ordinate indicates flash and probe threshold, respectively. Each data point is the median of at least four daily sessions. The horizontal dashed lines serve as a reference for the effectiveness of the flash at raising probe threshold.
chophysical studies. The inferred difference in the cone vulnerability to retinal disease comes from psychophysical studies by Greenstein, Hood, and Campbell on retinal disease. This inferred difference between the pathways is schematized in Figure 3A; the response of each pathway is shown as a function of flash intensity. Notice that the response range [R max] is smaller for the S-cone pathways. The dotted curves show the response function after retinal disease. Here the difference in the normal L- and S-cone pathway thresholds can yield a consistent with a difference in R max or detection criterion (see Greenstein and Hood and Greenstein).

The only difference between the curves is R max. R max is smaller for the S-cone pathways. The same value of σ was used for both pathways. In reality, the S-cone pathways also may have a larger σ. If true, then the difference in the normal L- and S-cone pathway thresholds measured psychophysically is due to both a difference in σ and R max.

In either case, the nature of the arguments in the text remains the same. The data are plotted in a form which adjusts for a difference in σ. If the only difference between pathways was a difference in σ, the probe-flash data in Figure 2 would coincide. The misfits are consistent with a difference in R max or detection criterion (see Hood and Greenstein and ). If L-receptor damage increases responsiveness of both pathways, the same value of R max is used for both pathways. The dotted curves show the response function after retinal disease. Here retinal damage is shown decreasing the responsiveness of both pathways by the same multiplicative factor at all intensities (see Greenstein et al). Notice that the response range [R max] is smaller for the S-cone pathway. Other forms of decreased responsiveness (eg, an even greater loss at higher intensities than at low intensities) can yield qualitatively similar results.

This inferred difference between the pathways is schematized in Figure 3A; the response of each pathway is shown as a function of flash intensity. Notice that the response range [R max] is smaller for the S-cone pathways. (For more details see the figure caption).

**Blue cone vulnerability:** The evidence for blue (S) cone vulnerability to retinal disease comes from psychophysical studies. The inferred difference in the response range (Fig. 3A) will affect the degree of vulnerability assessed psychophysically. Assume that retinal disease does not differentially affect the S-cone pathways, but rather decreases the responsiveness of all pathways equally. This is shown in Figure 3B as an equal decrease in the log response of both S- and L-cone pathways at all intensities. (We chose this form of response reduction because of the results of a study by Greenstein, Hood, and Campbell on retinal disease and changes in the L-cone pathway. The exact form of response reduction is not critical; see figure caption.) Because of the difference in the response functions, an equivalent change in log R max affects the threshold of the S-cone pathways, as measured psychophysically, far more than that of the L-cone pathways. The difference in the response range makes the S-cones appear more vulnerable to retinal disease.

In what additional ways might the S-cones be more vulnerable? The two most common explanations found in the literature assume either that the S-cones are scarce, or that they are more fragile. The fragile hypothesis implies that something in the S-cones' biophysical makeup renders them more susceptible to damage by light, chemicals, or retinal disease. The evidence for the S-cones being more fragile in the face of retinal disease is rather indirectly provided by their vulnerability to light damage and their selectivity for procion yellow. The limited response range indicated by the present experiment makes the S-cones functionally more vulnerable even in the absence of any other differences. If they are more fragile as well, then this will simply exaggerate the differences. (To see this, picture Figure 3B redrawn with a larger decrease in responsiveness for the S-cone pathways.)

It is well accepted that L- and M-receptors are more numerous than S-receptors; S-receptors are said to be scarce. Scarcity, per se, does not predict differential vulnerability, although it often is taken as if it does. Scarcity may be one of the factors contributing to the functionally restricted response range of the S-cone pathways. It simply does not follow that because there are fewer S-cones, the S-cone pathways will be more vulnerable to retinal disease. For example, one can imagine a linear system in which losing one-half of the receptors will lead to a 0.3 log unit sensitivity loss independent of how many receptors there were at the start. There are, however, nonlinear models that can be generated to explain the selective vulnerability of the S-cones based, in part, on the fact that they are scarce. Our hypothesized difference in the response range of the S-cone pathways is consistent with some of these models. Consequently, our results can be viewed as constraining explanations based on scarce receptors as opposed to being incompatible with them.

In conclusion, we have provided evidence that the S-cone pathways have a more limited response range than the L-cone pathways. A limited response range will have the effect of making the S-cone pathways more vulnerable to disease. This finding also serves to constrain other explanations of S-cone vulnerability.

**Key words:** blue cone, psychophysics, response range, retinal disease
Cyclosporine (CsA) has been shown to be effective in preventing S-antigen (S-Ag) induced experimental autoimmune uveitis (EAU) in Lewis rats. Alterations in the humoral immune responses associated with CsA therapy are illustrated by lower peak and delayed production of circulating anti-S-Ag antibodies in a proportional relationship to the dose of CsA in EAU. Circulating immune complexes were not detected in rats with EAU or in rats treated with CsA and without disease. These findings further support the important role of T-cells in EAU and further demonstrate the effect of CsA on helper T-cells. The kinetics of antibody production by S-Ag-immunized rats appears altered by CsA. Invest Ophthalmol Vis Sci 19:248, 1978.

Bovine S-Antigen (S-Ag), a soluble retinal protein, can induce experimental autoimmune uveitis (EAU), which can affect both the anterior and posterior ocular segments in lower mammals. The histopathology showed intensive acute and chronic inflammation in anterior chamber, choroid, retina, and vitreous.1,2 The induction and appearance of EAU can be inhibited successfully by treating the animals with Cyclosporine (CsA).2 The alterations of the cellular immune response induced in this inflammatory model with CsA include a modulation in the in vitro proliferative responses of lymphocytes from the lymph nodes and peripheral blood of S-Ag-immunized animals and the morphologic absence of active blastogenesis in the draining lymph nodes of S-Ag-immunized rats.1 In this report, we have examined the effects of CsA on the B-cell response in EAU, and whether circulating immune complexes could be correlated with the protected or diseased state.

**Materials and Methods.** Female Lewis rats, 150–200 g in weight, were used for all experiments. All rats in these studies were immunized with a single footpad injection of 30 μg of bovine S-Ag emulsified (1:1) in complete Freund's adjuvant (CFA, GIBCO; Grand Island, NY) supplemented with H37 RA Mycobacterium tuberculosis (Difco; Detroit, MI). The bovine S-Ag was prepared as reported elsewhere1 and kindly provided to us by Dr. Waldon B. Wacker (University of Kentucky, Lexington, KY). Sera of all animals were obtained at weekly intervals after immunization as indicated in the text. CsA, a gift from Sandoz (Basel, Switzerland) was dissolved in pure olive oil. Twenty treated rats were divided into three groups, and received 3 mg, 1 mg, or 0.1 mg CsA daily by subcutaneous injections into the thigh beginning on day 0 of immunization until killed. Twelve control animals received daily olive oil injections alone (Table 1).

References