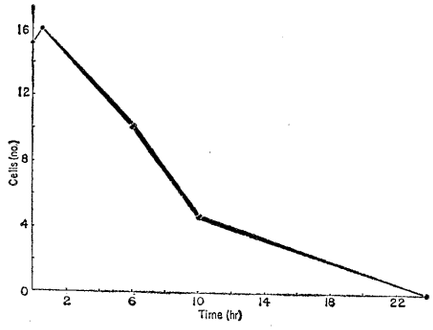


Fig. 1. Effect of aluminum sulfate ($10^{-3}M$) on the number (ordinate) of cells entering prophase in barley roots. Mean values from three "squash" preparations stained with acetocarmine.

(Table 1). In the aluminum-treated roots there are two apparent anomalies. The specific activity of the high-molecular-weight DNA is similar to that of the high-molecular-weight DNA from the actively dividing control roots. In the absence of cell division only slight incorporation of $^{32}P_i$ might have been expected. Also the newly synthesized DNA of this fraction is metabolically labile, resembling in this respect the low-molecular-weight DNA fraction. Thus it appears that the DNA synthesized during the period in which isotope was added is in fact of the low-molecular-weight form and that a part of this exists hybridized with the high-molecular-weight DNA. If this is so two requirements must be met. The base composition of newly synthesized DNA associated with the high-molecular-weight DNA must resemble that of the low-molecular-weight DNA in aluminum-treated roots, but not in untreated ones. This we have verified experimentally by determining base compositions by distribution of $^{32}P$ in the deoxynucleotides. The percentages of guanine plus cytosine in the control of low- and high-molecular-weight DNA's were 52 and 42, respectively; the corresponding percentages in the treated low- and high-molecular-weight DNA's were 51 and 53, respectively. For these determinations the barley DNA fractions were mixed with calf-thymus DNA as a carrier and treated with NaOH (0.3 *M*, 18 hours at 37°C, and 10 minutes at 70°C) to hydrolyze RNA. After acid precipitation, the DNA was digested with crystalline deoxyribonuclease (*p*H 5.0, 25°C) and then with purified snake-venom phosphodiesterase (*p*H 8.8, 37°C) (*9*). The deoxynucleotides were fractionated on Dowex-formate column (*10*), with a linear gradient of ammonium formate (*p*H 4.2, 300 ml of 0.1*M* to 300 ml of 1.0*M*). Five ml of 1*N* formic acid was added to the mixing vessel after the elution of deoxyadenylic acid. The distribution of $^{32}P$ followed that of deoxynucleotides, thus the radioactivity measured was derived from DNA.

Table 1. Specific activity of DNA fractions from aluminum-treated and untreated barley roots after a 4-hour label with carrier-free $^{32}P_i$ and again after a 48-hour "chase."

| DNA fraction | Radioactivity (count/sec) per μg DNA-P | |
|---|---|---|
| | Label | Chase |
| LMW control* | 107 | 10 |
| HMW control† | 24 | 22 |
| LMW treated | 71 | 18 |
| HMW treated | 26 | 7 |

* LMW, low-molecular-weight DNA having a base composition and chromatographic behavior on a MAK column similar to that of a wheat-root DNA fraction with a molecular weight of 2 to 3 × $10^5$. † HMW, high-molecular-weight DNA which, by the same two criteria, resembles a wheat-root DNA fraction with a molecular weight of 4 to 6 × $10^6$ (*5*).

Table 2. Refractionation of heat denatured DNA fractions labeled with $^{32}P$ from aluminum-treated and untreated barley roots. G, guanine; C, cytosine.

| DNA fraction | Al treatment | Amount μg DNA-P | Radioactivity per μg DNA-P (count/sec) | G+C (mole %) |
|---|---|---|---|---|
| | | *Eluted by NaCl* | | |
| LMW | — | 2.9 | 22 | 52 |
| HMW | — | 1.6 | 5.6 | |
| LMW | + | 4.8 | 15 | 51 |
| HMW | + | 5.3 | 28 | 52 |
| | | *Eluted by NaOH* | | |
| LMW | — | 0.5 | 24 | |
| HMW | — | 11.2 | 6 | 42 |
| LMW | + | 0.5 | 16 | |
| HMW | + | 18.5 | 1.2 | 51 |

The second requirement is that after separation of the hybrid into its components, these parts should behave chromatographically as two distinct molecular species. One should contain most of the total radioactivity and behave like low-molecular-weight DNA, the other should contain most of the total DNA, and behave like high-molecular-weight DNA. That this is in fact so has been verified. DNA may be rendered single-stranded by heating and subsequent cooling (*11*). We have heated DNA (3 μg DNA-P per ml) in distilled water for 2 minutes at 100°C. After rapid cooling the solution was stirred with MAK in a chromatography column containing one volume of NaCl (0.1*M*) in phosphate buffer (0.05*M*, *p*H 6.7). The column outlet was closed while the DNA adsorbed to the MAK. After 5 minutes the column was allowed to drain and it was then used in the normal manner. In this way the tendency of the DNA to become renatured is reduced. Low-molecular-weight DNA treated in this manner can be eluted with NaCl (2.5*M*) in phosphate buffer (0.05*M*, *p*H 6.8) whereas the high-molecular-weight DNA, which is resistant to elution with NaCl, can be eluted with NaOH (0.5*M*). Table 2 shows the results of such refractionation of high- and low-molecular weight DNA extracted from aluminium treated and untreated barley roots and meets the second requirement. Base compositions were determined for all fractions as described above and are given in Table 2.

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### References and Notes

1. E. Hutchinson, *Soil Sci.* **60**, 29 (1945).
2. D. Clarkson, *Ann. Bot.*, in press.
3. B. L. Hartwell and F. R. Pember, *Soil Sci.* **6**, 259 (1918).
4. D. I. Arnon and C. M. Johnson, *Plant Physiol.* **17**, 525 (1942).
5. M. Sampson, A. Katoh, Y. Hotta, H. Stern, *Proc. Nat. Acad. Sci. U.S.* **50**, 459 (1963).
6. M. Sampson and D. D. Davies, in preparation.
7. J. D. Mandell and A. D. Hershey, *Anal. Biochem.* **1**, 66 (1956).
8. D. I. Arnon, *Amer. J. Bot.* **25**, 322 (1938).
9. R. Sinsheimer, *J. Biol. Chem.* **198**, 293 (1952).
10. E. S. Canellakis and R. Mantsavinos, *Biochim. Biophys. Acta* **27**, 643 (1958).
11. J. Marmur and D. Lane, *Proc. Nat. Acad. Sci. U.S.* **46**, 453 (1960).
12. Aided by fellowships, to M.S. from Shell Chemicals, and to D.C. from the Department of Scientific and Industrial Research.

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## Particles Resembling Papova Viruses in Human Cerebral Demyelinating Disease

Abstract. *Degenerated brain tissue obtained from a deceased patient who had progressive multifocal leukoencephalopathy had been fixed in formalin prior to processing for electron microscopy. In ultrathin sections virus-like particles resembling papova virions were frequently observed in glial nuclei. Correlation with light-microscopy findings suggests that demyelination resulted from the cytocidal effect of the virus on oligodendroglia.*

Progressive multifocal leukoencephalopathy (PML) is a rare human demyelinating disease first reported in 1958 by Åström, Mancall, and Richardson (*1*). Only about 35 cases have

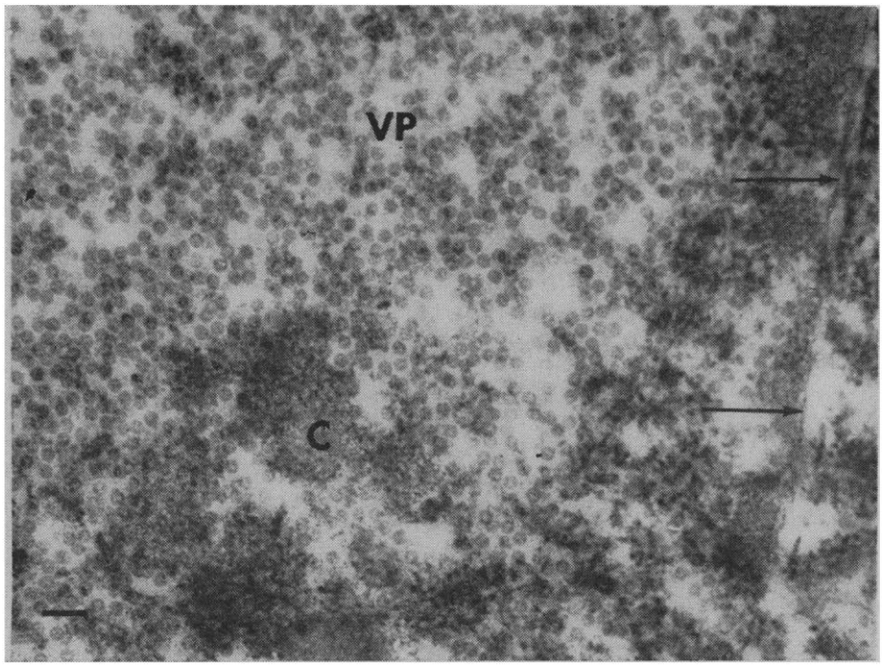


Fig. 1. Ultrathin section of portion of glial nucleus in degenerated cerebral white matter. Arrows indicate nuclear membrane. *VP*, virus-like particles scattered throughout major portion of nucleoplasm. *C*, residual nuclear chromatin. The bar equals 100 mμ (about × 56,100).

been reported from North America, Europe, and Australia. With few exceptions, this disease is superimposed, during the last several months of the patient's life, upon a chronic disease, such as leukemia, malignant tumors, or tuberculosis (*2*). A unique combination of glial cytopathology has led several authors to suggest a viral etiology for the disease (*2, 3*). Impairment of normal immunologic responses secondary to the chronic background diseases has been considered (*2, 3*). In the absence of the correct diagnosis prior to death, no fresh material has so far become available for virus isolation.

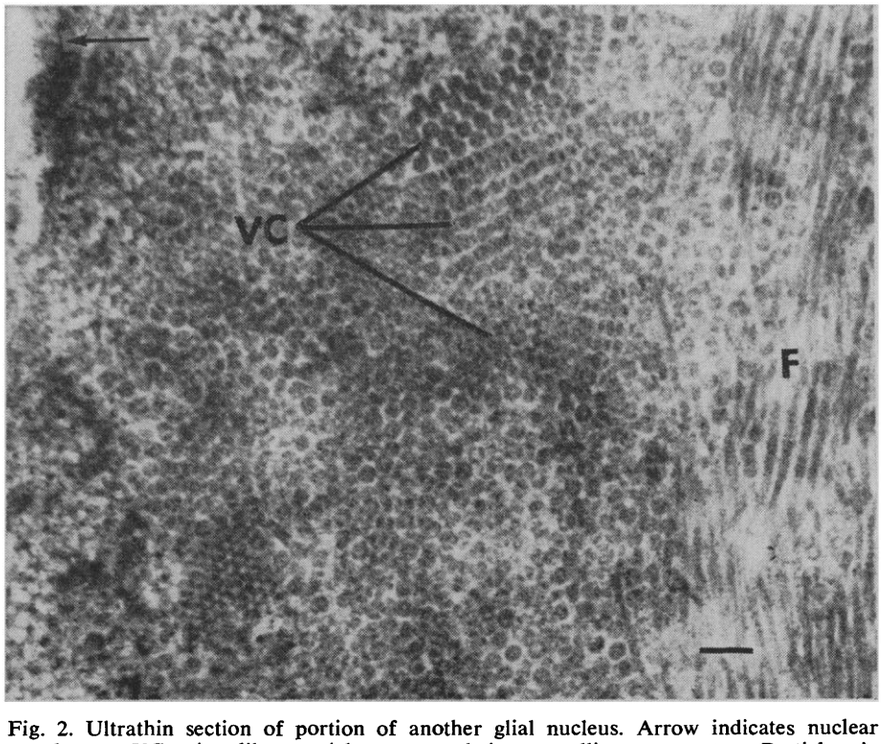


Fig. 2. Ultrathin section of portion of another glial nucleus. Arrow indicates nuclear membrane. *VC*, virus-like particles arranged in crystalline aggregates. Particles in two crystals blurred in contour due to partial overlapping. *F*, filamentous structures arranged in a bundle. The bar equals 100 mμ (about × 71,400).

We checked for the presence of a virus by performing, for the first time, an electron-microscope examination of sections from such a diseased brain. The autopsy material, obtained 1½ hours after death, originated from a 67-year-old woman afflicted with bronchial asthma for 30 years and with progressive paralysis of her left limbs, mental deterioration, and blurred speech for the last 7 months of life. At the time of death from pulmonary complications, multiple small and large areas of cerebral white matter were degenerated. Prior to electron microscopy the brain slices had been kept for 2 years in 10 percent formalin (*p*H 6.0).

Discrete 1-mm demyelinated grey areas beyond the periphery of major lesions were selected for study. With light-microscope examination the former had yielded the highest concentration of oligoglial cells with enlarged, deeply basophilic nuclei, suggestive of viral infection. The pieces were fixed again for 2½ hours in phosphate-buffered osmium tetroxide, dehydrated in an ethanol series, and embedded in Epon 812. Sections were cut on a Porter-Blum microtome, stained with either lead citrate or uranyl acetate, and examined in RCA EMU 3D and 3G electron microscopes (*4*).

The glial nuclei were the best-preserved tissue components. At magnifications ranging from 18,700 to 35,700 virus-like particles were detected in a majority of these nuclei (*5*). The shape of the particles varied, as did the arrangement and the density of distribution in a given nucleus. Most frequently, spherical particles of rather uniform size (33 to 36 mμ) and medium electron opacity filled the nuclei in random distribution and in abundance, with residual chromatin mostly marginated along the nuclear membrane (Fig. 1).

Multiple collections of tens or hundreds of such particles were scattered in other nuclei with unusually opaque and coarsely granular chromatin. Crystalloid arrays of particles (Fig. 2)

were seen less often. If multiple, they were usually set at sharp angles to each other. On rare occasions, single, large, centrally located crystalloid arrangements of innumerable particles occupied from one-half to two-thirds of the sectioned plane of a nucleus.

The peculiar streaked pattern of such a formation was the first abnormality observed when sections were screened at a magnification of about 6000. Particles in crystalloids were either oblong or hexagonal and frequently showed an electron-lucent center. Further variations in shape suggested overlapping or tilting of particles (Fig. 2). The distance from center to center was 40 mμ. Filamentous structures about one-half to two-thirds the diameter of the spherical particles were found in combination with the above forms (Fig. 2). They were arranged in strands or in whorls, or were scattered singly. Electron-lucent centers were distinct on cross sections.

Subsequent electron-microscope studies performed on grossly nondegenerated cerebral white matter of this patient and on white matter of another elderly woman's brain, kept for 2 years in 10 percent formalin, revealed none of the aforementioned nuclear abnormalities.

The morphology of the particles described, as well as their arrangement and intracellular location, resembles most closely the members of the papova virus group. This virus group was proposed as a separate entity by Melnick in 1962; it was enlarged and further defined in 1963 by a study group of the virus subcommittee of the International Nomenclature Committee for Bacteria and Viruses (see *6*).

Man is considered the natural host of only one established member of the papova virus group: the human wart virus. Aside from cutaneous papillomata, no lesions have been attributed to this agent. The mouse is the natural host of two members of the group, polyoma virus and K-virus; and the rabbit and monkey are hosts of one member each, the Shope papilloma virus and the simian vacuolating agent, respectively.

Serologic studies for polyoma antibodies in human volunteers, conducted by Huebner parallel to serology of local mouse populations, yielded no significant results (see *7*). Short-term effects on man produced by simian virus 40 (SV 40), injected or ingested inadvertently with poliomyelitis vaccines, were summarized by Fraumeni (*8*). Melnick commented in 1962 that no illness possibly related to this virus had been reported so far (*9*).

Numerous, randomly distributed, spherical virus particles (30 to 50 mμ) are present in nuclei of tissues infected with any member of the papova virus group (*10, 11*). Intranuclear, ordered, crystalline arrays have been reported for four of the five members (*10, 11*), and elongated structures, seen in purified preparations of all papova viruses, were found in cell nuclei infected by polyoma virus (*11*). Tumorigenicity has been proven for the four initial members of the group (*6*). Under laboratory conditions, inflammation or degeneration may result from infection (*12*), but no cerebral demyelinization has been observed in animals.

The case history of this patient indicated (*13*) that warts had been removed from her fingers four times during the last 6 years of her life, that she had lived about 1500 feet (500 m) from the city dump, and that an old shed on her premises was probably at times frequented by rats and mice. The woman had not received any polio vaccine.

In the light microscope, presence of abnormal oligoglial cells characterized the earliest incompletely demyelinated foci. Neither normal nor abnormal oligoglia were found within the older, larger, totally demyelinated lesions. Previously, authors have concluded that the primary alteration of progressive multifocal leukoencephalopathy occurred in the oligoglial cells with secondary demyelination (*14*). The role of oligodendrocytes as sole maintainers of adult mammalian central myelin has not been proven. Studies on myelin formation and experimental demyelination has led some electron microscopists to advocate such metabolic relationship (*15*). On the assumption that the particles observed actually are virions, it is suggested that the demyelination is produced by the metabolic and eventually cytocidal effect of the virus on oligoglial cells and that this demyelination follows massive intranuclear replication. The suggested virus may be an established member of the papova group or an as yet biologically undiscovered agent. The possibility of a chance presence of virus seems slight because of the excessive number of intranuclear particles in diseased areas, their absence in nondiseased tissues, and their observation in all three cases of this disease which have been studied by means of the electron microscope (*4, 5*).

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### References and Notes

1. K. E. Åström, E. L. Mancall, E. P. Richardson, Jr., *Brain* **81**, 93 (1958).
2. E. P. Richardson, Jr., *New Engl. J. Med.* **265**, 815 (1961).
3. J. B. Cavanagh, D. Greenbaum, A. H. E. Marshall, L. J. Rubinstein, *Lancet* **1959-II**, 524 (1959); B. H. Waksman and R. D. Adams, *J. Neuropathol. Exptl. Neurol.* **21**, 491 (1962).
4. More detailed information about this case, as well as the clinical history and the light- and electron-microscope findings of a second case of PML, will be reported in the forthcoming vol. 44 of the Association for Research in Nervous and Mental Disease, proceedings of the 1964 conference on infections of the nervous system (Williams and Wilkins, Baltimore, Md.).
5. This finding has been independently confirmed by L. Silverman and L. J. Rubinstein, Stanford University School of Medicine, Palo Alto, Calif., in a study of nonformalinized brain tissue of a recent autopsy case.
6. J. L. Melnick, *Science* **135**, 1128 (1962); Sir C. Andrewes, *Viruses of Vertebrates* (Baillière, Tindall, and Cox, London, 1964), pp. 196–210.
7. R. J. Huebner, W. P. Rowe, J. W. Hartley, W. T. Lane, in *Proceedings, Ciba Foundation Symposium on Tumour Viruses of Murine Origin*, G. E. W. Wolstenholme and M. O'Connor, Eds. (Little, Brown, Boston; Churchill, London; 1962), p. 329.
8. J. F. Fraumeni, F. Ederer, R. W. Miller, *J. Am. Med. Assoc.* **185**, 713 (1963).
9. J. L. Melnick and S. Stinebaugh, *Proc. Soc. Exptl. Biol. Med.* **109**, 965 (1962).
10. R. S. Stone, R. E. Shope, D. H. Moore, *J. Exptl. Med.* **110**, 543 (1959); G. B. Chapman, L. M. Drusin, J. E. Todd, *Am. J. Pathol.* **42**, 619 (1963); N. Granboulan, P. Tournier, R. Wicker, W. Bernhard, *J. Cell Biol.* **17**, 423 (1963); A. J. Dalton, L. Kilham, R. F. Zeigel, *Virology* **20**, 391 (1963).
11. A. F. Howatson and J. D. Almeida, *J. Biophys. Biochem. Cytol.* **7**, 753 (1960).
12. S. E. Stewart, B. E. Eddy, N. Borgese, *J. Natl. Cancer Inst.* **20**, 1223 (1958); E. L. Jungherr, V. J. Cabasso, M. Stebbins, *J. Neuropathol. Exptl. Neurol.* **22**, 512 (1963).
13. We are indebted for this information to Frank C. Iber, M.D., Stevens Point, Wis.
14. E. Christensen and M. Fog, *Acta Psychiat. Scand.* **30**, 141 (1955); A. N. D'Agostino, G. L. Pease, J. W. Kernohan, *J. Neuropathol. Exptl. Neurol.* **22**, 138 (1963).
15. E. De Robertis and H. M. Gerschenfeld, *Intern. Rev. Neurobiol.* **3**, 1 (1961); S. A. Luse, *Res. Publ. Assoc. Nervous Mental Disease* **40**, 1 (1962).
16. Supported in part by the Multiple Sclerosis Society (Fellowship 94–2) and aided by grant GM 09387 from the National Institutes of Health and by Public Health Service research grant B-3533.
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