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20 September 1968; revised 30 December 1968

Subacute Sclerosing Panencephalitis: Propagation of Measles Virus from Brain Biopsy in Tissue Culture

Abstract. *Measles virus was propagated in monolayer cultures established from brain tissue of a patient with subacute sclerosing panencephalitis. Syncytial cells were rendered fluorescent with measles specific antiserums only, by means of an indirect technique. The ultrastructural appearance of the microtubular aggregates was identical in brain tissue and in the cultured cells. Fusion experiments produced a cytopathic effect in human embryonic kidney and VERO cell cultures. The virus was identified by hemagglutination-inhibition, but only in the supernatant of disrupted cultured cells.*

Subacute sclerosing panencephalitis (SSPE) is a disease of children and adolescents. Intellectual deterioration or convulsive disorders are the usual presenting signs followed by motor disturbances of the extrapyramidal type and myoclonic jerks. The electroencephalogram presents synchronous, slow-frequency, high-voltage waves. Spinal fluid and serum contain increased amounts of immunoglobulin G, frequently associated with M-gradients and with abnormalities in the precipitation arc of gamma globulin (*1*). Although Dawson suspected a viral etiology as early as 1933 (*2*), it was not until 1965 that measles virus was incriminated on the basis of electron microscopic observations (*3*). This assumption has been supported by immunological findings: high levels of measles antibodies in serum, spinal fluid, and brain tissue extracts (*4*); and demonstration of intracellular measles antigen with fluorescein-labeled measles antibody (*5*).

Two groups of investigators succeeded in transmitting an encephalitogenic agent from brains of patients to experimental animals, but the agent was not identified as measles virus (*6*). Recently, propagation of measles virus has been achieved in tissue culture (*7*). We now confirm and extend these observations.

The patient, an 8-year-old white boy, exhibited the characteristic evolution and clinical picture of SSPE, except that he allegedly had neither been exposed to measles nor vaccinated against the virus. He had serum titers of measles antibodies of 1 : 512 for hemagglutination-inhibition and 1 : 64 for complement-fixation. The patient developed delayed hypersensitivity to homologous skin grafts and to dinitrochlorobenzene, but no delayed response was observed after intradermal injections of live and of killed measles virus (*8*).

A sample of brain tissue was obtained through a right frontal craniotomy on 4 October 1968. The brain appeared grossly normal, but histologic examination revealed the diagnostic findings of SSPE: intranuclear inclusion bodies of Cowdry type A, lymphoplasmocytic perivascular and interstitial infiltration, and microglial proliferation. Staining of acetone-fixed frozen sections with fluorescein-labeled rabbit antiserum to human gamma globulin (*9*) produced fluorescent staining of perivascular and interstitial mononuclear cells. No immunofluorescence studies were done on the biopsy tissue. Intranuclear and intracytoplasmic aggregates of microtubules, having a diameter of 20 to 22 nm, were identified by electron microscopy (Fig. 1).

A piece of brain tissue measuring 0.8 by 0.5 by 0.4 cm, including cortex and white matter, was washed four times in Puck's saline A (*10*) and minced into small pieces. Tissue fragments were incubated in 0.25 percent trypsin (*11*) at room temperature and stirred slowly. After 15 minutes the supernatant was transferred to a test tube containing an equal amount of culture medium NCTC 109 with 20 percent fetal calf serum (*10*). Fresh

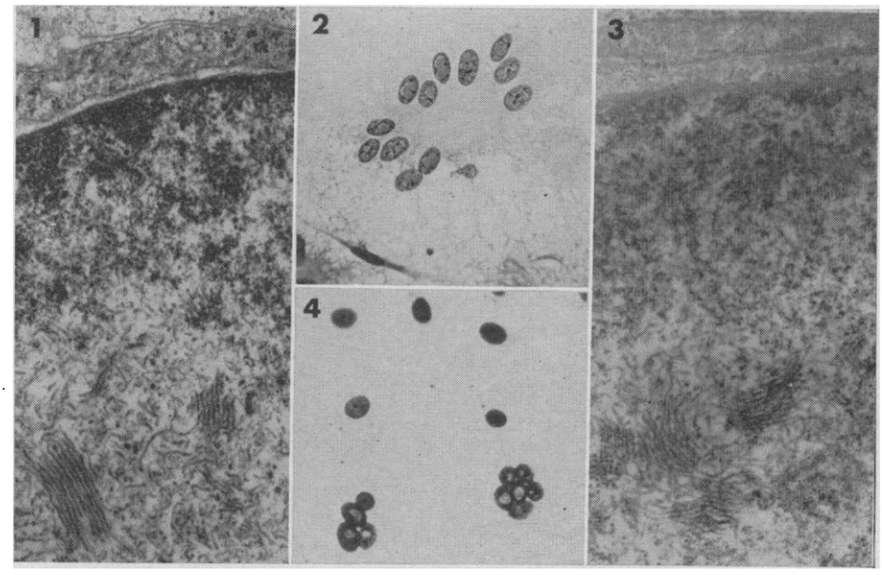

Fig. 1. Electron micrograph of brain biopsy showing oligodendrocytic nucleus with microtubules representing the ultrastructural correlate of the Cowdry type A inclusion body (× 18,100). Fig. 2. Syncytial giant cell in secondary culture of brain cells from biopsy specimen showing eosinophilic intranuclear inclusion bodies (hematoxylin and eosin stain; × 240). Fig. 3. Electron micrograph of syncytial giant cell from secondary culture of brain cells showing intranuclear aggregates of microtubules (× 21,300). Fig. 4. Two syncytial giant cells with intranuclear inclusion bodies of Cowdry type A. VERO cell culture inoculated with infected brain cells (Giemsa stain; × 160).

trypsin solution was added to the remaining tissue fragments, which were incubated for another 15 minutes and stirred. The supernatant was again transferred to test tubes containing an equal amount of culture medium with 20 percent calf serum. Both samples were centrifuged gently at 800 rev/min for 2 to 3 minutes. Pellets were resuspended in the culture medium and left standing to allow settling of undigested fragments; the supernatant was then decanted and isolated cells were counted in a hemocytometer. The suspension was adjusted to 10^5 cells per milliliter. Leighton tubes were filled with 1 ml of cell suspension each and incubated at 37°C in a gas phase of 95 percent air and 5 percent CO_2. The medium was replaced every 2 to 3 days.

After 1 week of incubation, complete monolayers were formed, and 1 week later, in all 20 cultures, multiple areas of lysis developed, lined by multinucleated syncytial giant cells. Their nuclei contained from one to five eosinophilic inclusion bodies (Fig. 2). Some primary cultures were subcultured on the 10th day and these secondary cultures showed the same cytopathic effect as did subsequent cultures, at present in the eighth passage, usually by the 4th day of incubation. With the use of electron microscopy, intranuclear and cytoplasmic aggregates of microtubules were demonstrated which had the same dimensions and a strikingly similar arrangement to those observed in the biopsy specimen (Fig. 3).

Fresh primary and secondary cultures of brain cells were fixed in absolute ethanol, incubated with the serum of the patient, monkey and goat antiserums to measles, and with the serum of another patient with SSPE having measles antibody titers of 1 : 128 (hemagglutination-inhibition) and of 1 : 1024 (complement-fixation). The slides were washed in phosphate buffer and incubated with fluorescein-labeled rabbit antiserum to human gamma globulin. The intranuclear and perinuclear inclusion bodies of the syncytial giant cells displayed selective fluorescence, except in goat serum preparations.

In fusion experiments, 10^5 isolated VERO and human embryonic kidney cells per milliliter were mixed with 10^3 infected brain cells per milliliter and cultured without addition of promoting agents. These experiments produced a cytopathic effect in the foreign cell lines after approximately 10 days (Fig. 4), but neither infectivity nor hemagglutination was shown in secondary cultures by transmission of the supernatant of brain cell and VERO or human kidney cell cultures. Hemagglutination of the red cells of green monkeys with a titer up to 1 : 32 occurred when the supernatant of infected cultures, repeatedly frozen and thawed, was used as the antigen, and the effect could be inhibited by dilute human, monkey, and goat antiserums to measles. Subcutaneous or intraperitoneal injection of disrupted cultured VERO cells produced hindleg paralysis and loss of hair in three of 18 gnotobiotic mice (*12*).

In the meantime, free infective virus, transmissible to HeLa cells, has been obtained from the brain biopsy of our second patient with SSPE by mixing brain cells, cultured in the same way as described, and fused with HeLa cells (*13*).

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10. Grand Island Biological Corp., Grand Island, N.Y.
11. Diluted 1:250 in Puck's Saline A (Difco Laboratories).
12. We thank Dr. J. Lukemeyer, Indiana University Medical Center, for this information.
13. The procedure for isolation of infective measles virus will be published soon (L. Horta-Barbosa, D. A. Fucillo, J. L. Sever, W. Zeman, *Nature*, in press).
14. We thank Dr. Klaus Schell of Pitman-Moore for providing antiserums and VERO cells, and for his encouragement. We are grateful to Dr. A. S. Levine, Dept. of Microbiology, Indiana University Medical Center, and Dr. J. L. Sever, National Institute of Neurological Diseases and Strokes, for helpful discussions. Supported in part by PHS grants CA 06145 and NB 04607. I.W. is a special trainee in neuropathology, training grant NB 05450.

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Purines: Active Transport by Isolated Choroid Plexus

Abstract. *Purines are actively accumulated by the isolated rabbit choroid plexus by a specific saturable transport mechanism. In vivo this system probably serves to excrete the purine catabolities of brain from cerebrospinal fluid into blood.*

A specific deficiency of the hypoxanthine phosphoribosyltransferase (E.C. 2.4.2.8), which catalyzes the conversion of hypoxanthine to nucleotide, is associated with profound neurological and behavioral disturbances (Lesch-Nyhan syndrome) (*1*). It has been speculated that uric acid concentrations in blood (and brain) are positively correlated with intelligence (*2*). However, little is known of the mechanisms by which the amounts of purine in brain and cerebrospinal fluid are regulated.

The study of transport by isolated choroid plexus has provided evidence for the existence of specific transport processes which export drugs and certain anions from cerebrospinal fluid to blood (*3*, *4*). Our data obtained with the isolated choroid plexus of the rabbit indicate that this tissue possesses a specific, saturable transport system for purines of high capacity.

Choroid plexuses from the lateral ventricles of female rabbits (1 kg) were incubated at 37°C in Krebs-Henseleit bicarbonate buffer containing C^{14}-labeled purines and other additives. The incubation was performed at 37°C in small stoppered tubes. The tubes were rotated to promote mixing of the tissue

Table 1. Inhibition of xanthine accumulation by various chemical analogs.

Analog	Inhibition (%)
Hypoxanthine	89
Guanine	80
Adenine	76
6-Methyluracil	79
Uracil	66
Pentobarbital	0
Allantoin	0
Allopurinol*	35
Urate	20

* [4-Hydroxypyrazolo(3,4-d)pyrimidine].