of neuronal transmission to the pineal, possibly secondary to an exhaustion of norepinephrine from nerve endings in the gland. This explanation seems unlikely for the following reasons. (i) The sympathetically mediated, exophthalmic response was maintained during the 3 hours of stimulation, and (ii) with the use of this preparation a linear fall in HIOMT activity over an 8-hour period of electrical stimulation was demonstrated (*5*). If neuronal transmission indeed remains intact, then the reduction in *N*-acetyltransferase activity after the stimulation-induced increase may be due to a process intrinsic to the pineal and not dependent on continued nerve stimulation. The response of pineal *N*-acetyltransferase to electrical stimulation may be relevant to the observation that a rhythm in *N*-acetyltransferase activity persists in blinded rats and in rats kept in constant darkness (*3*). The physiological response of pineal *N*-acetyltransferase to darkness and to electrical stimulation appears similar; after the initial rise in activity that occurs in both cases, enzymatic activity returns toward basal levels without any apparent change in afferent input to the pineal.

In vitro studies of pineal function (*3, 4*) have demonstrated that norepinephrine and dibutyryl cyclic adenosine monophosphate cause an increase in *N*-acetyltransferase activity. Norepinephrine also elevates pineal adenyl cyclase activity in vitro (*9*). However, serotonin, which is present in pineal nerves (*10*), is ineffective in raising *N*-acetyltransferase activity in vitro (*3*). Stimulation of *N*-acetyltransferase activity produced by norepinephrine and dibutyryl cyclic adenosine monophosphate can be blocked by cycloheximide (*4, 11*), an inhibitor of protein synthesis. Therefore, the rise in *N*-acetyltransferase activity in vitro is presumably mediated by a norepinephrine-stimulated adenyl cyclase–cyclic adenosine monophosphate mechanism that is dependent on protein synthesis (*11*). Our in vivo results are consistent with this hypothesis. The postganglionic sympathetic fibers to the pineal undoubtedly contain norepinephrine (*12*). Excitation of these nerve fibers by preganglionic sympathetic stimulation presumably releases norepinephrine from pineal nerve endings and, as predicted, increases activity of pineal *N*-acetyltransferase.

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References and Notes

1. R. J. Wurtman, J. Axelrod, D. E. Kelly, *The Pineal* (Academic Press, New York, 1968), pp. 58–59.
2. H. Weissbach, G. B. Redfield, J. Axelrod, *Biochim. Biophys. Acta* **43**, 352 (1960).
3. D. C. Klein and J. L. Weller, *Science* **169**, 1093 (1970).
4. D. C. Klein, G. R. Berg, J. L. Weller, *ibid.* **168**, 979 (1970).
5. M. J. Brownstein and A. Heller, *ibid.* **162**, 367 (1968).
6. C. Bernard, *Leçons sur la Physiologie et la Pathologie du Système Nerveux* (Baillière, New York, 1857), vol. 2, pp. 498–9; H. Müller, *Z. Wiss. Zool.* **9**, 541 (1858); *Verh. Phys. Med. Ges. Würzburg* 244 (1859); W. Turner, *Nat. Hist. Rev.* **2**, 106 (1862).
7. The increase in *N*-acetylated product formed can be attributed primarily to increased *N*-acetyltransferase activity and not to increased HIOMT activity. Although there were significantly greater amounts of [^{14}C]melatonin formed, *N*-acetyl[^{14}C]serotonin accounted for the major portion of the increase in *N*-acetylated product. For example, comparing 2-hour stimulated animals to decentralized controls, the amount of [^{14}C]melatonin formed showed a 36 percent increase ($P < .02$), but the amount of *N*-acetyl[^{14}C]serotonin formed showed a 249 percent increase ($P < .001$), and the ratio of *N*-acetyl[^{14}C]serotonin to [^{14}C]melatonin formed showed a 123 percent increase ($P < .01$). A decrease in HIOMT activity could also have accounted for the increase in *N*-acetyl[^{14}C]serotonin, but HIOMT activity was certainly not decreased since there was an increase in [^{14}C]melatonin formed.
8. J. A. Kappers, *Z. Zellforsch.* **52**, 163 (1960).
9. B. Weiss and E. Costa, *J. Pharmacol. Exp. Ther.* **161**, 310 (1968); *Science* **156**, 1750 (1967).
10. C. Owman, *Acta Physiol. Scand.* **63**, Suppl. 240, 1 (1964).
11. D. C. Klein and G. R. Berg, *Adv. Biochem. Psychopharmacol.* **3**, 241 (1970).
12. A. Bertler, B. Falck, C. Owman, *Acta Physiol. Scand.* **63**, Suppl. 239, 1 (1964); D. E. Wolf, L. T. Potter, K. C. Richardson, J. Axelrod, *Science* **138**, 440 (1962); W. Bondareff and B. Gordon, *J. Pharmacol. Exp. Ther.* **153**, 42 (1966).
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Subacute Sclerosing Panencephalitis: Isolation of Suppressed Measles Virus from Lymph Node Biopsies

Abstract. *Measles virus was isolated in mixed cultures of lymph node cells and HeLa cells. The agent was isolated by cocultivation from biopsy specimens of two of five patients with subacute sclerosing panencephalitis. The virus was identified by hemagglutination-inhibition, immunofluorescent, and neutralization tests. Biopsies from controls did not show evidence of measles virus.*

Subacute sclerosing panencephalitis (SSPE), a fatal disease of children and young adults, has been shown to be associated with a suppressed measles virus infection of the central nervous system (*1–4*). One characteristic laboratory finding in SSPE is an extremely high concentration of measles antibodies in the serum and spinal fluid of virtually all known cases of this chronic neurological disease (*5, 6*). Despite the presence of circulating antibodies, the disease inexorably progresses to death.

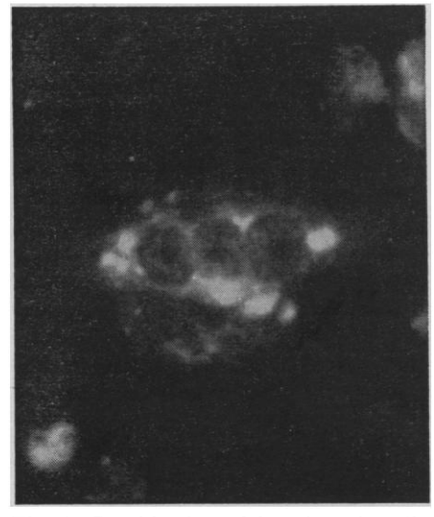

Fig. 1. Cocultivation of lymph node cells and HeLa cells demonstrating measles antigen by immunofluorescence (× 540).

The present report demonstrates that the infection is not limited to the central nervous system and suggests that SSPE may be associated with a specific defect of cellular immunity.

Lymph node biopsies from five patients with SSPE and five controls were included in this study (*7*). Although these specimens were obtained at different times, they were handled and processed in a similar fashion. Clinical and laboratory data supported the diagnosis of SSPE.

A part of each node was washed in Hanks balanced salt solution and minced into small fragments which were then suspended in Eagle's minimum essential medium (EMEM) enriched with 20 percent fetal bovine serum (FBS). After 3 days the cultures showed fibroblasts growing out of the edge of the explants and nonattaching cells which were presumed to be lymphocytes dispersed in the medium. Both types of cells appeared free of

virus inclusions or virus cytopathic effects, or both, and showed an overall normal morphology when fixed and stained with hematoxylin and eosin. The nonattached cells were decanted from the culture, centrifuged, and resuspended in Roswell Park Memorial Institute (RPMI-640) culture medium with 20 percent FBS. This cell suspension was used to prepare mixed cultures with the Ohio strain of HeLa cells (*8*) which is routinely grown in our laboratory in spinner cultures. The mixed cultures contained approximately five HeLa cells for each node cell, with a total of 10^5 cells per milliliter. These cultures were incubated at 37°C and, once confluence of the cells was observed, monolayers were maintained in EMEM with 2 percent FBS. The white cells appeared to be attached to or trapped by the HeLa cells and could be easily differentiated from the latter under phase microscopy. Table 1 lists the isolations obtained from the patients included in this study.

With the cultures from SSPE patient No. 3, foci of syncytia were seen scattered throughout the monolayers and developed into characteristic measles-produced giant cells within 2 to 3 days. In these cultures hemadsorption took place with rhesus monkey erythrocytes, while control cultures did not hemadsorb. Fluids from the mixed cultures hemagglutinated rhesus monkey erythrocytes with a hemagglutinating titer (HA) of 1 : 16. This HA activity was inhibited by serially diluted serums from SSPE patients as well as by antiserums from patients convalescing from measles, but not by serums from the same patients taken during early stages of the disease. In addition, immunofluorescent assays (FA) with these mixed cultures, in which paired serums from natural measles were used, showed specific intracellular viral fluorescence (Fig. 1). Disrupted cells and fluids from these cultures were centrifuged to remove cell debris and the resultant supernatant was used to inoculate HeLa monolayers. Typical and extensive cytopathic effects were produced on the second or third day of incubation. The virus suspension obtained therefrom was tested for hemagglutinins and infectivity, and showed an extremely high titer of 1 : 64 in HA and an infectivity titer of $10^{8.0}$ per milliliter or more in African green monkey kidney tissue culture.

Measles virus was also isolated from lymph node cells of SSPE patient No. 5, which were mixed with HeLa cells. However, in this case, the agent switched from its suppressed form to a fully replicative stage only after two subcultures of the mixed culture, hence suggesting that this isolate was somewhat less virulent than the previous one. In fact, this proved to be the case by HA and infectivity assays in which the virus isolated by cocultivation from patient No. 5 yielded an HA titer of 1 : 2 and an infectious titer of $10^{3.5}$ per milliliter, even after two additional passages in HeLa cultures.

Table 1. Isolations of measles virus from lymph node biopsies of SSPE patients.

Patient	Age	Sex	Isolation
No. 1 (D.F.)	13	F	−
No. 2 (R.S.)	7	M	−
No. 3 (M.H.)	12	F	+
No. 4 (C.T.)	11	F	−
No. 5 (K.F.)	10	F	+

Filtration experiments performed with both isolates established that the released viruses from the mixed cultures could pass Millipore membranes with pore sizes of 0.8 and 0.45 μm. Membranes with a pore size of 0.22 μm retained most of the virus. Neutralization tests, in which we used serial dilutions of SSPE and normal serum samples with 10 and 100 $TCID_{50}$ (tissue culture infective dose, 50 percent effective) of these isolates and a standard Edmonston strain of measles virus, showed that all these viruses were equally neutralized by equivalent dilutions of serums.

Tissue from each node was prepared for electron microscopy by fixation in cacodylate-buffered 2 percent glutaraldehyde, postfixing in Dalton's chrome-osmium, and embedding in Epon and Araldite. Ultrathin sections were cut and double-stained with uranyl acetate and lead citrate. Intracytoplasmic filamentous structures, suggestive of paramyxovirus nucleoprotein, were seen in the node tissue from SSPE patient No. 3. These filaments, however, could not be considered as conclusive evidence of myxoviruses since reticular fibers may have similar configurations.

Tissue culture and electron microscopy studies performed with the five control lymph node biopsies from children and young adults failed to show evidence of measles virus.

It is of interest that the two isolations were obtained from patients showing early clinical symptoms of the disease, while the patients from which measles virus could not be isolated were in later stages of SSPE, with severe mental and motor impairment.

One hypothesis that emerges from this observation is that a chronic intracellular viremia persists during the incubation period of SSPE. Since naturally occurring measles produces viremia, it is not unreasonable to postulate that in SSPE patients this viremia may continue intracellularly by virtue of a specific deficiency in cell immunity, either congenital or measles-induced. These measles-carrier white cells would eventually move into the lymph node and then finally into the central nervous system to initiate the neurological phase of SSPE.

The data presented here reveal that the suppressed measles virus infection observed in SSPE is not restricted to the brain alone. Moreover, the presence of a latent, intracellular measles virus in the lymph node cells of two patients suggests a tolerant infection with specific defectiveness of the cellular immunity. The presence of an aberrant immunological condition warrants further and detailed examination of the immune competence of SSPE immunocytes, particularly in the presence of measles antigens.

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References and Notes

1. L. Horta-Barbosa, D. A. Fuccillo, J. L. Sever, W. Zeman, *Nature* **221**, 974 (1969).
2. L. Horta-Barbosa, D. A. Fuccillo, W. T. London, J. T. Jabbour, W. Zeman, J. L. Sever, *Proc. Soc. Exp. Biol. Med.* **132**, 272 (1969).
3. F. E. Payne, J. V. Baublis, H. H. Itabashi, *N. Engl. J. Med.* **281**, 585 (1969).
4. J. R. Lehrich, M. Katz, L. B. Rorke, G. Barbanti-Brodano, H. Koprowski, *Arch. Neurol.* **23**, 97 (1970).
5. J. L. Sever and W. Zeman Eds., *Neurology* **18** (No. 1, pt. 2), whole issue (1968).
6. L. Horta-Barbosa, H. Krebs, A. Ley, T. C. Chen, M. R. Gilkeson, J. L. Sever, *Pediatrics* **47**, 782 (1971).
7. The following persons provided biopsies of patients with SSPE: Dr. John L. Sever, National Institute of Neurological Diseases and Stroke (patient No. 1); Dr. Wolfgang Zeman, Indiana University Medical School (patient No. 2); Dr. Claude Ballenger, Hospital of the Infant Jesus, Quebec City (patient No. 3); Dr. Ray Duncan, University of Florida (patient No. 4); and Dr. Anthony Iannone, Medical College of Ohio (patient No. 5).
8. The Ohio strain of HeLa cells was supplied by Dr. Robert Buscho, National Institute of Arthritis and Infectious Diseases.

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