this type in some old MS plaques is further evidence that the putative antigen may be continuously expressed in such lesions (*5*). Also, depending on the efficiency of antigen trapping by this tissue, it is possible that little or no antigen escapes from this region to induce systemic immunity. The present findings further suggest that the antigen may be processed and exhibited to passing lymphocytes by perivascular macrophages in a manner similar to that proposed for lymph nodes where B lymphocytes are thought to be arrested at this point in their circulation by contact with macrophage-processed, membrane-associated antigen to develop locally into antibody-forming plasma cells (*13*).

The thin-walled channels observed in perivascular spaces in unaffected CNS tissue in each of the five patients studied were indistinguishable from lymphatic capillaries in other tissues in terms of both their structure and contents (*10, 14, 15*). The presence of such channels is not easily reconciled with the traditional view that the CNS lacks lymphatic vessels (*10, 16–19*) and that the perivascular spaces represent cul de sacs or backwaters of the subarachnoid space whose chief function is to act as a protective cushion between the expansile blood vessels and the parenchyma (*20–22*). The present findings are more in keeping with the view of Harriman and other neuropathologists that these spaces serve the same function in the CNS as lymphatic vessels serve in other tissues (*23, 24*). While this is not to say that the CNS has a lymphatic drainage which is equivalent to that in other tissues (*25*), it is not unreasonable to view the presence of lymphocyte-containing channels in the perivascular spaces in the CNS as evidence that lymphocytes normally circulate through these channels, possibly in the same manner and in the same numbers as lymphocytes circulate in other tissues, and that this may constitute the basis of immunological surveillance in the CNS.

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8. The CNS tissue in four of the five patients was fixed within 20 minutes of death by perfusion in situ with 3 percent glutaraldehyde (*7*). In the fifth case (MS), glutaraldehyde-fixed subcortical white matter obtained at biopsy was studied. The tissue was postfixed in Dalton's solution, embedded in Spurr's medium, and prepared for light and electron microscopy. Epoxy-embedded biopsy tissue and immersion-fixed CNS tissue obtained at routine autopsy were examined in six further MS cases but in none were perivascular structures sufficiently well-fixed for the purposes of the study.
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18. Previous descriptions of the normal histological appearance of the perivascular spaces in the CNS (*19, 21, 24*) refer chiefly to the presence of flattened mesenchymal or reticuloendothelial cells. Some authors also describe occasional phagocytic cells, but lymphocytes are rarely mentioned (*24*). With reticular stains, and in studies in which the perivascular spaces have been filled with India ink, it has been reported that the flattened cells line both sides of the perivascular space, and that this lining endothelium is continuous with the arachnoid and pia where the perivascular space opens into the subarachnoid space. Electron microscopical studies (mostly dealing with the cerebral cortex in rodents) also note that the perivascular spaces normally contain flattened cells—described as flat connective tissue cells or fibroblast-like cells—which ultrastructurally resemble piaarachnoid cells, some collagen, and occasional phagocytic cells "of pial origin." Lymphocytes seem not to have been described, and several of these studies report that no continuous endothelial lining could be demonstrated [see (*17, 22*); E. Nelson, K. Blinzinger, H. Hager, *Neurology* **11**, 285 (1961); E. G. Jones, *J. Anat.* **106**, 507 (1970); B. van Deurs, *J. Ultrastruct. Res.* **56**, 65 (1976)].
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25. The view that the CNS lacks lymphatic vessels (vessels through which tissue fluid and cells return toward the blood stream) is based not only on the fact that such vessels have not been seen in the brain by routine histological methods, but also because multiple injections into the brain substance of India ink or other suspensions of particulate material fail to show such vessels (*15*, p. 15). When particulate material is introduced in this way into the brain parenchyma, it does not pass freely into the perivascular spaces but accumulates outside the glial-limiting membrane in the artifact space of His. When the injections are made into the subarachnoid space, however, the particulate material can be induced to enter the perivascular spaces, and so pass deeply into the brain, if the brain is simultaneously dehydrated (by hypertonic intravenous infusions) (*19*). Regarding draining to regional lymph nodes, while most of the draining tissue (spinal) fluid enters the blood stream without passing first through a lymph node, as occurs in other tissues (*15*, p. 518), some passes to cervical, paravertebral, and mesenteric lymph nodes via lymphatics in the olfactory mucosa and along vessels and nerves entering and leaving the cranial and spinal cavities [see (*16, 20*); J. B. Brierley and E. J. Field, *J. Anat.* **82**, 153 (1948); D. H. M. Woollam and J. W. Millen, *Lancet* **1953-I**, 364 (1953); Yoffey and Courtice (*15*, p. 309)].
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Red-Absorbing Visual Pigment of Butterflies

Abstract. *Noninvasive photochemical and physiological experiments with intact butterflies of 17 species showed that nine species have a rhodopsin absorbing maximally at 610 nanometers, contained in retinular cells that are maximally sensitive at 610 nanometers. This is the longest-wavelength visual pigment known for an invertebrate. Eight species of butterflies lack the 610-nanometers rhodopsin. All species possess a rhodopsin absorbing maximally in the green region of the spectrum.*

Compared with humans, most invertebrates have very low sensitivity and poor color discrimination in the red and orange regions of the spectrum. However, judging from behavioral studies (*1*) some butterfly species are an exception. The physiological basis for this sensitivity to long wavelengths is still unknown because the methods (*2–4*) that have been applied to the problem have serious weaknesses. For example, the electrical mass response of an eye, the electroretinogram (ERG), can not in general be used to infer the spectral sensitivity of

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the different spectral types of receptors in a mixed retina (*5, 6*). Furthermore, ERG peaks in the red may be a consequence of spectral filtering by pigment granules (*6*) or tapeta (*3, 4, 7*). Electrophysiological measurements from visual interneurons can exhibit spurious peaks of sensitivity because of neural interactions of signals that originate from receptors of dissimilar spectral type (*8*). Microspectrophotometry and intracellular electrophysiology, the methods of choice for determining the spectral properties of photoreceptors, have not contributed to the solution of the problem because butterflies are such difficult subjects. I now present results of the application of newly developed, noninvasive techniques that exploit the fact that most butterfly eyes exhibit both eyeshine and a pupillary response (*7, 9–13*). Evidence from combined photochemical and physiological studies of the eyes of intact, living butterflies indicates that the retinas of some species possess a red-absorbing rhodopsin (*14*) that is maximally absorbing at about 610 nm, which is contained in retinular cells that are maximally sensitive at 610 nm.

Photochemical measurements were made by immobilizing a butterfly with wax and mounting on the universal stage of an incident-light microspectrophotometer, focusing on the deep pseudopupil (*10*), and measuring the reflectance spectrum with dim monochromatic flashes (fewer than 10^4 quanta per ommatidium per flash) that neither trigger the pupillary response nor alter the photochemical state. The spectrum of the dark-adapted eye is compared with spectra taken after treatment with actinic illumination (*11–15*). The logarithm of the ratio of reflectances before and several minutes after actinic illumination is a photochemical absorbance difference for light that propagates through the entire rhabdom, strikes the tracheolar tapetum and propagates back through the rhabdom and out of the eye. The difference spectra shown in Fig. 1A demonstrate the existence of a red-absorbing rhodopsin and its conversion by red light to a metarhodopsin that absorbs maximally in the green region of the spectrum. The isosbestic point for the conversion is at about 560 nm.

The negative peak of the difference spectrum relaxes with time in the dark (compare curves *A* through *D*) with a half-time that can be as short as 15 minutes or as long as 3 hours, depending on the actinic protocol. This result supports the work of Stavenga (*15*) on the dark-regeneration of invertebrate rhodopsins. Contributions to the difference spectra from movement of pigment granules or changes in tapetal reflectance is negligible (*16*). The relaxation of the negative peak is not caused by the dim flashes used to measure the reflectance spectrum, for dark-regeneration to within 0.1 log unit of baseline is routinely observed in many kinds of experiments with all species studied.

The change from curve *D* to curve *X* (Fig. 1A), caused by 500 nm actinic illumination, demonstrates photo-regeneration (*17*) of red-absorbing rhodopsin from green-absorbing metarhodopsin. Figure 1B demonstrates the presence of red-absorbing rhodopsin in five other butterfly species.

Spectral sensitivity functions for the red-sensitive receptors were obtained by measuring the action spectrum of their pupillary response (*12, 18*). This is accomplished by continuously illuminating a selected, localized region of the eye with a deep red adapting beam of an intensity chosen so that the pupillary granules of the red-sensitive receptors are gathered next to the rhabdom while granules of other spectral types of photoreceptor cells are dispersed (*13, 19*). Monochromatic flashes from the second, superimposed beam of the microspectrophotometer are periodically delivered to the eye and thus evoke pupillary responses (decreases in reflectance of the deep pseudopupil). Criterion responses of 10 percent are achieved at

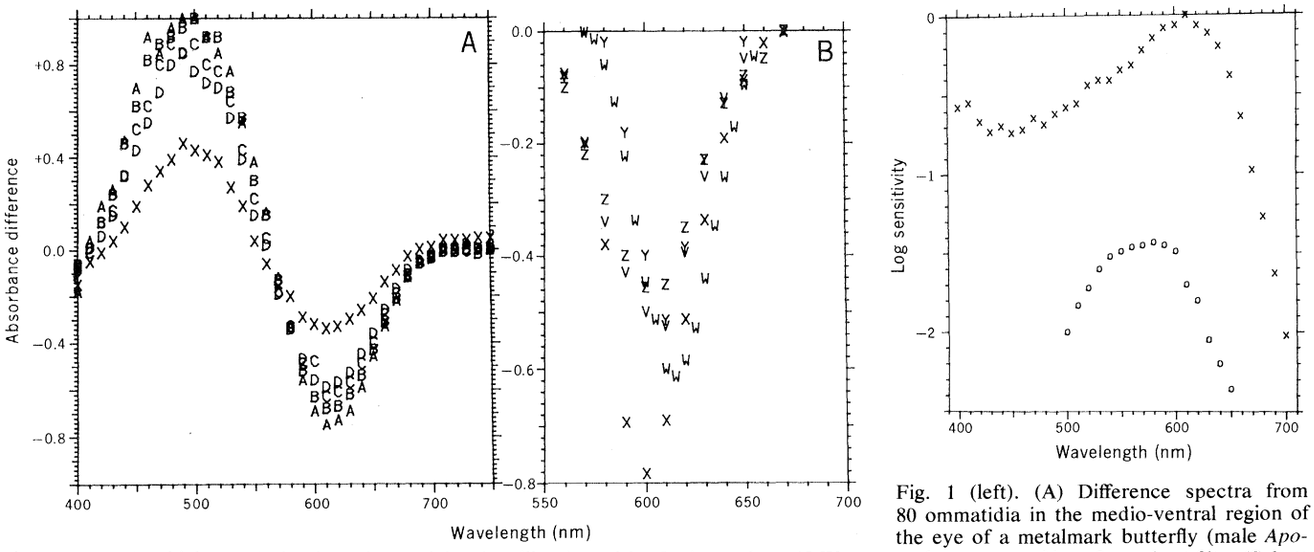

Fig. 1 (left). (A) Difference spectra from 80 ommatidia in the medio-ventral region of the eye of a metalmark butterfly (male *Apodemia mormo*), which was dark-adapted overnight, then illuminated for 5 minutes by a 45-W tungsten lamp covered by a 3-mm heat filter (Schott (KG-3) and an interference filter (Optics Technology) with peak at 695 nm. Curves *A* through *D* were taken at various times after cessation of the actinic illumination: *A*, 2 minutes, *B*, 16 minutes, *C*, 46 minutes, and *D*, 62 minutes. After completing measurements for curve *D*, the 695-nm filter was replaced by an interference filter with peak at 500 nm, then the eye was illuminated for 3 minutes; 2 minutes later the measurements for curve *X* were taken. (B) Difference spectra from the medio-ventral region of the eyes of five butterfly species. Actinic illumination was from the heat-filtered tungsten lamp covered by a cutoff filter (Schott). The filters and species are: *V* = RG630 and male *Anartia amathea*; *W* = RG665 and female *Phoebis sennae*; *X* = RG645 and *Pieris rapae*; *Y* = RG630 and *Eurema nicippe*; *Z* = RG665 and *Everes comyntas*. The positive peaks of the difference spectra are not shown because the reflectance is too low to permit accurate measurements in that spectral region. Fig. 2 (right). Pupillary action spectra of the medio-equatorial region of the eye of a male *A. amathea*. The steady, deep red adapting beam is from a 45-W tungsten source covered by 2.3 log units of neutral density, and 3 mm filters (Schott KG-3 and UG-3). The quantum flux of monochromatic flashes of 3-second duration and 5-nm bandwidth was adjusted to yield 10 percent pupillary responses. Quantum flux at peak sensitivity was about 2×10^{11} quanta cm^{-2} sec^{-1} over a numerical aperture of 0.18. After measuring curve *X*, the eye was treated overnight with RG645 actinic illumination, and the action spectrum was remeasured, yielding curve *O*.

each wavelength by adjusting the quantum flux of a flash, and spectral sensitivity functions are computed as the reciprocal of the quantum flux necessary to achieve the criterion response. Curve *X* of Fig. 2. shows the spectral sensitivity function for the red-sensitive receptors. Photochemical measurements taken both during and after this experiment show that the adapting beam and measuring flashes cause a decrease in titer of rhodopsin that is less than 0.1 log unit.

Curve *O* of Fig. 2 is from a similar experiment, except that the eye was first treated with bright, long-wavelength illumination to substantially reduce the titer of red-absorbing rhodopsin, thereby revealing the presence of green-sensitive receptors by their contribution to the pupillary response.

Experiments similar to those described above provide evidence that the red-absorbing rhodopsin is present in three species of Nymphalidae (*Anartia amathea*, *A. fatima*, *Polygonia interrogationis*), four species of Pieridae (*Eurema mexicana*, *E. nicippe*, *Phoebis sennae*, *Pieris rapae*), one of Riodinidae (*Apodemia mormo*), and one Lycaenidae (*Everes comyntas*). Both photochemical and physiological experiments show that all of these species possess a green-absorbing rhodopsin (*18*). The presence of both red-sensitive and green-sensitive receptors in butterfly eyes provides a functional basis for excellent discrimination (*1*) between similar orange and yellow colors.

The red-absorbing rhodopsin is not found in all species of butterflies. There is no evidence for its presence in seven species of Nymphalidae (*Adelphia bredowii*, *Asterocampa celtis*, *Nymphalis antiopa*, *N. j-album*, *N. urticae*, *Precis lavinia*, *Siproeta steneles*), and one Satyridae (*Euptychia cymela*). Photochemical experiments with these species yield difference spectra with negative peaks at less than 570 nm. Furthermore, the log-linear, long-wavelength portion of the spectral sensitivity functions are shifted to shorter wavelengths by at least 50 nm when compared with Fig. 2.

The agreement between results of photochemical and physiological experiments is compelling evidence that some butterfly species are exceptionally sensitive to long wavelengths because one spectral type of retinal photoreceptor contains a red-absorbing rhodopsin with peak at about 610 nm. This is by far the invertebrate visual pigment of greatest lambda-max. As dehydroretinal is not known from any invertebrate, the chromophore is most likely retinal (*17*), which would make 610 nm the greatest lambda-max for any retinal-based visual pigment, vertebrate or invertebrate. Considering that some butterfly eyes also contain receptors sensitive to ultraviolet (*3, 12, 13*) and to blue (*12*), their visible spectrum is the broadest known of any animal.

These are important results for those interested in the ecology and behavior of butterflies, for it is now clear that the red, orange, and yellow markings on butterflies can do more than warn vertebrate predators. They can also be important for communication among butterflies.

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14. "Red-absorbing rhodopsin" refers to a retinal-based visual pigment that absorbs well at wavelengths greater than about 600 nm. For example, the human red-absorbing visual pigment absorbs maximally at 575 nm. Adjectives such as "red-absorbing" and "green-sensitive" refer only to regions of the spectrum and do not imply that color vision of butterflies is the same as color vision of man.
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Semliki Forest Virus: Cause of a Fatal Case of Human Encephalitis

Abstract. *A fatal case of human encephalitis has been observed for which our results indicate that Semliki Forest virus (SFV) was the etiologic agent. This is surprising in view of the fact that this virus, which has been widely studied, was believed to be one of the arboviruses nonpathogenic for man. Described are the clinical course, the virological examinations performed, and the histopathological findings in the central nervous system.*

The classification of arboviruses according to their potential biohazard places Semliki Forest virus (SFV) among the agents for which strict precautions are not required (*1*). It may, therefore, be of interest that we observed a fatal case of a clinically peracute viral encephalitis and have obtained evidence that SFV was etiologically involved.

The case concerned a 26-year-old female scientist who worked with SFV, strain Osterrieth (*2*) in a German virological institute. She had been suffering for about 1 year from a purulent bronchitis and became ill on 10 June 1978 with severe headache and elevated temperature. The picture did not change until the morning of 13 June, when she began to have difficulties with speaking. During that day she showed attacks of general convulsions, after the first of which she was admitted to a hospital. In the course of the following night she became increasingly somnolent. On 14 June she developed neurological signs mainly from the left hemisphere, namely hemiplegia of the right side and generalized as well as focal epileptic seizures. On 16 June, the patient's respiration had to be assisted for 1 hour; from 17 June on, the respiration had to be assisted continuously. The patient was in a deep coma;

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