velop specific antigens at the cell surface and, further, that the antigens induced by a given virus in different hosts are similar or identical. Tompkins *et al.* (*7*) using serums from rabbits with regressed fibroma-induced tumors were able by immunofluorescence to detect a surface antigen on cells infected with fibroma virus. This antibody was specific for fibroma and unreactive with cells infected with other viruses. Their studies showed that the appearance of the antigen was dependent on synthesis of viral DNA. The nonreactivity of C.B., Jr.'s, remission cells in this study could be an indication that viral nucleic acid was not being synthesized.

The hybridization experiments of Spiegelman *et al.* (*8*), Kufe *et al.* (*9*), and Hehlmann *et al.* (*10*) demonstrated sequence homologies between RNA's from various human tumors (including leukemia) and DNA synthesized from mouse RNA tumor viruses and reverse transcriptase. Their experiments showed that a particular tumor contains RNA homologous to the animal virus from the same type of tumor but not to other oncogenic RNA viruses.

A further suggestion of viral etiology is the report of Gallo *et al.* (*11*) of the presence of RNA-dependent DNA polymerase in the lymphoblasts of three patients with acute lymphocytic leukemia. This enzyme, analogous to that of RNA tumor viruses, was not found in the lymphocytes of 48 normal subjects.

Of interest also is the report of Failkow *et al.* (*12*) of the recurrence of leukemia in a female patient who had received a marrow graft from her brother. In this case, the leukemic cells possessed the male karyotype of the donor.

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Herpesvirus hominis: Isolation from Human Trigeminal Ganglion

Abstract. Herpesvirus hominis *was isolated from the trigeminal ganglion obtained at autopsy from 1 of 22 patients with no clinical evidence of active herpetic disease, and from one patient with malignant lymphoma who died with herpes zoster on the abdomen, pulmonary cytomegalic inclusion disease, and possible oral herpes simplex. Virus was isolated by cocultivation of explants of ganglion with monolayers of Vero green monkey kidney cells and required 3 weeks of culture before viral cytopathic effects were evident. These observations support the concept that latent infection of sensory ganglia may be the source of virus in recurrent herpetic disease in man.*

Recurrent infections with *Herpesvirus hominis* (herpes simplex virus, HSV) in man are thought by some investigators to result from virus released from latently infected sensory ganglia (*1*, *2*). Although inflammatory changes were described in the trigeminal ganglia in association with active facial herpetic disease (*3*, *4*), several attempts to isolate virus from the ganglia were unsuccessful (*4*, *5*), and virus particles were not identified in ganglia by electron microscopy (*2*). However, Stevens and his associates provided evidence for latency in sensory ganglia. They showed that HSV can induce latent infection in spinal ganglia of mice (*6*) and in the trigeminal ganglia of rabbits (*7*). Although virus could not be recovered from samples of mouse or rabbit ganglia ground in a homogenizer, it could be isolated from supernatant fluids of organ cultures of the ganglia. Virus could also be isolated by cocultivating explants of mouse ganglia with monolayers of rabbit kidney cells (*6*). In order to determine whether HSV can produce a similar latent infection in man, we attempted to isolate virus from the trigeminal ganglia obtained at autopsy of 23 humans. We placed explants of the ganglia directly on monolayers of cells that are susceptible to lytic infection by HSV and observed these cultures for cytopathic effects. To determine whether viruses might be latent in the human choroid plexus, we obtained samples of choroid plexus and

Table 1. Viral isolation studies from human trigeminal ganglia. Positive isolates were obtained after 3 weeks in culture with Vero cell monolayers.

Patient			Diagnosis	Virus isolation	Serum antibody to HSV
No.	Age (years)	Sex			
1	72	M	Cirrhosis, nutritional	−	
2	70	F	Multiple sclerosis	−	
3	54	M	Histiocytic medullary reticulosis	−	
4	65	M	Trauma	−	
5	43	F	Atrial septal defect	−	
6	17	M	Primary pulmonary hypertension	−	
7	18	F	Acute myelocytic leukemia	−	
8	24	M	Acute myelocytic leukemia	−	
9	29	M	Chronic myelocytic leukemia	−	
10	45	M	Astrocytoma	−	
11	51	F	Adenocarcinoma, breast	−	
12	47	F	Acute myelocytic leukemia	−	+
13	57	M	Saphenous vein bypass	−	
14	60	F	Rheumatic heart disease	−	+
15	72	M	Brain tumor	−	
16	59	F	Ovarian carcinoma	−	+
17	56	M	Cirrhosis, nutritional	+	
18	16	M	Ependymoblastoma	−	
19	11	M	Cystic fibrosis	−	−
20	27	M	Hodgkin's disease	−	+
21	23	F	Malignant lymphoma, histiocytic	−	
22	70	F	Parkinson's disease	−	
23	54	F	Malignant lymphoma, unclassified	+	

attempted to isolate viruses with the same methods used for the ganglia.

At autopsy, trigeminal ganglia were removed aseptically from trigeminal cavities, and choroid plexuses from the lateral ventricles. The age, sex, and major diagnosis of each patient are listed in Table 1. Samples of blood for antibody studies were collected from the heart in five cases (Table 1).

The tissues were minced into 1-mm^3 explants, and five to ten explants were placed in each of four flasks of Vero cells (a continuous line of African green monkey kidney cells) and four roller tubes of primary human embryo kidney cells. Two of each type of cultures were fed with a medium composed of 20 percent fetal bovine serum (FBS) and 80 percent RPMI 1640 (*8*), and the other two were fed with 2 percent FBS and 98 percent RPMI 1640. All of the cultures were fed weekly and observed for cytopathic effects twice weekly. The cultures with no evidence of cytopathic effects were observed for 3 months before they were discarded.

Virus was recovered from the ganglia of 2 of the 23 patients (Nos. 17 and 23 in Table 1), and no viruses were recovered from the choroid plexuses. After tissues from patient 17 were cultured for 3 weeks, areas of rounded refractile Vero cells were noted around the ganglionic explants in the two flasks maintained in the 20 percent FBS medium. This change spread throughout the monolayers in the subsequent 3 days, and a cytopathic agent could be passed to new Vero flasks with supernatants. This agent produced a herpes-type cytopathic effect in 24 hours, and electron microscopy of infected Vero cells showed typical herpesvirus particles (Fig. 1). Pooled virus was prepared in Vero cells and contained 10^7 plaque-forming units (PFU) per milliliter. Neutralization tests were performed on Vero cells against approximately 100 PFU of the virus with standard techniques (*9*). There was 100 percent neutralization of the virus by a 1 : 100 dilution of rabbit antiserum to HSV type 1 or by a 1 : 100 dilution of human immune globulin (Lederle). To determine whether the virus was HSV type 1 or 2, we attempted to produce plaques on primary chick embryo fibroblast cultures. A known strain of HSV type 2 produced plaques, but none were produced by a strain of HSV type 1 or by the isolate from the trigeminal ganglion. The high titer of the virus and the failure to produce plaques on chick embryo fibroblasts strongly suggest that the agent we isolated is HSV type 1 (*10*); however, serological typing is not complete.

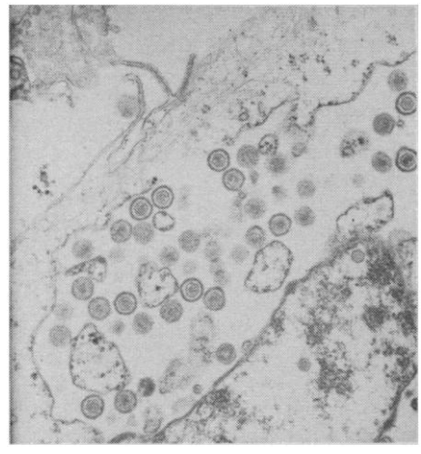

Fig. 1. Typical herpesvirus virions in nucleus and cytoplasm of Vero cells infected with virus isolate from trigeminal ganglion (×19,500).

The second virus isolation was made from the trigeminal ganglion of patient 23, a 54-year-old woman with malignant lymphoma. She died with pancytopenia, herpes zoster of the skin of the abdomen, and pulmonary cytomegalic inclusion disease. She had multiple oral ulcers, some of which may have been due to HSV, but no viral isolation studies were done with material from these lesions. Again, 3 weeks after initiation of the cultures, the two flasks with explants of ganglion on Vero monolayers in 20 percent FBS medium showed cytopathic effects. The supernatant was passed to new Vero flasks, and an agent with morphological and immunological properties of HSV, as described for the isolate from patient 17, was identified. Serological typing to determine whether the isolate is type 1 or 2 is not complete.

Although unfortunately we did not collect serum from the two cases from which the virus was isolated, we did have serum from five others. These were tested for neutralizing antibody against 100 PFU of HSV type 1 at a 1 : 10 dilution, and four of the five were positive. Despite the presence of antibody in these four, virus could not be isolated from ganglia or choroid plexus by cocultivation. If sensory ganglia are important sites for latent herpesvirus infection, it is possible that the virus was latent in one of the many other sensory ganglia not tested.

The possibility that the virus we recovered was not actually latent in the trigeminal ganglia but rather present in the blood is unlikely, because no virus could be isolated from the highly vascular choroid plexus. The 3-week period before cytopathic effects were seen does suggest that the virus was present in latent form in the ganglion and that it was activated in culture. In each case the virus was recovered only from explants in the Vero flasks in the 20 percent FBS medium and not from similar flasks in 2 percent FBS or from the human embryo kidney roller tubes. Although the differences in media and cells may have been the cause, it is also possible that the virus may be latent in only focal areas of the ganglia and that by chance these were in flasks that developed cytopathic effects.

The cell type containing virus in the human ganglion is not known; however, virions were detected in neurons of the rabbit ganglion by electron microscopy after 17 days in culture (*7*). The mechanisms involved in latency are unknown; other members of the herpesvirus family, such as *Herpesvirus saimiri*, may be latent in lymphoid cells and can be isolated by cocultivation of the lymphoid cells with Vero monolayers (*11*). The Epstein-Barr virus, a lymphocytotropic herpesvirus of man, can be activated by culture of lymphoid cells in media containing bromodeoxyuridine; similar methods of induction might be of value in studying latency of HSV in human neural tissues (*12*).

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