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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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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subsequently she had to be reanimated after a temporary cardiac arrest. From 18 June on, the electroencephalogram showed no further brain activity, and after due time, respiratory assistance was stopped.

Cerebrospinal fluid samples taken on 13 and 14 June yielded a cytopathogenic effect (CPE) typical for arboviruses after inoculation on primary chicken embryo fibroblasts (CEF). Five swabs were taken at autopsy, each from a different region of the brain, and these were used for inoculation on CEF. Three of them yielded the same type of CPE as obtained with the cerebrospinal fluid.

For the first step toward identification of the virus we used electron microscopy. The supernatants of the cultures exhibiting a heavy CPE were examined directly, after negative-staining. Particles with the typical morphology of alphaviruses could be observed. Semliki Forest virus, strain Osterrieth, was used for purpose of comparison (Fig. 1).

Final typing was achieved serologically. First, a micro neutralization test (*3*) was performed with two serums: one from a rabbit that had not been immunized (control serum), and another from a hyperimmune rabbit that had been immunized with SFV, strain Osterrieth (SFV was the only alphavirus that was worked with in the patient's institute at that time). The isolated strain and the Osterrieth strain were tested in parallel. Both were neutralized specifically by the antiserum to SFV (Table 1).

Second, the proteins of the isolate were labeled with [^{3}H]leucine, precipitated by antibodies, and coelectrophoresed on a polyacrylamide gel with ^{14}C-labeled SFV (strain Osterrieth) proteins (Fig. 2). The precursor-62 molecule and the complex of the structural proteins E_1 and E_2 (*4*) migrated identically in both preparations. Only traces of the

Table 1. Results of micro neutralization tests with SFV (strain Osterrieth) and the isolate. Both were tested against a normal rabbit serum and a rabbit antiserum to SFV (Osterrieth) and against the two serum specimens obtained from the patient.

	SFV, standard strain (Osterrieth)	Isolated virus strain
Normal (rabbit) serum	1 : <4	1 : <4
Rabbit antiserum to SFV (Osterrieth)	1 : 128	1 : 96
Patient's serum (June 13)	1 : <8	1 : <8
Patient's serum (June 20)	1 : 128	1 : 192

Table 2. Results of hemagglutination inhibition tests in the microtiter system (at *p*H 6.3) with four hemagglutinating units of antigen. The tests were done according to published procedures (*9*). The ascitic fluid was obtained from mice infected with SFV (Smithburn and Haddow prototype strain) (*5*).

Antibody source	Antigens		
	SFV (Osterrieth)	Isolate from patient	Smithburn and Haddow prototype strain
Antiserum to SFV (Osterrieth)	1 : ≥640	1 : ≥640	1 : 320
Ascitic fluid	1 : 320	1 : ≥640	1 : 160
Patient's serum (June 13)	1 : <10	1 : <10	1 : <10
Patient's serum (June 20)	1 : 30	1 : 60	1 : 15

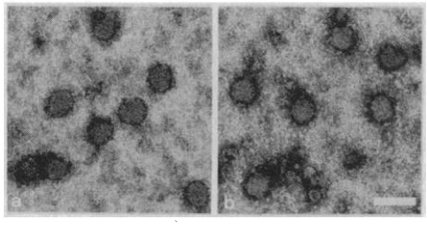

Fig. 1 (above). Electron micrographs of (a) SFV (strain Osterrieth) and (b) particles of the isolate. (Scale bar, 100 nm. Contrast enhanced with 1 percent uranyl acetate applied for 1 minute on hydrophilic carbon-coated Pioloform films.)

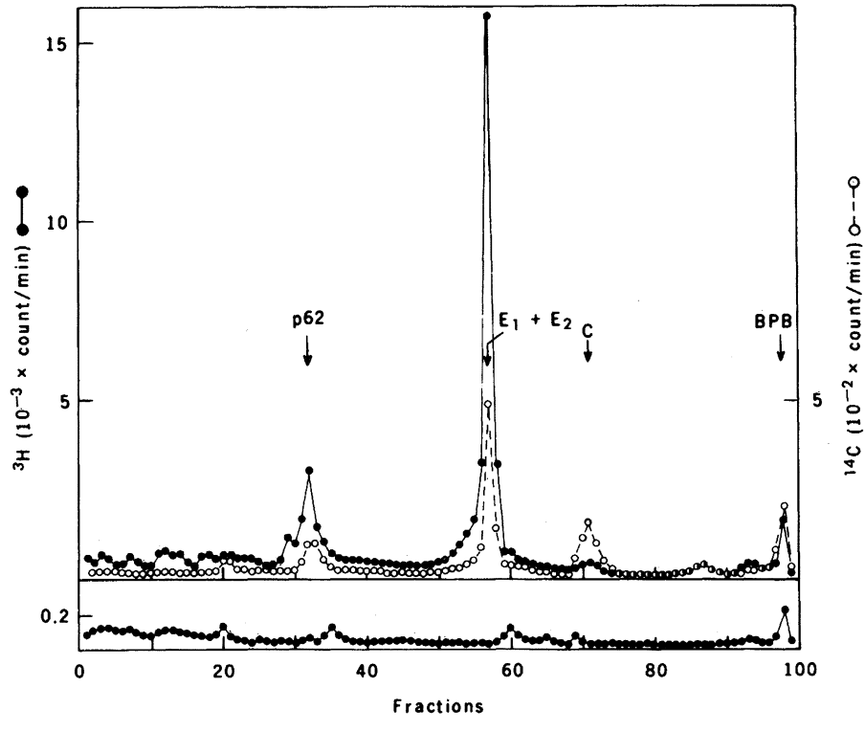

Fig. 2 (right). Analysis of the SFV proteins on a sodium dodecyl sulfate polyacrylamide gel. 10^7 BHK cells on a 9.5-cm plastic petri dish were infected with the isolated virus (second passage in BHK cells). After adsorption for 1 hour at 37°C, the inoculum was removed and the cells fed with Eagle's minimum essential medium (Dulbecco's modification) lacking leucine. Five hours after infection the cells were labeled for 90 minutes with tritiated leucine (Radiochemical Centre, Amersham; 40 μCi/ml; 38 Ci/mmole), then immediately lysed with detergent and subjected to indirect immune precipitation (*4*). Portions of the lysate were incubated with rabbit antiserum to the structural proteins of SFV, strain Osterrieth (upper graph, •) and with normal rabbit serum (lower graph). The rabbit immunoglobulin G (IgG) was precipitated with a sheep antiserum to rabbit IgG, and the immune complexes were washed and dissolved in sample buffer (*10*). The sample containing antiserum to SFV was coelectrophoresed with BHK cells that had been infected with SFV (strain Osterrieth), labeled with ^{14}C protein hydrolysate, and lysed with sample buffer (upper graph, ○). Electrophoresis, gel-slicing, and radioactivity-counting were done according to established procedures (*4*).

core protein are visible in the gel pattern of the isolate, because of the method of lysate preparation used, during which most of the core protein is lost by centrifugation. Under these conditions the E_3 structural protein is not sufficiently labeled to appear in the gel.

Two serum specimens were obtained from the patient, the first taken on 13 June, the second on 20 June, the day of death. They were tested in a micro neutralization test against the isolate and the Osterrieth strain of SFV. A significant increase in antibody titer could be observed with both antigens (Table 1). For control purposes, the two serum samples from the patient were tested in a micro neutralization test against herpes simplex virus: both samples were negative (1: <8).

Hemagglutination inhibition tests were also performed, with antigen preparations of the isolated strain, the Osterrieth strain, and the Smithburn and Haddow prototype strain (*5*). The patient's serum, our rabbit antiserum to SFV (Osterrieth), and ascitic fluid of mice infected with the Smithburn and Haddow prototype strain (*5*) were examined for the presence of cross-reacting hemagglutination-inhibiting antibodies. The results (Table 2) show an increase in antibody titer from the first to the second sample of the patient's serum as well as cross-reactions between the antigens, as would only be expected between related strains of one and the same virus.

It is noteworthy that the Osterrieth strain of SFV used here for comparative identification of the isolate was obtained in 1969 by one of us (G.K.) from the same institute in which the patient was later employed. It has recently been unambiguously characterized in this institute as a strain of SFV by "fingerprinting" (oligonucleotide mapping) of the viral ribonucleic acids (RNA's) (*6*). In that work, the RNA's of the Osterrieth strain were found to be almost identical to those of the Smithburn and Haddow prototype strain. The RNA fingerprints of three other alphaviruses (Sindbis, O'nyong-nyong, and Chikungunya) examined, however, were completely different not only from those of the SFV strains but also from each other.

When the patient's brain and spinal cord were examined neuropathologically, a meningoencephalomyelitis was found. The histopathologic picture consisted essentially of gliomesenchymal nodules scattered throughout all parts of the central nervous system (Fig. 3) and foci of spongy necrosis, corresponding

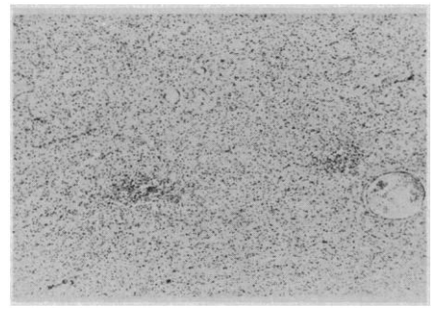

Fig. 3. Histopathological picture of the patient's cerebral white matter, stained by cresyl violet. Two gliomesenchymal nodules can be seen.

to the cellular nodules. The meninges and the perivascular spaces showed mild to moderate infiltration by lymphocytes and histiocytes. These features correspond to those described for panencephalitides caused by arboviruses (*7*).

To summarize the above findings, we conclude that SFV played an important role in the etiology of this fatal encephalitis. To our knowledge this is the first case of disease caused by SFV infection of a human being, although antibodies against this virus can be demonstrated in the serum of many laboratory personnel working with it (*8*).

We consider three possible explanations for the extraordinary course of this infection. First, host-specific factors may have been influential, for example, the preexisting bronchitis. Second, the patient may have been infected via an unusual route or by an unusually high viral dosage. Neither of these possibilities can be verified. The third possibility is the evolution of a mutated strain of SFV, either before or after infection of the patient took place. This question is being investigated.

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Opioid Peptides Modulate Luteinizing Hormone Secretion During Sexual Maturation

Abstract. *Subcutaneous injections of naloxone, an opiate antagonist, lead to an increase in serum luteinizing hormone concentrations in female but not in male rats before they reach puberty. In addition, estradiol benzoate specifically blocks the luteinizing hormone response to naloxone in prepubertal female rats, suggesting that the opioid peptides have a physiological role in the endocrine events leading to sexual maturation.*

Several recent studies have implicated the endogenous opioid peptides in the regulation of growth hormone, prolactin (PRL), and luteinizing hormone (LH) secretion. Both morphine and opioid peptides induce rapid changes in the concentrations of circulating anterior pituitary hormones (*1*), whereas the antagonists naloxone or naltrexone reverse these effects. Moreover, the administration of antagonists such as naloxone alone leads to an increase in serum LH and a decrease in serum PRL levels (*1*, *2*). These last findings constitute the most direct evidence that the endogenous morphine-like peptides are involved in the control of LH and PRL secretion. The effect of opiate antagonists has been examined in

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