ing the creation of a memory were otherwise identical for the two groups. A second experimenter interviewed the subjects posthypnotically in order to ascertain their perceptions of the study and to obtain their reports of what had happened during the hypnosis session. Since there was no difference between the two groups in frequency of their response to the memory creation item, the data were pooled.

Of the 27 highly hypnotizable subjects tested, 13 accepted the suggestion and stated after hypnosis that the suggested event had actually taken place on the night they had chosen, whereas 14 did not. The latter subjects stated correctly that the noises had been suggested by the hypnotist, but a few of them reached this conclusion by quite idiosyncratic means. One, for instance, decided that the event was suggested since the noise was far more vivid than any noise that he felt could occur in reality.

Of the 13 subjects who reported the suggested memory as real, six of them were unequivocal in their certainty that the suggested event had actually occurred; the remaining subjects came to this conclusion on the basis of a reconstruction of events. One subject, for example, recalled being physically startled. She stated:

> "I'm pretty sure it happened because I can remember being startled. It's the physical thing I remember. . . . I'm making an assumption that it was a noise but I was conscious of the different cars. It must have been something like that. I can remember the startle."

Even when they were told that the hypnotist had actually suggested the noises to them during hypnosis, these subjects still maintained that the noises had actually occurred. One subject stated, "I'm pretty certain I heard them. As a matter of fact, I'm pretty damned certain. I'm positive I heard these noises."

The results support Orne's contention that the memories of victims and witnesses of crime can be modified unsuspectingly through the use of hypnosis. They suggest, further, that an initially unsure witness or victim can become highly credible in court after a hypnotic memory "refreshment" procedure. Although Orne's procedural safeguards permit evaluation of the degree to which a hypnotic procedure may have inadvertently altered a person's memory, such safeguards do not prevent such memory modification from occurring. Indeed, there is no way to differentiate what actually happened during a crime from what a person recalls of it during hypnosis, other than the obtaining of independent verification of the hypnotically elicited recall (*17*).

The pseudomemory of some loud noises is harmless when suggested in the laboratory. In the more emotionally charged investigative situation, where motivation to please is enhanced, it assumes greater importance; a pseudomemory of a trivial event that has become inadvertently connected with the events of a crime is more likely to persist in permanent memory storage and not decay in the manner of a posthypnotic suggestion. Such "recall" could lead to a false but positive identification and to all of the legal procedures and penalties that this implies. Accordingly, the utmost caution should be exercised whenever hypnosis is used as an investigative tool.

JEAN-ROCH LAURENCE*
CAMPBELL PERRY
Department of Psychology,
Concordia University,
Montreal, Quebec H3G 1M8, Canada

References and Notes

1. M. Reiser, *Handbook of Investigative Hypnosis* (Law Enforcement Hypnosis Institute, Los Angeles, 1980).
2. M. Barnes, "Hypnosis on trial" (BBC television program, London, October 1982).
3. Resolution adopted August 1979 by the International Society of Hypnosis [*Int. J. Clin. Exp. Hypn.* **27**, 453 (1979)].
4. B. Diamond, *Calif. Law Rev.* **68**, 313 (1980); D. J. Carter, *Wash. U. Law Q.* **60**, 1059 (1982).
5. C. Perry and J.-R. Laurence, *Can. Psychol.* **24**, 155 (1983).
6. J. Dywan Segalowitz and K. S. Bowers, paper presented at the 34th annual meeting of the Society for Clinical and Experimental Hypnosis, Indianapolis, October 1982.
7. M. T. Orne, affidavit to California Supreme Court on *People* v. *Shirley*, 1982 (mimeograph); J. M. Stalnaker and E. E. Riddle, *J. Gen. Psychol.* **6**, 429 (1932).
8. M. M. Gill and M. Brenman, *Hypnosis and Related States* (International Univ. Press, New York, 1959); E. R. Hilgard, *Divided Consciousness: Multiple Controls in Human Thought and Action* (Wiley, New York, 1977).
9. M. T. Orne, in *Handbook of Hypnosis and Psychosomatic Medicine*, G. D. Burrows and L. Dennerstein, Eds. (Elsevier/North-Holland, Amsterdam, 1980), pp. 29–51.
10. J. P. Sutcliffe *J. Abnorm. Soc. Psychol.* **62**, 189 (1961).
11. T. R. Sarbin and W. C. Coe, *Hypnosis: A Social-Psychological Analysis of Influence Communication* (Holt, Rinehart & Winston, New York, 1972).
12. J. R. Hilgard, *Personality and Hypnosis: A Study of Imaginative Involvement* (Univ. of Chicago Press, Chicago, ed. 2, 1979).
13. M. T. Orne, *J. Abnorm. Soc. Psychol.* **58**, 277 (1959).
14. In a recent reported legal case, a witness of a murder provided identification during hypnosis of a youth, who was subsequently prosecuted. The case was rejected by the court because of testimony that the witness had been 270 feet away from the incident in conditions of semidarkness; an ophthalmologist testified that positive identification would not have been possible beyond 25 feet under the prevailing light conditions [*People* v. *Kempinski*, No. W80CF 352 (Circuit Court, 12th District, Will County, Illinois, 21 October 1980; unreported); (*2*)].
15. M. T. Orne, *Int. J. Clin. Exp. Hypn.* **27**, 311 (1979).
16. H. Bernheim, *Hypnosis and Suggestion in Psychotherapy* (Aronson, New York, 1973; originally published in 1888).
17. C. Perry and J.-R. Laurence, *Int. J. Clin. Exp. Hypn.* **30**, 443 (1982).
18. G. Wagstaff, *Percept. Mot. Skills* **55**, 816 (1982).
19. J. P. Sutcliffe, C. Perry, P. W. Sheehan, *J. Abnorm. Psychol.* **76**, 279 (1970); C. Perry, *J. Pers. Soc. Psychol.* **26**, 217 (1973).
20. R. E. Shor and E. C. Orne. *The Harvard Group Scale of Hypnotic Susceptibility, Form A* (Consulting Psychologists, Palo Alto, Calif., 1962).
21. A. M. Weitzenhoffer and E. R. Hilgard, *The Stanford Hypnotic Susceptibility Scale, Form C* (Consulting Psychologists, Palo Alto, Calif., 1962).
22. The SHSS:C Kuder-Richardson reliability coefficient; $r = 0.85$ (*21*).
23. Hypnotizability is a stable characteristic of the individual, with from 10 to 15 percent of the population having high susceptibility, 10 to 15 percent low susceptibility, and the remaining 70 to 80 percent moderate susceptibility to various degrees [E. R. Hilgard, *Hypnotic Susceptibility* (Harcourt, Brace & World, New York, 1965); C. Perry, *Int. J. Clin. Exp. Hypn.* **25**, 125 (1977)].
24. Supported by Natural Sciences and Engineering Research Council (NSERC) of Canada grant A6361 to C.P.
* Present address: Department of Psychology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada.

4 April 1983; accepted 29 July 1983

Detection of Antibodies to Herpes Simplex Virus with a Continuous Cell Line Expressing Cloned Glycoprotein D

Abstract. *The gene for glycoprotein D of herpes simplex virus type 1 (HSV-1) was expressed in stable mammalian cell lines. Glycoprotein D produced in these cells has a number of antigenic determinants in common with the native glycoprotein. Cell lines expressing glycoprotein D were used in an enzyme-linked immunosorbent assay to detect human antibodies to glycoprotein D. This strategy should prove useful in determining the extent to which the immune response to HSV-1 is directed toward glycoprotein D.*

Analysis of the immune response to a number of infectious agents has been limited by the fact that it is often difficult to culture pathogens in quantities sufficient to permit the isolation of important cell surface antigens. The advent of molecular cloning has overcome some of these limitations by providing a means whereby gene products from pathogenic agents can be expressed in virtually unlimited quantities in a nonpathogenic form. Surface antigens from such viruses as influenza (*1*), foot-and-mouth disease (*2*), hepatitis B (*3*), vesicular stomatitis virus (*4*), rabies (*5*), and herpes simplex viruses (*6, 7*) have now been expressed in *Escherichia coli* and *Saccharomyces cerevisiae*, and, in the future, promise to provide improved subunit vaccines. However, the expression of surface anti-

gens in lower organisms is not entirely satisfactory in that potentially significant antigenic determinants may be lost through incomplete processing (for example, proteolysis or glycosylation) or by denaturation during the purification of the cloned gene product. This is particularly true of membrane proteins, which—because of hydrophobic transmembrane domains—tend to aggregate and become insoluble when expressed in *E. coli*. One solution to this problem has been to express cloned genes coding for membrane proteins in mammalian cells, where the host cell provides the factors necessary for proper processing, polypeptide folding, and incorporation into the cell membrane (*8*). We have applied this method to the expression of the glycoprotein D (gD), which is common to herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) (*9*, *10*). In this report we describe the construction of stable cell lines that constitutively express a membrane-bound form of the cloned gD protein. We used these cell lines to begin analysis of the immune response to gD in humans and animals infected with HSV-1 and HSV-2. Our results suggest that cell lines of this type may be useful for the clinical diagnosis of HSV-1 and HSV-2 infections.

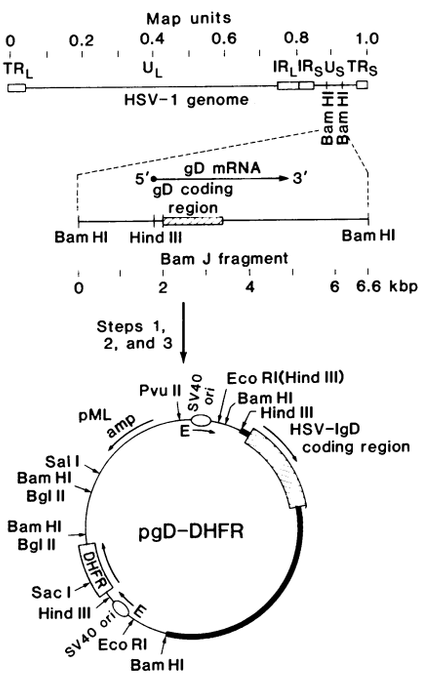

Fig. 1. Diagram of the plasmid, pgD-DHFR, constructed for the expression of HSV-1 glycoprotein D. The expression plasmid consisted of the origin of replication and the ampicillin-resistance β-lactamase gene derived from the *E. coli* plasmid pBR322 (*33*), a complementary DNA insert encoding mouse *dhfr* (*15*, *16*) under control of the SV40 early promoter and a 4.6-kbp Hind III to Bam HI fragment containing the gD gene also under control of the SV40 early promoter. The Hind III end of this fragment lies 74 bp to the 5′ side of the initiator methionine codon and includes the messenger RNA (*mRNA*) cap site. The Hind III site lies 250 bp to the 3′ side of the Goldberg-Hogness box of the SV40 promoter. The coding region of the gD-containing fragment is 1179 bp long and adjoins a large (2.9-kb) 3′ region that contains at least part of the glycoprotein E gene (*13*, *34*), a translational stop codon, and a polyadenylation site. Steps *1*, *2*, and *3* are, respectively, digestion with Hind III and Bam HI, isolation of the 4.6-kbp-encoding fragment, and ligation into the Hind III–Bam HI–cleaved DHFR vector.

The strategy we used to clone, sequence, and express the HSV-1 gD protein is briefly described in this report and presented in detail elsewhere (*7*, *11*). HSV-1 DNA was prepared from the clinical isolate Hzt p4 and was digested with the restriction endonuclease Bam HI. The genes coding for gD are localized on the short segment of the HSV-1 and HSV-2 genomes (*6*, *12*, *13*). The 6.6-kilobase pair (kbp) Bam HI fragment J, which contains the gD gene, was isolated from an agarose gel and was ligated to Bam HI–digested pBR322. The complete nucleotide sequence of the gD gene was determined by the dideoxynucleotide sequencing method after the gD-containing Bam HI fragment was subcloned into phage M-13 (*14*). The sequence obtained agrees, with the exception of five base changes, with that described by Watson *et al.* (*6*).

In order to establish cell lines that constitutively express gD, we ligated the gD gene to a mammalian expression vector (Fig. 1) that incorporated the selectable marker dihydrofolate reductase (*dhfr*) (*15*, *16*). The construction of this vector was similar to that described by Simonsen and Levinson (*15*) and included complementary DNA encoding a murine *dhfr* (*16*). The resultant vector, pgD-DHFR, was introduced into Chinese hamster ovary (CHO) cells deficient in the production of *dhfr* (*17*) by the calcium phosphate precipitation method (*18*), and colonies capable of growth in medium lacking hypoxanthine, glycine, and thymidine were selected (*15*). Nine *dhfr*-containing colonies were analyzed. Of these, gD could be detected in five by the use of antibodies to HSV-1 in radioimmunoprecipitation and indirect immunofluorescence assays (see legend to Fig. 2). One of the five lines (gD12) was designated for further study. This cell line has been kept in continuous culture for more than 5 months.

To characterize the cloned gD gene product, we metabolically labeled gD12 cells with [^{35}S]methionine and analyzed them by radioimmunoprecipitation (*19*, *20*). Autoradiographs obtained with the gD12 cell line and HSV-1–infected cells are compared in Fig. 2A. A diffuse band of 59 to 60 kilodaltons (kD) was specifically precipitated from the gD12 cell line either with rabbit polyclonal antibodies to HSV-1 (lane 4 in Fig. 2A) or with 55-S (lane 5), a monoclonal antibody to gD that is specific for the HSV-1 protein (*21*). This molecular size (59 to 60 kD) agrees with that reported for gD isolated from HSV-1–infected KB cells (*22*). The same monoclonal antibody precipitated proteins of similar molecular sizes from HSV-1–infected human cell lines. The molecular size of the major product precipitated from the HEL human embryonic lung cell line (lane 2) was 56 kD and

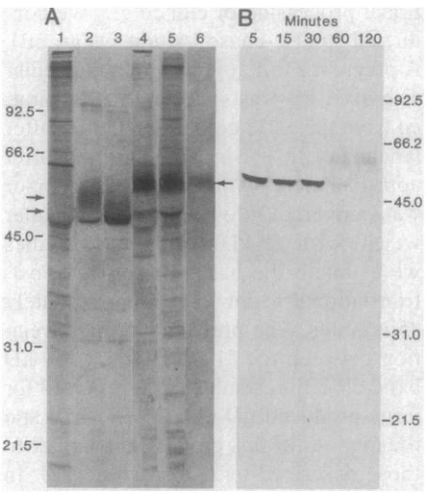

Fig. 2. Radioimmunoprecipitation of cloned gD from the gD12 cell line and native gD from HSV-1–infected human cells. (A) Control precipitation from the gD12 cell lysate with normal rabbit serum (lane 1); precipitation of native gD grown in HEL cells (lane 2) and A549 cells (lane 3) with 55-S, the monoclonal antibody to gD (*21*); precipitation of cloned gD from the gD12 cell lysate with rabbit polyclonal antibodies (Dako) to HSV-1 (lane 4), and the monoclonal antibody 55-S (lane 5); precipitation of cloned gD from the gD12 cells metabolically labeled with [^{3}H]glucosamine with rabbit polyclonal antibodies to HSV-1 (lane 6). Numbers in the margins indicate the molecular sizes (in kilodaltons) of protein standards; arrows on the left indicate the apparent molecular sizes of gD precipitated from HSV-1–infected HEL cells (56 kD) and A549 cells (53 kD); arrow on the right indicates the apparent molecular size of gD precipitated from gD12 cells (59 kD). (B) Immunoprecipitation of cloned gD from gD12 cells with rabbit antibodies to HSV-1 (Dako) at various times after pulse labeling with [^{35}S]methionine. Cells were grown to confluence in 10-cm tissue culture dishes and labeled with [^{35}S]methionine for 15 minutes at 37°C (*19*); the cells were then washed twice with fresh medium and incubated at 37°C for the indicated times. The reactions were terminated by solubilizing the cells as described above. Proteins immunoprecipitated at the following times after short-term labeling: lane 1, 5 minutes; lane 2, 15 minutes; lane 3, 30 minutes; lane 4, 60 minutes; and lane 5, 120 minutes.

that precipitated from the human lung carcinoma (A549) was 53 kD (lane 3). Earlier studies (*23*) have shown that the molecular size of HSV glycoproteins varies depending on the host cell and that these differences arise from differences in glycosylation. To determine whether the gD protein produced in CHO cells was, in fact, glycosylated, we metabolically labeled the cells with [^{3}H]glucosamine. Because bands of identical molecular size (lanes 5 and 6) were precipitated after metabolic labeling with [^{35}S]methionine or [^{3}H]glucosamine, we concluded that the gD protein produced in CHO cells is glycosylated.

To explore further the posttranslational processing of cloned gD, we conducted "pulse-chase" studies (Fig. 2B). A precursor form of gD with a molecular size of 51 kD was specifically precipitated from the gD12 cell line 5 minutes after labeling with [^{35}S]methionine and after approximately 60 minutes this precursor was converted into the higher molecular weight form (59 kD). From these studies we estimate the half-time for this posttranslational event to be approximately 45 minutes. The precursor-product relation between the 51-kD band and 59-kD band closely resembles that reported for virus-produced gD (*10, 22, 24, 25*), and the kinetics of this process are similar to those described by Cohen *et al.* (*22*). In virus-infected cells the difference in molecular weights between the precursor and the product has been attributed to both *N*-linked and *O*-linked oligosaccharides (*26*).

To determine whether gD was exported to the cell surface, we conducted indirect immunofluorescence studies. In these studies, rabbit, mouse, and human antibodies to HSV were allowed to react with unfixed cells under conditions that do not disrupt the cell membrane (*27*). Comparison of the phase-contrast images with the fluorescence images showed that the gD12 cells were heavily labeled, whereas the parental CHO cells bound little or no labeled antibody (data not shown). In control experiments with normal mouse serums, normal rabbit serums, or human serums known to be negative for HSV antibodies, no specific labeling of the cells could be detected. These studies suggested that the gD was exported to the cell surface.

To determine whether gD12 cells expressed antigenic determinants relevant to human HSV-1 and HSV-2 infections, we examined the binding of antibodies from individuals known to have antibodies to HSV-1 or HSV-2 (*28*). Radioimmunoprecipitation of lysates from metabolically labeled gD12 cells gave results comparable to those obtained with rodent antiserums to HSV. Similarly, human antiserums to HSV-1 showed specific labeling of gD12 cells in an indirect immunofluorescence assay and did not label the parental CHO cell line. Taken together, the results obtained with various rodent antiserums to HSV-1 and HSV-2, monoclonal antibodies to gD, and human antiserums to HSV provide evidence that gD expressed on the surface of gD12 cells has a number of antigenic determinants in common with the native virus and that the structure of these determinants is not dependent on interactions with other HSV-1 proteins. The fact that one of the monoclonal antibodies tested (1-S) neutralizes HSV-2 in vitro (*21*) and in vivo (*29*) demonstrates that the gD produced in CHO cells has at least one of the neutralizing antigenic determinants in common with the native virus.

A quantitative measure of the binding of antibodies against HSV to gD12 cells was attained with the development of an enzyme-linked immunosorbent assay (ELISA) (*30, 31*). In these studies, gD12 cells and CHO cells were plated into alternate wells of 96-well microtiter tissue culture plates and chemically fixed. Various antiserums known to contain antibodies to HSV were then serially diluted and allowed to react with the fixed cells (*30*). The bound antibodies were detected by use of a second antibody (immunoglobulin G) labeled with horseradish peroxidase. At the end of the assay, the absorbance of the material in each well was measured, and normal binding curves were constructed. The specific binding of antibodies to the gD12 cells was determined by subtracting the values obtained with the parental CHO cells from those obtained from the gD12 cells. Specific binding by high-titer serums could be detected at dilutions of 1:10,000.

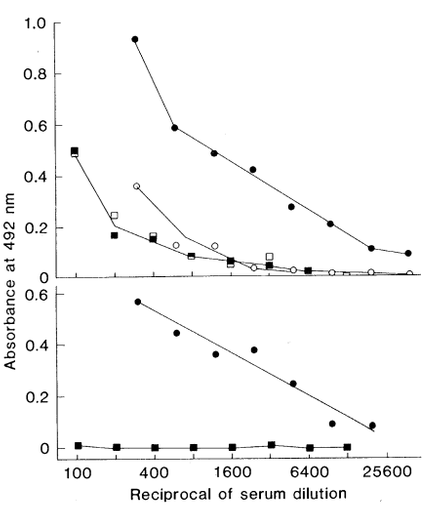

Fig. 3. Binding of human antibodies against HSV to gD12 cells and the parental CHO cell line. Human serums previously titered (*28*) against HSV by conventional assays [inhibition of hemagglutination (IHA) or complement fixation (CF)] were serially diluted into wells of microtiter plates containing either gD12 cells or the parental CHO cell line; the binding of antibodies to gD was monitored in an ELISA assay (*30*). The serum represented by the open and closed circles exhibited an HSV-1 CF titer of 128 and HSV-1 and HSV-2 IHA titers of 4096. The serum represented by open and closed squares exhibited an HSV-1 CF titer of < 8 and HSV-1 and HSV-2 IHA titers of < 8; (A) (●) and (■) binding to gD12 cells; (○) and (□) binding to CHO cells; (B) (●) and (■) specific binding to gD12 cells calculated by subtraction of the values in (A).

To determine whether the gD12 ELISA assay might serve as the basis for a clinical assay to monitor the serum concentration of antibodies to HSV, we have begun to compare serum titers determined by the ELISA assay with HSV-1 and HSV-2 antibody titers determined by conventional methods (*32*). Figure 3 shows that a serum with a high titer for antibody to HSV determined by conventional assays gave a high ELISA titer, whereas another serum with a low titer for antibody to HSV gave no detectable binding in the gD12 ELISA. In preliminary studies with ten authentic herpetic serums (*28*), we found a good correlation between the titers determined by the ELISA and the titers determined by inhibition of hemagglutination and by complement fixation. These studies suggested that the titers of antibodies to the HSV gD protein alone parallel the titers of antibodies to the spectrum of HSV proteins monitored in the conventional virus-based assays.

The studies described show that stable cell lines which constitutively express on their surface a transfected gene product can be used to analyze the immune response to an integral membrane protein. The gD12 cell line will be useful in determining the extent to which the immune response to herpes simplex viruses are keyed to the gD protein and may prove useful in the clinical diagnosis of HSV-1 and HSV-2 infections. The possibility of developing diagnostic reagents based on clonal cell lines is appealing because it eliminates the need for the culture and containment of infectious agents while providing a stable, well-defined, reproducible source of antigen. When a cell-based diagnostic system is configured in the form of an ELISA, antibody determination can be performed in 2 hours or

less and require less than 50 μl of serum. It is expected that cell lines expressing the surface antigens of infectious agents can be configured into simple chlorometric tests that could be performed in a physician's office or a clinical laboratory. In principle, the strategy we have described could be applied to any situation where the expression of a membrane protein is desired.

PHILLIP W. BERMAN
DONALD DOWBENKO
LAURENCE A. LASKY
Department of Vaccine Development, Genentech, Inc., South San Francisco, California 94080
CHRISTIAN C. SIMONSEN
Department of Molecular Biology, Genentech, Inc.

References and Notes

1. J. S. Emtage, W. C. A. Tacon, G. H. Catlin, B. Jenkins, A. G. Porter, N. H. Cary, *Nature (London)* **283**, 171 (1980); A. R. Davis, D. P. Nayak, M. Veda, A. L. Hiti, D. Dowbenko, D. G. Kleid, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5376 (1981); I. Weiland and M. J. Gething, *Nature (London)* **292**, 851 (1981).
2. H. Kupper *et al.*, *Nature (London)* **289**, 555 (1981); D. G. Kleid *et al.*, *Science* **214**, 1125 (1981).
3. P. Charnay, E. Mandart, A. Hampe, F. Fitoussi, P. Tiollais, F. Galibert, *Nucleic Acids Res.* **7**, 335 (1979); P. Valenzuela, A. Medina, W. J. Rutter, G. Ammerer, B. D. Hall, *Nature (London)* **298**, 347 (1982); R. A. Hitzeman *et al.*, *Nucleic Acids Res.* **11**, 2743 (1983).
4. J. K. Rose and A. Shafferman, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6670 (1981).
5. E. Yelverton, S. Norton, J. F. Obijeski, D. V. Goeddel, *Science* **219**, 614 (1983).
6. R. J. Watson, J. H. Weis, J. S. Salstrom, L. W. Enquist, *ibid.* **218**, 381 (1982).
7. L. Lasky and D. Dowbenko, in preparation.
8. M. J. Gething and J. Sambrook, *Nature (London)* **293**, 620 (1981); J. K. Rose and J. E. Bergman, *Cell* **30**, 753 (1982); R. S. Goodenow, M. McMillan, A. Orn, M. Nicolson, N. Davidson, J. A. Frelinger, L. Hood, *Science* **215**, 677 (1982); C. W. Crowley, C-C. Liu, A. D. Levinson, *Mol. Cell. Biol.* **3**, 44 (1983).
9. C. Sim and D. H. Watson, *J. Gen. Virol.* **19**, 217 (1973).
10. G. H. Cohen, M. Katze, C. Hydrean-Stern, R. J. Eisenberg, *J. Virol.* **27**, 172 (1978).
11. L. A. Lasky, D. Dowbenko, C. Simonsen, P. W. Berman, in preparation.
12. W. T. Ruyechan, L. S. Morse, D. N. Knipe, B. Roizman, *J. Virol.* **29**, 677 (1979).
13. G. T.-Y. Lee, M. F. Para, P. G. Spear, *ibid.* **43**, 41 (1982).
14. F. Sanger, S. Nicklen, A. R. Colson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977); J. Messing, R. Crea, P. H. Seeburg, *Nucleic Acids Res.* **9**, 309 (1981).
15. C. C. Simonsen and A. D. Levinson, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2495 (1983).
16. J. H. Nunberg, R. J. Kaufman, A. C. Y. Chang, S. N. Cohen, R. T. Schimke, *Cell* **19**, 355 (1980).
17. G. Urlaub and L. A. Chasin, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4216 (1980).
18. F. Graham and A. van der Eb, *Virology* **52**, 456 (1973).
19. The human cell lines A549 and HEL were grown to confluence in 3.5-cm tissue culture dishes and infected with HSV-1 at a multiplicity of 10 plaque-forming units per cell. Virus-infected cells were labeled by a method similar to that described by Cohen *et al.* (*10*). The medium was removed 4 hours after infection, and the cells were washed once with fresh medium (Dulbecco's modified Eagle's medium) and once with phosphate-buffered saline (PBS). Fresh medium containing one-tenth the normal concentration of methionine was then added to the cells along with [^{35}S]methionine (~1450 Ci/mmole) to a final concentration of 75 μCi per milliliter of medium. The cells were grown an additional 20 hours and then harvested by treatment of washed cells with PBS containing EDTA (0.02 percent). Viral proteins were solubilized in lysis buffer consisting of PBS, 3 percent NP-40, 1 percent bovine serum albumin, 5×10^{-5} *M* phenylmethylsulfonyl fluoride, and apoprotinin at 0.017 trypsin international unit per milliliter. The resultant lysate was clarified by centrifugation at 12,000*g* in a microcentrifuge. The gD12 cells were grown to confluence in 10-cm tissue culture dishes, washed twice with PBS, labeled with [^{35}S]methionine, harvested, and solubilized in lysis buffer as described above. For immunoprecipitation reactions the cell or virus lysates were diluted threefold with PBS, mixed with 2 to 5 μl of the appropriate antiserum and incubated for 30 minutes at 4°C. Antibody-antigen complexes were removed from the reaction medium by the addition of 25 μl of fixed *Staphylococcus aureus* (10 percent solution) and were precipitated by centrifugation at 12,000*g* for 30 seconds (*20*). The *S. aureus* cells were then washed three times with wash buffer (PBS, 1 percent NP-40, 0.3 percent sodium dodecyl sulfate), and the cells were suspended in 20 μl of polyacrylamide gel sample buffer (10 percent glycerol, 5 percent 2-mercaptoethanol, 0.0625*M* in *p*H 6.8 tris buffer, and 0.01 percent bromophenol blue) and incubated at 90°C for 3 minutes. After centrifugation (12,000*g*) for 30 seconds the supernatants were applied to 10 percent polyacrylamide slab gels (*20*).
20. S. Kessler, *J. Immunol.* **115**, 1617 (1975); U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
21. S. D. Showalter, M. Zweig, B. Hamper, *Infect. Immun.* **34**, 684 (1981). Monoclonal antibodies to gD, 1-S and 55-S, were provided by Dr. M. Zweig of the Laboratory of Molecular Oncology, National Cancer Institute, Frederick, Md. 21701.
22. G. H. Cohen, D. Long, R. J. Eisenberg, *J. Virol.* **36**, 429 (1980).
23. L. Pereira, D. Dondero, B. Norrild, B. Roizman, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5202 (1981).
24. R. W. Honess and B. Roizman, *J. Virol.* **16**, 1308 (1975).
25. P. Spear, *ibid.* **17**, 991 (1976).
26. G. Campadelli-Fiume, L. Poletti, F. Serafini-Cessi, *ibid.* **43**, 1061 (1982); D. C. Johnson and P. G. Spear, *Cell* **32**, 987 (1983); G. H. Cohen, D. Long, J. T. Matthews, M. May, R. Eisenberg, *J. Virol.* **46**, 679 (1983).
27. R. J. Bloch, *J. Cell. Biol.* **82**, 629 (1979).
28. Human herpetic serums titered against HSV-1 and HSV-2 by inhibition of hemagglutination and by complement fixation assays were provided by J. A. Stewart of the Centers for Disease Control, Atlanta, Ga.
29. J. T. Rector, R. N. Lausek, J. E. Oakes, *Infect. Immun.* **38**, 168 (1982).
30. The gD12 cells and parental CHO cells were seeded into alternate wells of 96-well microtiter tissue culture plates (Falcon Labware) and were grown to confluence in F12 medium (Gibco) containing 10 percent fetal bovine serum (dialyzed). The cells were washed three times with PBS and then were fixed with 0.0625 percent glutaraldehyde in PBS. The cells were again washed three times with PBS and stored at 4°C in PBS containing 1 percent bovine serum albumin, 100 m*M* glycine, and 1 m*M* NaN_3. To measure antibody to gD titers, the cells were washed with PBS, and serially diluted antiserums were allowed to react with the fixed cells (50 μl final volume) for 1 hour at room temperature. Unbound antibody was washed away and the cells were incubated with 50 μl of a 1:2000 dilution of goat antibody to human immunoglobulin G coupled to horseradish peroxidase (Tago). The enzyme-linked antibody was allowed to react for 1 hour at room temperature, and the cells were then washed three times with PBS. After incubation, the peroxidase substrate, *o*-phenylene diamine, was added (200 μl), and the reaction was allowed to proceed for 10 minutes. The reaction was terminated by the addition of 2.5*M* H_2SO_4 (50 μl), and the absorbance of the reaction medium from each well was determined with an automated plate-reading spectrophotometer (Titertek).
31. R. H. Kennett, in *Monoclonal Antibodies*, K. Kerrett, T. McKearn, B. Bechtel, Eds. (Plenum, New York, 1980), pp. 376–377.
32. K. L. Herrmon and J. A. Stewart, in *Manual of Clinical Immunology*, N. R. Rose and H. Friedman, Eds. (American Society of Microbiology, Washington, D.C., 1980) p. 343.
33. M. Lusky and M. Botchan, *Nature (London)* **293**, 79 (1981).
34. R. J. Watson, A. M. Colberg-Poley, C. J. Marcus-Sekura, B. J. Carter, L. W. Enquist, *Nucleic Acids Res.* **11**, 1507 (1983).
35. This is Genentech contribution No. 219.

1 September 1983; accepted 21 September 1983

Electrical Brain Stimulation and the Localization of Cardiopulmonary Function

Eldridge *et al.* (*1*) found that electrical stimulation of a subthalamic locus could induce locomotion and augment respiration simultaneously. This finding does not support their conclusion that: "Hypothalamic command signals are thus primarily responsible for the proportional driving of locomotion and respiration during exercise." Two erroneous assumptions were made in drawing this conclusion. The first is that the responses elicited by focal stimulation could be assumed to be generated by the region stimulated. The second is that the stimulus-induced hyperpnea was functionally related to the locomotion because both responses could be evoked from the same site.

The subthalamic locomotor region is located within a large, ill-defined area, encompassing much of the hypothalamus, medial thalamus, and brainstem reticular core, all of which can give pressor responses (*2*, *3*) and hyperpnea (*3–5*) when stimulated. Cohen and Hugelin (*5*), among others, found that ventilation rate increased in proportion to the strength of electrical stimulation at many widespread sites in this area. It thus seems unlikely that the hyperpnea can be ascribed specifically to stimulation of the subthalamic locomotor region.

The second issue is whether a causal or functional relation between evoked hyperpnea and evoked locomotion can be assumed. One or both responses could have arisen from stimulation of fibers of passage, from an overlapping spatial distribution of structures subserving locomotion with those subserving cardiac and respiratory functions (*6*), or from current spread to adjacent structures (*7*). For instance, urination can be evoked in conjunction with locomotion by brainstem stimulation (*8*), even though these two responses are not functionally related. A functional relation may be suggested under the circumstances if, regardless of stimulus strength and location, the locomotion