Proc. Natl. Acad. Sci. USA
Vol. 78, No. 10, pp. 6461–6465, October 1981
Medical Sciences

Herpes simplex type 1 DNA in human brain tissue

(latent virus/Southern blot/cloned DNA fragment/multiple sclerosis)

NIGEL W. FRASER, WILLIAM C. LAWRENCE*, ZOFIA WROBLEWSKA, DONALD H. GILDEN, AND HILARY KOPROWSKI

The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104

Contributed by Hilary Koprowski, June 26, 1981

ABSTRACT **Herpes simplex virus type 1 (HSV-1) is known to reside latently in the trigeminal ganglia of man. Reactivation of this virus causes skin lesions and may occasionally infect other tissues, including the brain. To determine whether the brain tissue of humans free of clinical signs of HSV-1 infection contains any trace of HSV-1, we examined the DNA from brain tissue by endonuclease digestion, separation of the fragments by gel electrophoresis, and hybridization with labeled HSV-1 DNA probes. Hybrid bands were detected autoradiographically in experiments using cloned and virion-purified fragments of the HSV-1 genome. HSV-1 DNA sequences were found in 6 of 11 human brain DNA samples tested. In some cases, these bands corresponded to the bands expected for the complete viral genome, whereas others contained bands representing only a part of the genome. In some cases, the terminal fragments could be found, suggesting that the DNA was in a linear, nonintegrated form.**

Four types of human herpesviruses can be identified: cytomegalovirus, Epstein–Barr virus, varicella zoster virus, and herpes simplex virus (types 1 and 2; HSV-1 and HSV-2, respectively). Of these, herpes simplex virus (HSV) has been studied most and, almost since its discovery, has been known by its ability to form latent infections (1). The genome of HSV-1 is a linear double-stranded DNA of 10^8 daltons (2) and consists of two unique segments bounded by inverted repeats (3). The G+C content of the genome is unusually high (67%; ref. 2), and it is cut by restriction endonuclease *Bam*HI (G↓G-A-T-C-C) 40 times.

There have been many reports that HSV is present in the trigeminal ganglia of animals (4, 5) including humans (6–8). Isolation of HSV from superior cervical and vagus ganglia of humans has also been reported (9). HSV sequences have been detected in human brain by *in situ* hybridization, although no virus could be isolated nor was any detected by immunofluorescence (10). Recently, Cabrera *et al.* (11) have shown that, in mice experimentally infected with HSV-1, infectious virus can be recovered from brain tissue of only 5% of the latently infected animals whereas, by reassociation kinetics analysis, HSV-1 DNA sequences can be detected in the brains of 30% of mice harboring latent HSV.

Although some work has been done on the analysis of HSV-1 recovered from tissue, little has been done on the viral nucleic acid present in that tissue. Probably this is because only a small amount of material is present in latently infected tissue. Instead of the classical reassociation kinetics analysis, we have used restriction endonuclease digestion, gel electrophoresis, and Southern blotting (12) to produce filters that can be hybridized with ^{32}P-labeled nick-translated DNA of the highest possible specific activity. The small amount of radioactivity in hybrids could be detected autoradiographically. This technique was used by Botchan *et al.* (13) in their study of simian virus 40 sequences in transformed cells. The detection of positive results is simplified because such results appear as a pattern of discrete bands against a background smear on an autoradiograph.

By using blot hybridization techniques, we demonstrate here the presence of HSV-1 sequences in the central nervous systems of humans who died from neurological and nonneurological causes.

MATERIALS AND METHODS

Cells and Tissue. CV-1 and baby hamster kidney (BHK)-21 cells were used to grow HSV-1 strain F (kindly supplied by B. Roizman, University of Chicago, Viral Oncology Laboratories). Tissues from cadavers of patients who had had multiple sclerosis or died of some accidental death with no record of neurological disease were obtained within 18 hr of death and stored frozen (−70°C) until DNA extraction.

DNA Extraction. Approximately 1 or 2 cm^3 of tissue was minced in 0.15 M NaCl/0.05 M EDTA/0.01 M Tris, pH 8.0, at 4°C. This material was thoroughly homogenized with a Dounce homogenizer, brought to a final concentration of 0.5% in $NaDodSO_4$, and incubated overnight with self-digested Pronase at 500 μg/ml. The DNA was extracted with phenol/chloroform and precipitated with 2 vol of ethanol. The precipitate was centrifuged out and dissolved in 0.01 M Tris·HCl/0.01 M EDTA, pH 7.4. Ribonuclease A was added to 40 mg/ml, the solution was incubated at 37°C for 2 hr, extracted with phenol, and the DNA was precipitated with ethanol. The DNA recovered was quantitated by measuring the A_{260} (1 μg/ml = 0.02 *A* unit) and was ≈0.1 mg.

Probe DNA was prepared from extracellular virions essentially by the technique of Pellicer *et al.* (14) and was subjected to two cycles of sodium iodide/ethidium bromide gradients (15). Plasmid and phage DNA were prepared by standard techniques (16, 17).

Preparation of Blots. The technique was that of Southern (12) modified according to Wahl *et al.* (18), using acid hydrolysis in 0.25 M HCl for 15–20 min. Twenty units of restriction endonuclease *Bam*HI (Bethesda Research Laboratories, Rockville, MD) was used to digest 10 μg of DNA in 20 μl of 100 mM Tris·HCl, pH 8.0/7 mM $MgCl_2$. After incubation for 2 hr at 37°C, an additional 20 units of enzyme was added and digestion was continued for a further 2 hr tc minimize the possibility of partial digestion products. Electrophoresis was done on 0.5% agarose gels (Bio-Rad) in a borate buffer (0.09 M Tris·HCl, pH

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: HSV, herpes simplex virus; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; BHK, baby hamster kidney; NaCl/Cit, standard saline citrate.

* Present address: University of Pennsylvania, School of Veterinary Medicine, 3800 Spruce St., Philadelphia, PA 19104.

8.3/0.9 M boric acid/0.25 M EDTA containing ethidium bromide at 0.05 μg/ml). Gels were then blotted onto nitrocellulose filters (12, 18).

Preparation of Nick-Translated Probes. The technique was essentially that of Maniatis *et al.* (19) and Rigby *et al.* (20). To 0.25 μg of HSV-1 DNA purified from virions in 25 μl of 50 mM Tris·HCl/5 mM $MgCl_2$/bovine serum albumin (50 μg/ml)/1 mM dGTP/1 mM dTTP was added 100 μCi (1 Ci = 3.7×10^{10} becquerels) of [α-^{32}P]dATP, 100 μCi of [α-^{32}P]dCTP (Amersham; 200–300 Ci/mM), 2×10^{-4} Kunitz units of DNase 1 (Worthington), and 2 units of DNA polymerase purified from *Escherichia coli* (Boehringer Mannheim). The reaction mixture was incubated at 15°C for 2 hr, and then 100 μl of 0.1 M NaCl/10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.1% $NaDodSO_4$ containing 50 μg of sonicated salmon sperm DNA was added. The solution was passed through a Sephadex G-50 column, and the peak fractions of DNA were ethanol precipitated. Specific activities of 3–7 × 10^8 cpm/μg were achieved with 30–50% incorporation of label.

Hybridization of Blots. The "blot" filters were maintained in 10 ml of 20% formamide/0.6 M NaCl/0.06 M sodium citrate/0.01 M EDTA/0.1% $NaDodSO_4$/5-fold Denhardt's solution (21) containing sonicated denatured salmon sperm DNA at 50 μg/ml in ½-gal plastic bottles at 50°C on a standard tissue culture roller bottle apparatus for 5–16 hr prior to the addition of denatured radioactive probe (1–5 × 10^7 cpm per filter).

Hybridization was for 36 hr at 50°C in 50% formamide/10% dextran sulfate (18)/Denhardt's solution/4-fold standard saline citrate (NaCl/Cit)/0.1 M EDTA/0.1% $NaDodSO_4$ containing sonicated denatured salmon sperm at 25 μg/ml.

To remove unhybridized labeled material, filters were rinsed twice with 5-fold NaCl/Cit/0.1% $NaDodSO_4$ at 50°C for 10 min each and then for 20 min with hybridization mixture without salmon sperm DNA and dextran sulfate at 50°C. The filters were rinsed once in NaCl/Cit/0.1% $NaDodSO_4$ at 37°C for 20 min, once in NaCl/Cit/0.1% $NaDodSO_4$ at 65°C for 20 min, once in half-strength NaCl/Cit/0.1% $NaDodSO_4$ at 65°C for 20 min, and finally in quarter-strength NaCl/Cit/0.1% $NaDodSO_4$ at 65°C for 15 min. Filters were blotted dry and autoradiographed with XR film and DuPont Cronex Lightning Plus screens at −70°C for 1–10 days.

RESULTS

To detect viral DNA sequences which may occur at <1 copy per cell in infected tissue (22), radioactive HSV-1 probes of the highest specific activity and purity must be produced. Traditionally, virions have been purified from infected cell media on sucrose density gradients and the DNA has been extracted by fluid extraction. The extracted DNA was then subjected to CsCl or, more recently, sodium iodide/ethidium bromide gradients (15) to separate the small amounts of contaminating cellular DNA from viral DNA. We have used the latter gradients to purify viral DNA, which was then nick translated and used to detect HSV-1 sequences in infected cells. Fig. 1*A* shows the pattern of bands occurring after restriction endonuclease *Bam*HI cleavage of DNA from CV-1 cells infected with HSV-1 strain F.

Fig. 1*B* shows a comparison of viral probes from different cell sources. For the detection of HSV-1 sequences in human tissue, DNA purified from virus grown on BHK-21 cells is superior to that of CV-1 cells. Thus, in Fig. 1*B*, lanes 1 and 2 show a cleaner hybridization pattern and control than lanes 3 and 4. Similarly, lanes 5 and 6 are superior to lanes 7 and 8 because there is a higher background on 7 and 8. Thus, it is likely that, even after extensive purification, the virion DNA used for nick translation still contains small amounts of contaminating cellular DNA. This

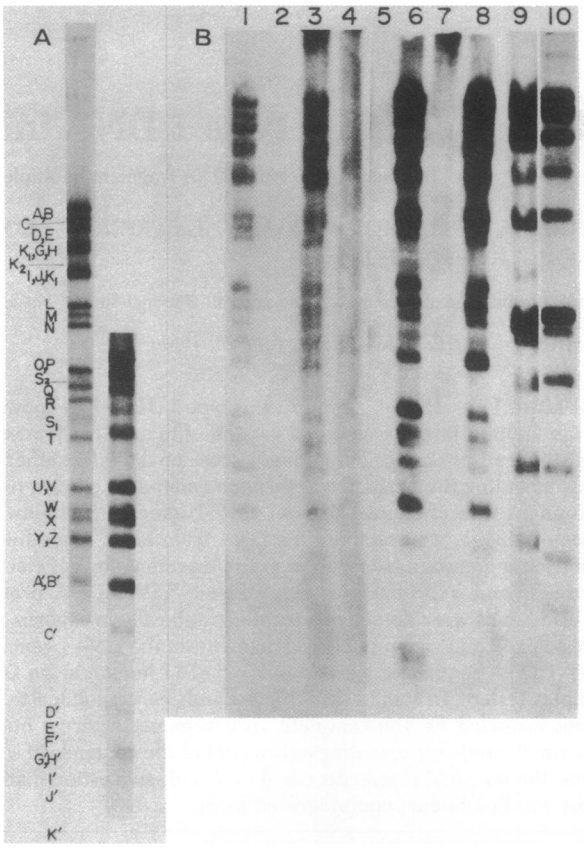

FIG. 1. (*A*) Blot hybridization of nick-translated ^{32}P-labeled HSV-1 strain F DNA probe to *Bam*HI digests of DNA from HSV-1-infected CV-1 cells. *Bam*HI fragments can be detected in a pattern similar to that of Locker and Frankel (23). (*B*) Comparison of ^{32}P-labeled HSV-1 DNA probes prepared from different sources. DNA from noninfected and HSV-1-infected BHK and CV-1 cells was digested with *Bam*HI, subjected to electrophoresis, blotted, and hybridized with nick-translated HSV-1 strain F DNA prepared from virions from infected BHK or CV-1 cells or from cloned HSV-1 DNA fragments. Lanes: 1, HSV-1 DNA from CV-1 cell-produced virions hybridized to infected BHK cell DNA; 2, HSV-1 DNA from CV-1 cell-produced virions hybridized to noninfected BHK cell DNA; 3, HSV-1 DNA (CV-1 cells) hybridized to infected CV-1 cell DNA; 4, HSV-1 (CV-1 cells) hybridized to noninfected CV-1 cell DNA; 5, HSV-1 DNA from virions produced in BHK cells hybridized to noninfected CV-1 cell DNA; 6, HSV-1 (BHK cells) DNA hybridized to infected CV-1 cell DNA; 7, HSV-1 (BHK cells) DNA hybridized to noninfected BHK cell DNA; 8, HSV-1 (BHK cells) DNA hybridized to infected CV-1 cells; 9, cloned HSV-1 DNA fragments (*Eco*RI D, G, I, L, M, N and *Bam*HI Q) hybridized to infected CV-1 cell DNA; 10, cloned HSV-1 DNA fragments (see lane 9) hybridized to infected BHK cell DNA.

is seen in Fig. 1*B* (lanes 1–8), where the probe DNA is made from virions purified from CV-1- or BHK-infected cells and hybridized to DNA of CV-1 or BHK cells that are uninfected or infected with HSV-1 strain F.

Furthermore, nick-translated probes made to a mixture of HSV-1 restriction endonuclease fragments cloned in λWES bacteriophage (kindly provided by Lynn Enquist, National Institutes of Health; ref. 17) had equally low backgrounds when hybridized with DNA extracted from either human or mouse cells. Unlike the virion DNA, the cloned probes could be used individually or in groups of fragments from selected regions of the genome (Fig. 1*B*, lanes 9 and 10).

In reconstruction experiments, when 10 μg of human brain DNA (previously shown to be negative for HSV-1 DNA) was mixed with known amounts of HSV-1 DNA and subjected to endonuclease digestion, gel electrophoresis, Southern blotting,

hybridization, and autoradiography, HSV-1 DNA could be detected at 10 pg and occasionally less.

Detection of HSV-1 DNA Sequences in Human Brain. DNA was extracted from pieces of human brain, and 10-μg samples were digested with restriction endonuclease *Bam*HI. The digests were separated on 0.5% agarose gels, which were transferred to nitrocellulose sheets and hybridized with nick-translated HSV-1 DNA probe. Fig. 2*A* shows brain DNA hybridized to ^{32}P-labeled whole virion DNA purified from the media of infected BHK cells. Lanes 1–4 contain band patterns that are similar to the patterns found in Fig. 1*B*. Fig. 2*B* shows the same brain samples hybridized to ^{32}P-labeled cloned HSV-1 DNA containing a pool of *Eco*RI fragments D, E, I, L, M, and N and the *Bam*HI Q fragment. In autoradiography, samples 1–3 are shown to be positive. Fig. 2*C* shows autoradiographs of filters hybridized only to the *Bam*HI Q fragment (containing the thymidine kinase gene). Again, lanes 1–3 show positive bands of hybridization. Because the pattern of bands corresponds to the type of labeled probe used in the experiment, we conclude that the viral sequences are hybridizing with the brain DNA and not with some weakly homologous cell sequences. Note that brain 3 appears to have two bands hybridizing with the *Bam*HI Q probe.

Detection of Terminal Regions of the Genome. From the results described above, it appeared that most, if not all, of the HSV-1 genome was present in the HSV-1-positive human brains. To determine whether the HSV-1 DNA was integrated or episomal, we used a probe to hybridize the terminal fragments of the HSV-1 genome. The clone pBR115 containing the *Bam*HI SP fragment was obtained from the laboratory of B. Roizman (24). Fig. 3 shows the band pattern obtained on hybridization of brain DNA with ^{32}P-labeled pBR115 DNA. In lanes 2, 5, and 6, the pattern resembles that expected of normal permissively infected cells. This pattern of three bands represents the two terminal *Bam*HI DNA fragments of the HSV-1 genome and the internal fragment at the junction of the long and short segment genome. However, one sample (lane 3) has only a single band of intermediate size between the two terminal fragment bands and the internal junction band. In this case, the form of the DNA is clearly not normal. It may be integrated into the cellular DNA or it may be from a fragment of the viral genome.

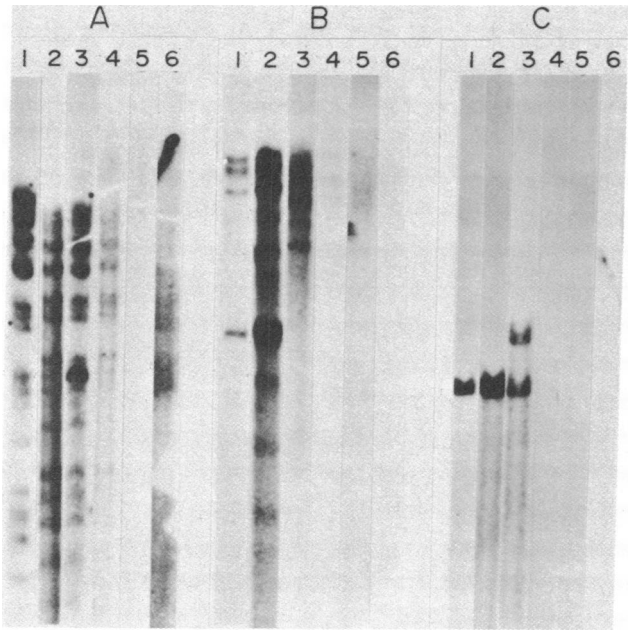

FIG. 2. Comparison of HSV-1 DNA sequences in different human brain DNAs. DNA was extracted from brain tissue and digested with *Bam*HI; the digests were subjected to electrophoresis and transferred to nitrocellulose sheets; and the sheets were hybridized with ^{32}P-labeled nick-translated HSV-1 strain F DNA (*A*), ^{32}P-labeled nick-translated DNA containing a pool of cloned fragments (see Fig. 1*B*) (*B*), and ^{32}P-labeled nick-translated DNA from a cloned *Bam*HI 3.4-kilobase fragment containing the viral thymidine kinase gene (*C*). Lanes: 1, male patient (2441) who died of trauma with no clinical evidence of HSV infection; 2, male patient (S-1133), diagnosis of multiple sclerosis; 3, female patient (S-1201), diagnosis of multiple sclerosis; 4, male patient (S-1107), diagnosis of multiple sclerosis; 5, male patient who died of trauma (SV78-18), no clinical evidence of HSV infection; 6, male patient, diagnosis of coronary artery disease.

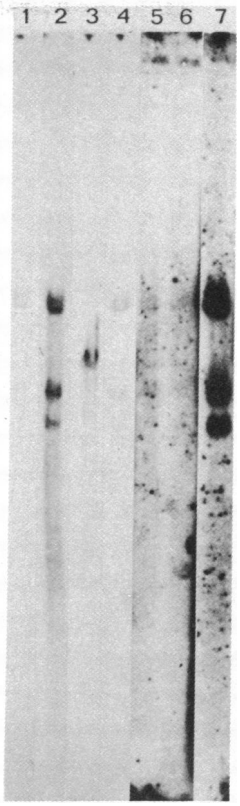

FIG. 3. Blot hybridization of nick-translated ^{32}P-labeled *Bam*HI SP probe DNA to *Bam*HI digests of DNA from human brains. Lanes: 1, 0.1 ng of HSV-1 DNA and 10 μg of uninfected DNA; 2, 10 μg of S-1201 DNA; 3, 10 μg of 1133 DNA; 4, 0.1 ng of HSV-1 DNA and 10 μg of uninfected DNA; 5, 10 μg of 1107 DNA; 6, 10 μg of 80-4 DNA; 7, 0.1 ng of HSV-1 DNA and 10 μg of uninfected DNA. Lanes 5–7 were exposed for 10 days and lanes 1–4 were exposed for 2 days.

Location of HSV-1 Sequences. Having demonstrated the presence of HSV-1 sequences in some brains, we attempted to localize the viral DNA to certain brain regions and to determine whether any other organ harbored HSV-1 DNA sequences. Fig. 4 shows the result of hybridizing DNA extracted from the parietal lobe white matter, pons, cerebellum, and cortex of brain 2441 (lanes 2–5, respectively) with ^{32}P-labeled HSV-1 DNA. Those tissues were obtained from a male patient who died of trauma without clinical evidence of HSV-1 infection. The parietal lobe white matter (lane 2) is the only region of the brain in which HSV-1 DNA sequences could be detected. In another study with other brains, different regions were found to be positive (Table 1).

Table 1 summarizes the results of blot hybridization analysis of 11 human brain samples and shows that HSV-1 DNA sequences were detected in 6 of 11 brains. From the 11 patients, 36 samples, mostly corresponding to defined anatomical regions of the brain, were analyzed. The data indicate that HSV-1 sequences were not consistently detected in any one area of the brain.

Hybridization of DNA extracted from the kidney and liver of cadaver 2441 showed that, although the kidney was negative, the liver did contain HSV-1 sequences (Fig. 5).

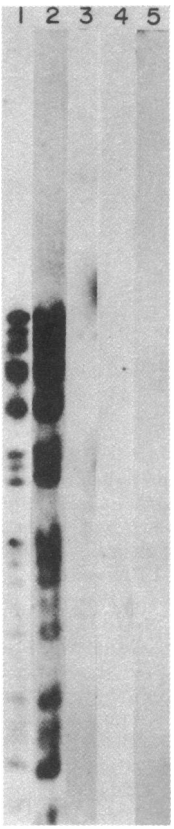

FIG. 4. Comparison of HSV-1 DNA sequences in different regions of the brain. Tissue from various areas of the brain was obtained from a male patient who died of trauma without clinical evidence of HSV-1 infection. DNA was extracted and digested with *Bam*HI, and the digests were subjected to electrophoresis, transferred to nitrocellulose, and hybridized to a ^{32}P-labeled nick-translated HSV-1 strain F DNA probe. Lanes: 1, control (10 μg of noninfected CV-1 cell DNA with 1 ng of HSV-1 DNA); 2, 10 μg of parietal lobe white matter DNA; 3, 10 μg of pons DNA; 4, 10 μg of cerebellum DNA; 5, 10 μg of cortex DNA.

DISCUSSION

Our results indicate that HSV-1 sequences are found in the brain of some normal and neuropathological humans. This is in contrast to the previous reports of Wetmur (25) and Aulakh *et al.* (26). However, we account for this discrepancy by the greater sensitivity of the blot hybridization technique as compared with reassociation kinetics analysis.

By using nick-translated viral DNA at an average specific activity of 5×10^8 cpm/μg and 10 μg of brain DNA per sample, we could detect $\approx 10^4$ cells containing viral DNA. This was possible because we could detect 10 pg of HSV-1 DNA in the presence of 10 μg of uninfected cell DNA. If the amount of DNA in the human genome is taken as $\approx$5 pg (27), then, in a 10-μg sample of brain DNA, we will have 2×10^6 copies of DNA. If the size of the HSV-1 genome is taken as 10^8 daltons (28), one can detect 10^4 viral genomes. However, these 10^4 viral genomes were detected in a sample of 10 μg or 2×10^6 cell copies of DNA. Thus, we can detect the viral genome at a level of 1 copy of HSV-1 in 200 cells. At this sensitivity, we can detect $\approx$50% of the expected DNA fragments because the largest fragments accumulate more activity on hybridization than the smaller fragments. In cases in which HSV-1 DNA occurs in large amounts, all of the bands are detectable whereas, with small amounts, only the largest fragments appear and thus the presence of the rest of the viral genome is inferred. By using nick-translated globin DNA at 6×10^7 cpm/μg specific activity, Jeffreys and Flavell (29) detected 0.03 copies of a 1.5-kilobase DNA fragment in a reconstruction experiment. Thus, it is not unreasonable to detect 0.005 copies of *Bam*HI fragments, some of which are >10 kilobases long, by using nick-translated HSV-1 DNA of 5×10^8 cpm/μg specific activity. It should also be remembered that the cloned fragments of HSV-1 used for probes contain considerable tails of λWES or pBR322 DNA sequences that may amplify the hybrid signal.

In detecting HSV-1 sequences in human brain tissue, we have also shown that apparently most of the HSV-1 genome is present. We can identify HSV-1 fragments of sizes similar to those that occur in virion HSV-1 DNA, including the terminal HSV-1 *Bam*HI fragments described by Locker and Frankel (23). This suggests that the viral DNA is not integrated into the cellular DNA.

Of the humans analyzed, some proved negative for HSV-1 sequences (Fig. 2). Because only a small amount of brain was sampled (1 cm^3 of white matter), it is possible that some of those shown negative were positive in other tissue samples. In an

Table 1. Summary details of patients whose brain tissues were examined for HSV-1 DNA

Patient	Age/sex	Disease	Autopsy, hr after death	HSV-DNA*	Brain Segment†
S-1201	56/female	Multiple sclerosis	17	Positive (N)	Not defined
SV78-18	?/male	Normal		Negative	—
80-4	54/male	Coronary artery	17	Negative	—
S-1107	65/male	Multiple sclerosis	8	Positive (N)	Not defined
S-1133	?/male	Multiple sclerosis	6	Positive (U)	Not defined
S-1207	82/female	Multiple sclerosis	15	Negative	—
S-1208	32/male	Multiple sclerosis	8	Positive (U)	Frontal lobe grey matter Periventricular white matter
3531	28/male	Normal	9	Positive (U)	Frontal lobe grey matter Cerebellum
3529	13/male	Normal	12	Positive (U)	Brainstem Frontal lobe grey matter
3500	18/male	Normal	6.5	Negative	—
2441	37/male	Normal	14	Positive (N)	Parietal lobe

Samples of brain (total of 36 segments from various anatomically defined regions) from 11 cadavers were analyzed for HSV-1 DNA.

* N, normal pattern of HSV-1 bands on autoradiographs; U, some bands were different from those of the marker HSV-1 strain F DNA.

† Not defined, undefined region of a brain for which only one sample was taken. Data are not presented for segments taken but negative for HSV-1.

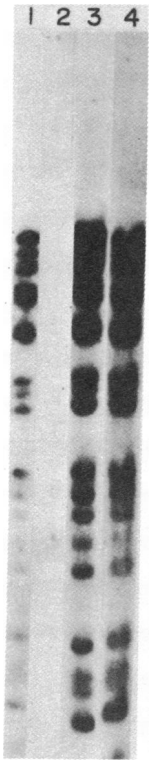

FIG. 5. HSV-1 DNA sequences in different tissues. Kidney, liver, and brain tissue were obtained from a male patient who died of trauma without clinical evidence of HSV-1 infection. Hybridization was as described in the legend to Fig. 4. Lanes: 1, control (10 μg of noninfected CV-1 cell DNA and 1 ng of HSV-1 DNA; 2, 10 μg of kidney DNA; 3, 10 μg of liver DNA; 4, 10 μg of brain DNA.

attempt to determine whether different anatomical sites harbored HSV-1 DNA, samples were analyzed from different regions of the brain. From the limited samples examined, it seems that no unique site for HSV-1 DNA exists within the central nervous system, nor is there an obvious correlation between neurological disease (multiple sclerosis) and the presence of HSV-1. Samples examined from each brain generally included cerebellum, frontal lobe, parietal lobe, cortex, and brainstem. The course of infection by HSV-1 has been thought to be spread along the nerve fibers (30, 31). However, the finding that the liver of patient 2441 was positive for the presence of HSV-1 sequences whereas the kidney was negative suggests a more traditional transport of HSV-1 from the site of invasion (skin) to the regional lymph nodes and via the bloodstream to the spleen and liver.

We thank Drs. L. Enquist and B. Roizman for providing clones of HSV-1 DNA, Jeffrey Faust and Veronica Ang for excellent technical assistance, and Dr. M. Miovic and Mses. R. Sinnis, E. Sutherland, and N. Weissbach for their contribution to the completion of this work. This work was supported by Grant NS-11037 from the National Institute of Neurological and Communicative Disorders and Stroke.

1. Goodpasture, E. W. (1929) *Medicine (Baltimore)* **8**, 223.
2. Kieff, E. D., Bachenheimer, S. L. & Roizman, B. (1971) *J. Virol.* **8**, 125–132.
3. Sheldrick, P. & Berthelot, N. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 667–678.
4. Stevens, J. G., Nesburn, A. B. & Cook, M. L. (1972) *Nature (London) New Biol.* **235**, 216–217.
5. Stevens, J. G. & Cook, M. L. (1971) *Science* **173**, 843–845.
6. Bastian, F. O., Rabson, A. S., Yee, C. L. & Tralka, T. S. (1972) *Science* **178**, 305–307.
7. Baringer, J. R. (1974) *N. Engl. J. Med.* **291**, 828.
8. Warren, K. G., Gilden, D. H., Brown, S. M., Devlin, M., Wroblewska, Z., Subak-Sharpe, J. H. & Koprowski, H. (1977) *Lancet* **ii**, 637–639.
9. Warren, K. G., Brown, S. M., Wroblewska, Z., Gilden, D. H., Koprowski, H. & Subak-Sharpe, J. H. (1973) *N. Engl. J. Med.* **192**, 282.
10. Sequiera, L., Sanasio, L., Curry, A., Jennings, L., Lord, M. & Sutton, R. (1979) *Lancet* **ii**, 609–612.
11. Cabrera, C. V., Wohlenberg, C., Openshaw, H., Rey-Mendez, M., Puga, A. & Notkins, A. L. (1980) *Nature (London)* **288**, 288–290.
12. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
13. Botchan, M., Topp, W. & Sambrook, J. (1976) *Cell* **9**, 269–287.
14. Pellicer, A., Wigler, M., Axel, R. & Silverstein, S. (1978) *Cell* **14**, 133–141.
15. Walboomers, J. M. M. & Schegget, J. T. (1976) *Virology* **74**, 256–258.
16. Clewell, D. B. & Helinski, D. B. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 1159–1166.
17. Enquist, L. W., Madden, M. J., Shiop-Stanley, P. & Vande Woude, G. F. (1979) *Science* **203**, 541–544.
18. Wahl, G. M., Stein, M. & Stark, G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3683–3687.
19. Maniatis, T., Kee, S. G., Efstratiadis, A. & Kafatos, F. C. (1976) *Cell* **8**, 163–182.
20. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Bera, P. (1977) *J. Mol. Biol.* **113**, 237.
21. Denhardt, D. (1966) *Biochem. Biophys. Res. Commun.* **23**, 641–646.
22. Walz, M. A., Mamamoto, H. & Notkins, A. L. (1976) *Nature (London)* **264**, 554–556.
23. Locker, H. & Frankel, N. (1979) *J. Virol.* **32**, 429–441.
24. Post, L. E., Conley, A. J., Mocarski, E. S. & Roizman, B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4201–4205.
25. Wetmur, J. G. (1976) *Annu. Rev. Biophys. Bioeng.* **5**, 337–361.
26. Aulakh, G. S., Albrecht, P. & Tourtellote, W. W. (1980) *Neurology* **10**, 530–532.
27. Luria, S. E., Darnell, J. E., Baltimore, D. & Campbell, A., eds. (1978) *General Virology* (Wiley, New York), p. 255.
28. Lampert, F., Bahr, G. F. & Rodson, A. S. (1969) *Science* **166**, 1163.
29. Jeffreys, A. J. & Flavell, R. A. (1977) *Cell* **12**, 1097–1108.
30. Cook, M. L. & Stevens, J. G. (1972) *Infect. Immun.* **7**, 272–288.
31. Johnson, R. T. (1964) *J. Exp. Med.* **119**, 343–356.