from 2-tetradecanone and octadecyl bromide by a Wittig reaction and hydrogenation of the olefin product. (Other compounds were similarly synthesized.) Its retention index (3132) closely matched that of the active material (3125) and therefore ruled out the possibility of dimethyltriacontane.

The results of bioassays of the synthesized monomethyl constituents of the active material and the closely related 12-methyl analog are given in Table 1. 13-Methylhentriacontane was the most active compound, its activity being significantly greater than that of its analogs. Also, additional bioassays proved it to be active at the 50-ng level, which compared favorably with the activity of the isolated material. Table 2 gives the results obtained with other analogs and with the olefin intermediates used in the synthesis. Activity of the compounds generally depended on the closeness of the structure to that of 13-methylhentriacontane. Moreover, the compound was considered remarkably specific, especially for a saturated hydrocarbon, a class of compounds which are usually considered physiologically inert. Activity dropped significantly when the methyl was moved to an adjacent carbon atom or when the chain was shortened or lengthened by a single methylene group. Straight-chain C_{28}, C_{30}, and C_{32} hydrocarbons (not listed in the table) produced no response at any concentration.

Since the presence of compounds similar to 13-methylhentriacontane has been demonstrated in the cuticle of other species (*17*), the surface of third-, fourth-, and fifth-instar larvae (ten of each) was washed with hexane (3×10 ml). These fractions all elicited a positive response when they were bioassayed. The presence of the active material in the feces and salivary secretions may be explained by noting that the hind gut and salivary glands are of ectodermal origin and thus may secrete some of the same material as the epidermal cells of the integument. The fact that larvae consume their cast-off exuviae may also explain the presence of the compounds in the feces and in the hemolymph.

The possibility that the components of the isolated material were synergistic was tested by bioassaying 13- and 15-methylhentriacontane separately and together. The results (Table 3) do not indicate synergism, but imply a dilution effect. For example, the score of 0.92 for a mixture of 50 ng each of 13- and 15-methylhentriacontane is about halfway between the scores for 50 ng of the individual compounds.

We hope the findings of this study will stimulate further research in this important area of insect biochemistry and lead to a better understanding of insect behavior mechanisms.

13-Methylhentriacontane is also potentially valuable in biological control programs aimed at reducing the amount of pesticide pollution, because it may retain parasites in fields requiring protection from the corn earworm.

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8. Silica gel, J. T. Baker Chemical Co., No. 3405. The silica gel contained 3.5 percent water. Mention of a proprietary product in this paper does not constitute an endorsement of the U.S. Department of Agriculture.
9. A glass column (outside diameter, 6 mm; inside diameter, 4 mm; length, 120 cm) containing 5 percent OV 101 on 80 to 100 mesh Gas Chrom Q (Applied Science Lab., State College, Pa.) was temperature programed at 5°C per minute between 200° and 300°C in an F&M model 700 instrument equipped with a flame ionization detector. Rate of flow of carrier gas (helium) was 85 ml/min.
10. Chromatography was conducted on a Darco KB (Atlas Chemical Industries, Inc., Wilmington, Del.) column (4 by 35 cm). Elution solvents were 500 ml of hexane followed by 2000 ml of 2 percent benzene in hexane. The active material emerged between 1500 and 1700 ml of the latter solvent.
11. The gas chromatograph and column described in (*9*) were fitted with a 10 : 1 splitter and operated isothermally at 250°C. The active material was collected on a stainless steel tube [21 by 0.6 (outside diameter) cm] packed with Gas Chrom Q and equipped with a male Luer fitting to permit its connection to the heated exit of the gas chromatograph. After the active material was washed from the tube with hexane, the solution was concentrated to 1 ml and rechromatographed in 10-μl amounts.
12. Conditions for gas chromatography on OV 101 were the same as those in (*9*). In the other chromatography, conditions were the same as in (*9*) except that the liquid phase was on OV 210.
13. A pentane solution of the sample was passed through a column (inside diameter, 5 mm) containing 0.5 g of silica gel (Baker, No. 3405) wet with pentane; the hydrocarbon was eluted with 3 ml of pentane.
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1 June 1971

Latent Herpes Simplex Virus in Spinal Ganglia of Mice

Abstract. *Herpes simplex virus establishes a persistent, latent infection in spinal ganglia after mice have recovered from posterior paralysis. Infectious virus is replicated when these ganglia are explanted and maintained as organ cultures in vitro.*

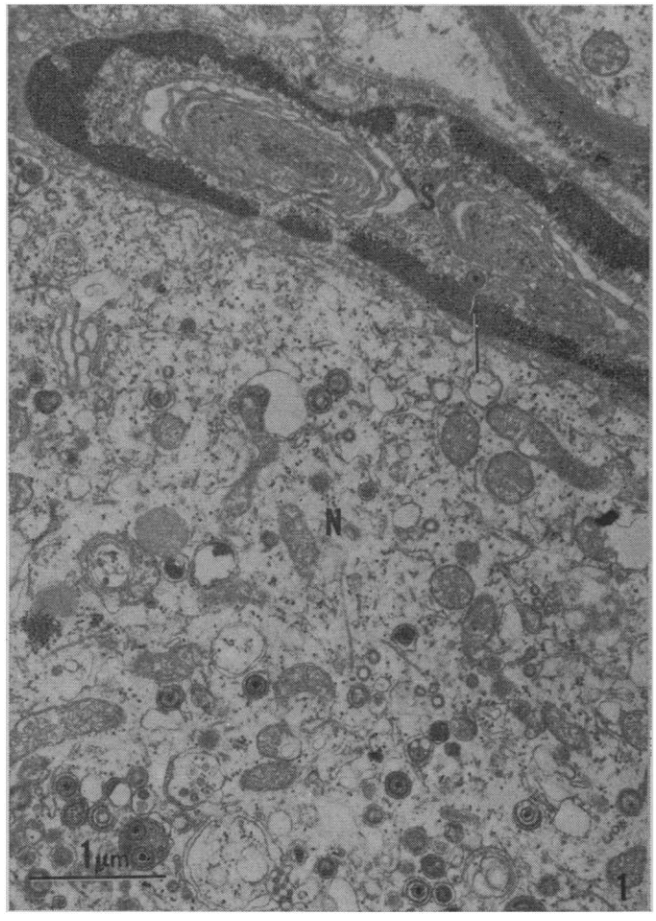
The viruses of herpes simplex (HSV) and herpes zoster are considered to be classic examples of agents that induce latent infections in man [see reviews by Roizman or Fenner (*1*)]. Thus it is considered probable that, following initial infection, these viruses are maintained in some "quiescent" state from which they may periodically be reactivated to produce overt disease. Additional data from several sources suggest that the virus persists in sensory ganglia between these episodes of clinical disease. However, all the evidence supporting these statements is indirect. A direct demonstration that the viral genome can persist in sensory ganglia would be of central importance in establishing the validity of the entire concept. In this report, we show that HSV can induce a latent infection in the spinal ganglia of mice.

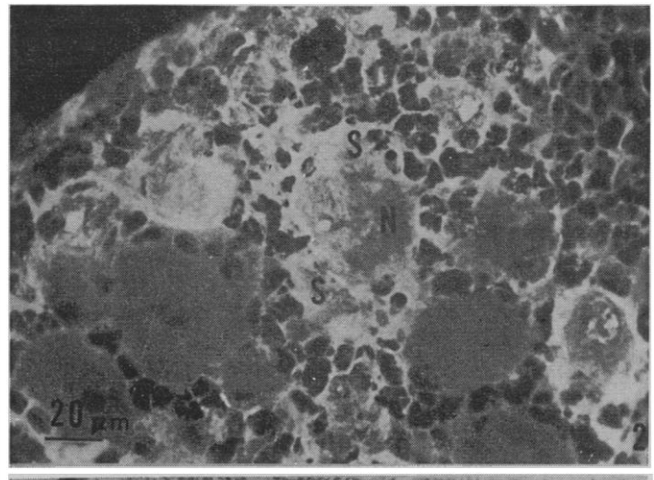
Four-week-old SJL mice (*2*) were inoculated in the left rear footpad with 4×10^3 RK_{13} cell plaque-forming units (PFU) (*3*) of HSV (*4*) according to the technique of Olitsky and Schlesinger (*5*). After inoculation in this manner, virus travels centripetally in the nervous system to the brain (*6*). In our experiments, about 80 percent of mice become paralyzed in one or both hind legs in 7 to 9 days. Of the mice paralyzed, about one half undergo a complete

Table 1. Recovery of infectious herpes simplex virus following in vitro cultivation of latently infected sciatic spinal ganglia from mice. Ganglia were explanted at the following time intervals after mice had recovered from paralysis induced by herpes simplex virus: Exp. 1, 2 months; Exp. 2, 3 months; and Exp. 3, 2 weeks. Eighty percent of the ganglia cultures producing virus were detected at 7 days after explantation; the remainder were identified at 14 days. Details concerning culture methods are given in the text.

Exp.	Mice positive/ mice tested	Mice with left ganglia positive/mice tested	Mice with right ganglia positive/mice tested
1	4/4	4/4	Not done
2	6/6	6/6	4/6
3	6/7	5/7	3/7

clinical recovery in an additional 2 to 3 weeks. The others either die with acute encephalitis by the 12th day or remain permanently paralyzed. During the acute infection, the titer of infectious virus in spinal ganglia reaches a maximum in 4 days [2×10^3 PFU per ganglion after homogenization of ganglia in a Ten Broeck grinder (*7*)] and decreases beyond the level of detection by 7 days after infection (fewer





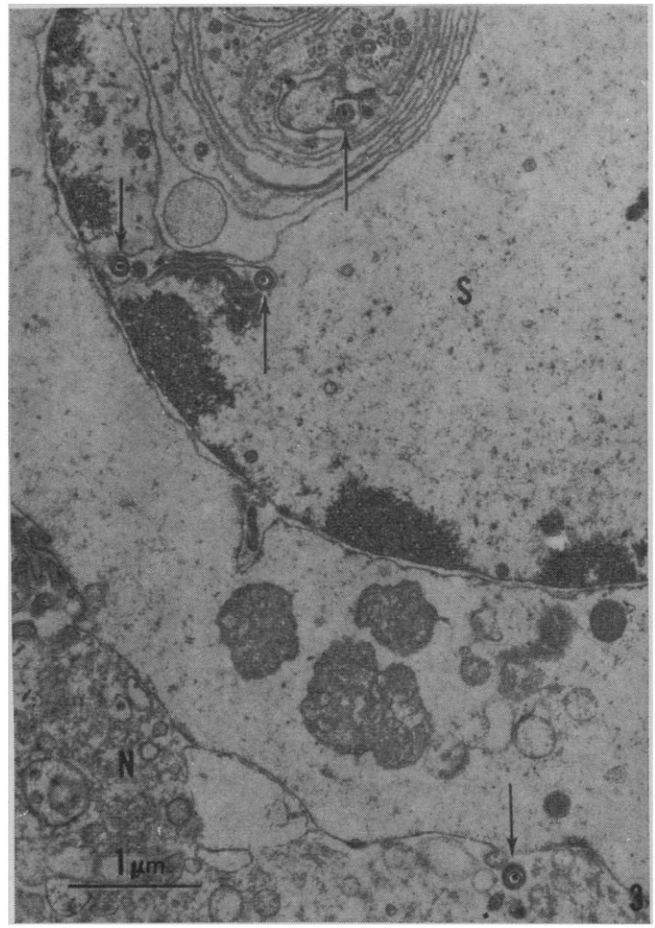


Fig. 1. Electron micrograph of a sectioned sciatic spinal ganglion from a mouse killed 4 days after inoculation of HSV in the ipsilateral footpad. Part of a satellite cell nucleus (*S*) contains empty capsids, a viron (arrow), and membranous whorls. The adjacent neuronal cytoplasm (*N*) contains mature virions (× 18,500). Fig. 2. Photomicrograph of a sciatic spinal ganglion from a mouse killed 4 days after inoculation of HSV in the ipsilateral footpad. The section was cut in a cryostat and stained by direct immunofluorescent methods, first employing an antiserum specific for herpes simplex antigens. This serum was prepared against maximally infected RK_{13} cells and possessed a neutralizing constant of 72 ml/min at 37°C against the virus. In the secondary reaction, sections were stained with fluorescein-labeled goat antiserum to rabbit gamma globulin. Specific staining of herpes antigens in a neuronal soma (*N*) and adjacent satellite cells (*S*) is evident. Adjacent somas are unstained. Control sections consisting of non-virus-containing ganglia stained with immune serum and virus-containing ganglia stained with preimmune serum demonstrated insignificant background fluorescence (× 400). Fig. 3. Electron micrograph of a spinal ganglion from a mouse with latent HSV processed after 10 days in culture. The mouse had recovered from viral-induced paralysis 2 weeks prior to being killed. A supporting cell nucleus (*S*) contains three mature virions (arrows) as well as membranous whorls and empty capsids. A single virion (arrow) is seen in the adjacent neuronal cytoplasm (*N*) (× 18,500).

than 1 PFU per ganglion). Typical ultrastructural features of the infection in neuronal somas and satellite cells from ganglia processed 4 days after infection are presented in Fig. 1. Morphologically complete and incomplete virions are present in both cell types. Figure 2 depicts a section from a similar ganglion stained by immunofluorescent methods with HSV-specific rabbit antibody. Viral antigen is present in several neuronal somas and satellite cells.

Animals that had recovered from paralysis for periods of 2 weeks to 3 months were killed, and the spinal ganglia associated with sciatic nerves were explanted and maintained as organ cultures on glass cover slips in 2-ounce French square bottles at 37°C, standard methods and media (*8*) being used. Supernatant fluids from the organ cultures were assayed on RK_{13} cells for infectious virus at 7 and 14 days after explantation. Similar ganglia were ground in a Ten Broeck homogenizer and directly assayed for virus. In these latter tissues, infectious virus could not be found. However, HSV was detected in supernatant fluids after ganglia had been cultured in vitro (Table 1). Thus some form of the virus was present in ganglia of essentially all mice tested at the time of explant, and it had persisted for at least 4 months after the time of initial infection (the maximum time interval that we have examined). In addition, ganglia contralateral to the inoculated foot from about one half the animals supported viral replication in vitro. Extensive morphologic study of ganglia from recovered mice with ultrastructural and immunofluorescent techniques has failed to reveal evidence that virions or virus-specified antigens persist. An electron micrograph of neuronal cytoplasm and a supporting cell in a ganglion explanted from a latently infected mouse and maintained for 10 days in vitro is presented in Fig. 3. Many morphologically complete virions are seen. Finally, it should be noted that these "recovered" mice possess significant levels of neutralizing antibody (a 1:25 dilution of serum neutralizes $\geqslant 75$ of 100 PFU of virus).

To determine whether other tissues in the nervous system also harbor latent virus, portions of left and right sciatic nerve trunks, thoracic spinal cord, and medulla oblongata from 13 mice with latent infection (six recovered for 3 months and seven for 2 weeks) were cultured as organ cultures by use of methods identical to those used for spinal ganglia. In no case were we able to recover infectious virus. In additional experiments, similar tissues from 11 mice that had been recovered for 2 weeks were minced and maintained on monolayer cultures of RK_{13} cells for 1 week. These cultures failed to reveal the presence of virus. When spinal ganglia from the same mice were cultured in this fashion, virus was replicated in ganglia and the RK_{13} cells were destroyed. From these results, we tentatively conclude that, in this system, latent infection is limited to spinal ganglia.

The immunologic relation between virus recovered from ganglia and the parental virus was compared by neutralization kinetics (*9*) by using the specific antiserum used in immunofluorescent studies. This serum possessed a neutralization constant of 74 ml/min at 37°C against the homologous virus, and the mean neutralization constant against six separate isolates from spinal ganglia was 78 ml/min (standard deviation, 12.6). Thus all virus isolates tested have similar if not identical neutralizable antigens.

Cell types involved and the physical state of virus in latently infected ganglia are unknown, and maintenance of infection by continued replication of a small amount of infectious virus is a possibility that certainly cannot be ruled out by our data. As noted above, our extensive searching of ganglia in latently infected mice by means of ultrastructural and immunofluorescent methods has given no evidence for the presence of viral-specific products in any cell. Thus, it is also possible that the viral genome is maintained in a subviral state in ganglionic neurons or supporting cells.

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Serum Parathyroid Hormone in X-Linked Hypophosphatemia

Abstract. *Serum immunoreactive parathyroid hormone (IPTH) is normal in patients with X-linked hypophosphatemic rickets who are not treated with phosphate salts. Phosphate raises IPTH in these patients. Endogenous IPTH does not influence the existing defect in tubular reabsorption of phosphate in male patients.*

X-linked hypophosphatemic rickets is a dominant disease in which a low concentration of orthophosphate in plasma is the most constant index of the phenotype (*1*). In males, the mutant allele also causes bone disease, while in carrier females it is variably expressed, some showing only hypophosphatemia, while others have bone disease as well. Impairment of renal tubular reabsorption of phosphate has long been recognized as another important phenotypic feature of this trait.

Two opposing views have evolved concerning the pathogenesis of X-linked hypophosphatemia. One opinion favors a primary disorder of intestinal calcium absorption due to impaired endogenous conversion of vitamin D (*2*) to normal biologically active polar derivatives (*3*). This would result in secondary hyperparathyroidism, renal loss of phosphate, hypophosphatemia, and bone disease as an ultimate consequence.

The well-known suppressive effect of intravenous calcium infusion upon the hyperphosphaturia (*4*) appeared to support this hypothesis. The failure of X-linked hypophosphatemia to respond