Neurotropic Virus–Host Relationship Alterations Due to Variation in Viral Genome as Studied by Electron Microscopy

Cedric S. Raine, PhD and Bernard N. Fields, MD

A series of experiments has been described in which litters of suckling rats were inoculated either with wild-type reovirus type III or one of two of its temperature-sensitive (*ts*) mutants. While the wild-type virus produced an acute, fatal syndrome, the *ts* mutants were substantially less neurovirulent. Of the *ts* mutant-inoculated animals, a large percentage of the surviving (chronic) animals given *ts* mutant *B* showed an unobstructive hydrocephalus *ex vacuo* whereas chronic *ts* mutant C animals showed no visible nervous system disease. The *ts* mutants persisted within the central nervous system (CNS) for 6 to 8 weeks, after which they could not be detected either virologically, immunologically or morphologically. In another set of experiments, organized CNS explants were studied following infection with either measles virus or the neuroadapted Mantooth strain of SSPE virus, a variant of measles. Wild measles (Edmonston strain) exerted an acute destructive effect, but SSPE virus had a tendency to enter into coexistence with the tissue without destroying its organotypic nature. These relationships are somewhat reminiscent of the neuropathologic conditions caused by these two viruses in man. Since the reovirus type III *ts* mutants possess both genetic and morphologic defects and in many instances cause CNS conditions different from that induced by the wild-type virus, it has been proposed that a comparable situation may exist after measles and SSPE virus infection. SSPE virions of the strain studied were found to be defective in certain viral components which may have contributed to the lower neurovirulence and its entering into a chronic relationship with the CNS, in contrast to the acute destructive nature of measles infection. The findings are discussed in terms of relevance to other chronic CNS diseases, particularly multiple sclerosis, in which the possiblity exists that a mutant virus is operative (Am J Pathol 75:119–138, 1974).

WITH THE ADVENT of more refined virologic, immunologic and morphologic technics for the demonstration and rescue of latent viruses, numerous reports have appeared etiologically linking a number

From the Departments of Pathology (Neuropathology), Medicine and Cell Biology, and The Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY.

Supported in part by Grants NS-08952, NS-03356, NS-06375 and 1 R01-A10326-03 from the National Institutes of Health; Grants 433-D-11, 867-A-1, 555-C-3 and 692-B-2 from the National Multiple Sclerosis Society; Grants GB-27390 from the National Science Foundation and a grant from the Alfred P. Sloan Foundation. Dr. Raine is the recipient of a Research Career Development Award NS-70265 from the National Institutes of Health; Dr. Fields is supported by a Faculty Research Associate Award PRA-63 from the American Cancer Society and a career scientist award from the Irma T. Hirschl Foundation.

Accepted for publication November 11, 1973.

Address reprint requests to Dr. Cedric S. Raine, Department of Pathology (Neuropathology), Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461.

of congenital and chronic disorders of the central nervous system (CNS) to virus infections.[1-6] An understanding of the manner in which these proven or suspected chronic viral infections might have become established depends upon relating a gamut of host and viral factors.[7] The net result of such host–virus relationships is a compromised long-term situation in which the agent and the host maintain a tenuous balance. At some point in time, this latent infection is frequently stimulated by some extrinsic or intrinsic factor, resulting in the reappearance of active virus and an immune response on the part of the host. In addition to alterations in the course of infection, the neurovirulence of a latent neurotropic virus can be modified by spontaneous viral or host changes. It is known, for instance, in some latent viral infections of the CNS, that there can be recrudescence of infectious virus material (*eg*, herpes zoster). Also, the prolonged degenerative effect of a virus upon the CNS can lead to an autoimmune response in which the CNS serves as the "battle-field." These events invariably lead to a decrease in effectiveness of the host immune system to the virus and ensuing degeneration of CNS tissue. Only rarely do CNS latent viral infections remain dormant and noncytopathic, *eg*, lymphocytic choriomeningitis virus.[8]

It is generally accepted that the difference in disease patterns between acute measles encephalitis and subacute sclerosing panencephalitis (SSPE), both etiologically related to a measles virus, are manifestations either of a variation in the virus or in the host response or in both. Acute measles infection of the CNS is usually typified by short duration of disease, the production of small foci of degeneration and a good prognosis. SSPE has been linked to a latent infection by a strain of measles[6] and is a uniformly fatal condition, from which measles-type virus has been isolated.[9-12] While ongoing virologic and genetic studies in these laboratories are attempting to locate the differences between measles and SSPE viruses, this report will present morphologic data taken from CNS tissue infected with these two viruses in which it appears that certain strains of SSPE virus are defective in some measles-type components.[13] Although this work presently lacks precise genetic and biochemical data, our thesis has been supported by a related series of experiments [14,15] in which another neurotropic virus, reovirus type III with a known predilection for neurons and an affinity to coat microtubules,[16-19] and two of its biochemically and genetically well-characterized temperature-sensitive (*ts*) mutants,[20,21] were studied *in vivo*. This system affords a somewhat innovative approach to the study of viral genome *in vivo*, since, in addition to the biochemical and genetic data,

the *ts* mutants are known to induce morphologically defective viral structures [22] when grown at 39 C, a temperature nonpermissive for multiplication. Attempts have also been made to correlate the encephalitogenic properties of the various viral components using this system.

The purpose of the present communication is to collate our data from the studies on reovirus type III and its *ts* mutants *in vivo*, and measles and SSPE virus in CNS tissue *in vitro*.[13,19–21,23–26] The latter system is particularly advantageous since it permits examination of specific viral effects upon CNS tssue in the absence of an immune system. The comparative effects of these viruses and their mutants upon the CNS will be related to other CNS conditions which may be caused by altered viruses.

Materials and Methods

Reovirus Type III Experiments

Litters of 3-day-old Sprague-Dawley rats were inoculated intracerebrally with 0.03 ml of a suspension containing doses of wild reovirus type III (Dearing strain) ranging from 3×10^5 to 1×10^8 plaque-forming units (PFU)/rat.

Other litters were inoculated by the same route with 0.03 ml containing between 3×10^6 and 1×10^8 PFU/rat reovirus type III *ts* mutant 352 (B), or *ts* mutant 447 (C).[20–22]

Some suckling animals from all three groups (wild virus, B mutant and C mutant) were sacrificed following the onset of acute disease (5 to 14 days postinoculation) and perfused with 2.5% glutaraldehyde in 0.2 M phosphate buffer at pH 7.3. Coronal slices of brain tissue, 1 mm thick, were postfixed in 1% osmic acid in phosphate buffer. The tissue was then dehydrated through a graded series of ethyl alcohols and embedded flat in Epon 812.

Many animals inoculated with *ts* mutants B and C showed no signs of acute disease and were maintained for periods ranging up to 15 months postinoculation. These animals were periodically sampled for electron microscopy by perfusion with 4% phosphate-buffered paraformaldehyde followed by 5% glutaraldehyde in phosphate buffer and slices of brain were postfixed in Dalton's chrome osmium. The tissue was dehydrated in ethanol and embedded in Epon. Fuller details of the virologic data and the electron microscopy preparation procedures will be given elsewhere.[14,15]

Measles and SSPE Virus Experiments

Cultures of organized hamster cerebellar tissue were grown according to the technics of Bornstein and Murray [27] on collagen-coated coverslips [28] in Maximow slide-assemblies. After 7 days *in vitro*, the explants were fed a nutrient medium containing either a) 3×10^5 PFU/ml Edmonston strain measles virus [29] or b) 2×10^4 PFU/ml Mantooth strain SSPE virus [8] which had been adapted to grow in hamster brain tissue.[30] In each case, the virus was allowed to adsorb at room temperature for 1 hour after which the cultures were washed and given fresh virus-free nutrient medium, a modified Eagle's minimum essential medium.[25] The cultures were then incubated at 34 to 35 C and sampled between 3 and 82 days postinoculation (measles), or 3 and 60 days postinoculation (SSPE). Both cell-associated and cell-free

(in supernatant fluid) virus were assayed throughout the course of infection. Fixation for electron microscopy was carried out by immersing cultures in 2.5% glutaraldehyde for 1 hour, followed by 1 hour in 1% osmic acid. The explants, still on their coverslips, were then dehydrated in ethyl alcohol. While in 70% alcohol, each cultures was removed from the coverslip on a "window" of collagen. The explant was then processed for electron microscopy and embedded flat in Epon 812. These embedded cultures were polymerized at 60 C, and later reoriented for sectioning at right-angles to the plane of growth. Complete details of the viruses and methods employed are given elsewhere.[13,25,26]

In both the *in vitro* and *in vivo* experiments, 1-μ Epon sections were taken and stained with toluidine blue for light microscopic examination. Selected areas of some blocks were trimmed for electron microscopy, sectioned and stained with uranyl acetate and lead citrate. Grids were carbon-coated and scanned in a Siemens 101 at 80 kV.

Results

Reovirus Type III Experiments

All animals given wild reovirus type III succumbed within 14 days post-inoculation. Of the animals given B and C mutants, approximately 50% survived. Light microscopy of one micron sections of the CNS tissue showed that in all cases, the infection was highly neuronotropic (Figures 1–3). Lesions were located within the cerebral and hippocampal cortices, were essentially identical from group to group, and involved hemorrhagic necrosis. Electron microscopy of those lesions produced by wild reovirus type III complemented light microscopy. Virus factories predominated in the perikarya of cortical neurons (Figure 4), were not present in glial cells, and each consisted of a collection of virions surrounded by a fibrillar matrix. Higher magnification showed individual virions to possess an electron-dense core, 45 nm in diameter, which was surrounded by an outer capsid and a subjacent shell. Each wild-type virion was 75 nm in diameter. The virions were embedded in a background of kinky filaments. Occasionally, neurotubules in the vicinity of viral factories were coated with a granular material. The structural characteristics of these inclusions are in accord wth those previously described *in vitro* and *in vivo*.[15,17] Glial microtubules were never affected in this manner.

B mutant is known from previous *in vitro* studies [21] to assemble whole virions at 31 C (permissive temperature) morphologically indistinguishable from the wild form. At 39 C (nonpermissive), however, B mutant virions contain only RNA core material surrounded by inner capsid, 60 nm in diameter. In the present *in vivo* system, factories of B mutant virions contained a mixed population of particles, many of which lacked the outer protein shell, but some of which were apparently com-

pletely assembled (Figure 5). Since the body temperature of the rat is some 2 C lower than the temperature at which the mutation is fully expressed morphologically (39 C), the virus was probably able to assemble more components. Virologic studies performed on virus recovered from infected brain showed that B mutant retained its properties and that the ability to assemble more coat proteins in the present situation was probably due to genetic leak, and not reversion to wild form.[14,15] Several of the apparently "complete" virions had a diameter somewhat less than that of wild virus.

Coated microtubules were present, but not common, in B mutant-infected neurons. The virus was considerably less cytopathic than wild virus, and the inflammatory response to infection was less. This decrease in inflammation was also in contrast to acutely sick C mutant-inoculated rats which showed areas of necrosis approximating those appearances encountered in wild virus-treated animals. C mutant, like B mutant, is known to assemble complete virions at 31 C, but at 39 C, assembles coat proteins only, and therefore appears as an empty shell, 75 nm in diameter.[22] In infected neurons, the viral factories were large and comprised mainly empty virions although a few particles did contain cores. As in B mutant rats, this was also probably due to leak brought about by the temperature difference (Figure 6). Equally as striking as the greater encephalitogenic effects of C mutant (in comparison to B mutant), was the enormous ability for C mutant factories to cause large numbers of neighboring neurotubules to be coated with electron-dense material (Figure 7). It appeared, therefore, that the coating of microtubules was related to properties of the viral capsid, a suggestion first proposed by Dales *et al.*[31]

While the CNS of acute C mutant animals displayed a more severe pathologic response, animals chronically infected with this virus showed no abnormalities within their CNS tissue. However, chronic B mutant animals in 22 out of 52 cases went on to develop a communicating hydrocephalus which eventually progressed to cavitation of the cortical mantle, suggestive of an indolent disease (Figures 8 and 9). No lesions were seen in these brains and the ependyma was intact. Virologic studies on chronic B and C mutant-infected brains showed that B and C virus could be recovered for about 6 to 8 weeks but the CNS of animals 2½ months or more postinoculation failed to yield virus material.

Measles and SSPE Virus Experiments

Measles virus (Edmonston strain) introduced into the organotypic cerebellar culture system produced a rapid development of cytopathic

effect (CPE) during the first 3 weeks of infection. The development of giant cells heralded the beginning of CPE after about 3 days postinoculation. These syncytia were located on the surface of cultures and appeared to derive from both neurons and glial cells, but after 21 days postinoculation and longer, were exclusively astroglial in origin (Figure 10), unlike chronic SSPS cultures (Figure 11). Ultrastructural examination of measles-infected cultures between 5 and 14 days postinoculation showed the presence of replicating virus from the surface of cells and cytoplasmic aggregates of viral nucleocapsid. The replicating measles virion has been well described.[25,26,32,33] It arises from an area of cell membrane along which the tubular viral nucleocapsid (18 nm in diameter) becomes aligned and on the outside of which ridges of granular electron-dense material develop, thus giving the membrane an outer coating (Figure 12). These specialized areas of plasmalemma are extruded into the extracellular space and pinched off to become virions with a diameter of about 200 nm (Figure 13). In these CNS cultures, the process of active replication and formation of cytoplasmic viral inclusions (Figure 14) continued throughout the entire period of study. Intranuclear aggregates of viral material first appeared after about 14 days postinoculation and were constant features thereafter (Figure 15).

Neurons showed the greatest tendency to degenerate and did so within the first 3 weeks postinoculation. Oligodendroglia and myelin also degenerated, and by 25 days postinoculation, the cultures largely comprised a population of hypertrophied fibrous astrocytes and giant cells. The measles-infected cultures were able to survive in this condition up to 82 days postinoculation.

Cultures of hamster cerebellum exposed to hamster brain-adapted SSPE virus, showed a much slower development of CPE. This virus, since its original isolation [9] from a human brain biopsy, had been passaged a total of 13 times through HeLa cells, BSC-1 cells and hamster brain,[30] and virologic study has shown that it grows well in CNS cultures [13] with a growth curve comparable to that of measles.[26] Neurons in these cultures showed the earliest changes after 3 to 5 days, and frequently could be seen fusing to form giant cells. Astroglia also formed giant cells. On occasion, both neurons and glial cells fused. The membranes of the fusing cells showed no presence of viral material but elsewhere in the cultures virus replication was seen. This occurred in a manner identical to that of measles in that coated areas of cell membrane were pinched off into the extracellular space (Figure 16). In contrast to measles virus-infected CNS cultures, however, the virus

particles possessed few, if any, nucleocapsids aligned beneath the envelope (Figure 17). Infectious particles were produced throughout the entire 60-day period, as shown by virologic assay, but virions were difficult to detect, morphologically, between 15 and 35 days postinoculation. When encountered, however, they were usually located in close proximity to syncytia (Figure 18).

SSPE virus was highly neuronotropic but less cytopathic than measles virus. For as long as 52 and 60 days postinoculation, granule cells (neurons) abounded in infected explants. Purkinje cells also survived. In those cultures which survived past 21 days postinoculation, intranuclear viral inclusions were found in addition to cytoplasmic forms. These inclusions showed a predilection for nerve cells (Figures 19 and 20). Myelin and oligodendroglia persisted for at least 28 days postinoculation.

Discussion

With the present *in vivo* system using reovirus type III, it has been demonstrated that viral mutation can effect an altered course of CNS disease after intracerebral inoculation. The *in vitro* experiments with the Edmonston strain of measles virus and Mantooth strain SSPE virus (considered a variant of measles) also indicate differences in disease-producing capability.

Wild reovirus type III, at the doses used, produced a uniformly fatal acute hemorrhagic encephalitis within 14 days postinoculation. Temperature-sensitive (*ts*) mutants B and C killed only about 50% of animals. The CNS of sick B mutant animals showed the same degree of neuronotropism of the virus but the lesions were less destructive, while acutely sick C mutant animals showed acute lesions as destructive as those produced by the wild virus. All three reoviruses retained their morphologic characteristics *in vivo* except that the mutants showed a tendency to assemble more at the animal's temperature of 37 C than they do *in vitro* at 39 C.[22] C mutant had a more profound effect upon neurotubules in the vicinity of viral factories than did the other viruses. These tubules became coated with granular material, a feature now synonymous with reovirus infection.[16,17] Since this propensity to coat microtubules occurred most with C mutant, the virus which assembled mainly outer capsid proteins only, it would appear that the coating is related to some antigenic property of the viral capsid, as suggested earlier.[31]

Of the strains of wild reovirus which have been tested *in vivo* from each of the three serotypes (types I, II and III), neuronotropism is a

universal feature.[18,19,34-36] This property is particularly prominent with type III which also demonstrates the greatest degree of neurovirulence. Reovirus type I, unlike type III, frequently induces a chronic CNS syndrome which manifests itself as an obstructive hydrocephalus. This occurs as a sequela to the infection and destruction of ependyma,[37-39] after which the virus is thought to move deeper into the parenchyma. The pathogenesis of the hydrocephalus produced in the present series of experiments after the inoculation of B mutant appeared to develop as the result of a subclinical infection which effected an indolent degeneration of CNS parenchyma. There was no obstruction of the ventricular system, the ependyma lining the ventricles although attenuated, was preserved, and subependymal white matter persisted.[15] In both chronic B and C mutant-inoculated animals it was not possible to recover virus after 2 to 3 months. Therefore, the late disease in B mutant animals may evolve subsequent to an earlier subclinical neuronotropic infection which subsided but initiated a slow dropout of CNS parenchyma. It appeared in chronic C mutant animals that the infection may have been more efficiently handled by the host without lasting ramifications.

The genetic variations responsible for temperature sensitivity between wild reovirus type III and the two *ts* mutants probably resides in a single gene.[40] The present results, therefore, demonstrate dramatically how relatively minor changes in viral genome can bring about vastly different conditions *in vivo*—wild type causing a 100% fatal acute encephalitis; B mutant, in approximately 50% of surviving animals, hydrocephalus *ex vacuo;* and C mutant causing no histologic abnormalities in chronically infected animals.

Using the present *in vitro* CNS system infected with Edmonston strain measles and Mantooth strain SSPE virus, it appears that we are visualizing a situation somewhat comparable to the reovirus *ts* mutant experiments. It is generally accepted on the basis of immunologic,[41] virologic [6] and morphologic [13,42] evidence that SSPE virus is a variant or mutant of measles virus. The manner in which SSPE virus produces a chronic disease *in vivo* appears not only to be related to viral genome alone, since it has also been shown that the immunologic status and age of the animal at the time of exposure to the virus are also crucial to the development of disease.[43,44] The present *in vitro* CNS system completely eliminated the effects of the host immune system and, in our hands, it was clear that wild measles virus caused rampant degeneration [25,26] in comparison to SSPE virus.[13] In addition, it appears that, despite the fact that SSPE virus was more neuronotropic in nature,

in long-term infected cultures many neurons were preserved, suggestive of a less cytopathic, more cell-associated situation. This situation is in sharp contrast to measles infection, which in about 3 weeks reduced the tissue to a mat of hypertrophied fibrous astrocytes and syncytia.

In addition to measles encephalitis and SSPE, myxoviruses as a group embrace many other agents capable of producing spontaneous and experimental CNS disease, *eg*, influenza, mumps, parainfluenza and distemper.[45–50] It is also well known that different strains of the same myxovirus can show variation in encephalitogenic effect.[46,51] Their great propensity to infect CNS tissue renders myxoviruses promising candidates in the study of virus–CNS relationships. In man, several chronic CNS diseases of suspected viral etiology, such as multiple sclerosis (MS), display elevated myxovirus antibodies, *eg*, to measles.[52] In MS, conventional virologic and morphologic approaches have traditionally been unsuccessful in demonstrating viral material. More recently, however, ter Meulen *et al*,[3] using cell-fusion technics, have isolated a parainfluenza type I virus from MS tissue. The failure of others to detect an agent in MS previously might suggest an infection by a defective, or mutant, myxovirus. Failure to rescue myxovirus subsequent to experimental inoculation and the induction of chronic disease is not an unique phenomenon.[53,54] While the results of ter Meulen *et al*[3] await further confirmation, especially since this virus is one of the most common laboratory contaminants, they have engendered a fresh impetus in the search for a causal agent in MS. Since this work, myxovirus-like particles *in situ* in MS tissue have also been described by electron microscopy.[55–57]

One question which can be directed towards the present findings on SSPE virus in cultures of CNS tissue is to what extent has the virus been changed since its isolation from the human CNS biopsy.[9] This SSPE strain has been passaged a total of 13 times through HeLa, BSC-1 and hamster brain cells, and it is far from remotely possible that its efficacy and possibly its assembly have been altered. We are currently testing the same Mantooth strain prior to its adaptation to brain tissue. This virus isolate has been passaged only twice in HeLa cells. Preliminary results indicate the production of a lesser degree of destructive effect and fewer syncytia than the hamster brain-adapted strain but a similar viral morphology. In addition, several strains of SSPE virus that were isolated from different patients by co-cultivation with HeLa cells but were not neuroadapted are under study in CNS tissue *in vitro*. In combination with ongoing genetic and virologic characterization of measles and SSPE virus strains, our work might allow

a detailed dissection of the various properties of the measles genome and the genomes of other viruses with known neurotropic properties.

References

1. Weiner LP, Herndon RM, Narayan O, Johnson RT, Shah K, Rubinstein LJ, Preziosi TJ, Conley FK: Isolation of virus related to SV40 from patients with progressive multifocal leukoencephalopathy. N Engl J Med 286:385–390, 1972
2. ter Meulen V, Koprowski H, Iwasaki Y, Kackell YM, Muller D: Fusion of cultured multiple-sclerosis brain cells with indicator cells: presence of nucleocapsids and virions and isolation of parainfluenza-type virus. Lancet II: 1–5, 1972
3. Grose C, Feorino PM: Epstein-Barr virus and Guillain-Barré Syndrome. Lancet II:1285–1287, 1972
4. Rawls WE: Congenital rubella: the significance of virus persistence. Prog Med Virol 10:238–285, 1968
5. Stevens JG, Cook MC: Latent herpes simplex virus in neural tissue, Multiple sclerosis, Immunology, Virology and Ultrastructure. Edited by F Wolfgram, GW Ellison, JG Stevens, JM Andrews. New York, Academic Press, 1972, pp 183–192
6. Horta-Barbosa L, Hamilton R, Wittig B, Fuccillo D, Sever J: Subacute sclerosing panencephalitis: isolation of suppressed measles virus from lymph node biopsies. Science 173:840–841, 1971
7. Raine CS, Fields BN: Neurotropic viruses and the developing brain. NY State Med J 73:1169–1179, 1973
8. Lehmann-Grube F: Lymphocytic choriomeningitis virus, Virology Monographs, Vol 10. New York, Springer, 1971, pp 1–173
9. Horta-Barbosa L, Fuccillo DA, Sever JL, Zeman W: Subacute sclerosing panencephalitis: Isolation of measles virus from a brain biopsy. Nature (Lond) 221:974, 1969
10. Katz M, Oyanagi S, Koprowski H: Subacute sclerosing panencephalitis: structures resembling myxovirus nucleocapsids in cells cultured from brain. Nature (Lond) 222:888–890, 1969
11. Chen TT, Watanabe I, Zeman W, Mealey J: Subacute sclerosing panencephalitis: Propagation of measles virus from brain biopsy in tissue culture. Science 163:1193–1194, 1969
12. Payne FE, Baublis JV, Itabashi HH: Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. N Engl J Med 281:585–589, 1969
13. Raine CS, Feldman LA, Sheppard RD, Bornstein MB: Subacute sclerosing panencephalitis virus in cultures of organized central nervous tissue. Lab Invest 28:627–640, 1973
14. Fields BN, Raine CS: Altered nervous system response to temperature-sensitive mutants of reovirus type III. I. Virology. Unpublished observations
15. Raine CS, Fields BN: Altered nervous system response to temperature-sensitive mutants of reovirus type III. II. Neuropathology. Unpublished observations
16. Dales S: Association between the spindle apparatus and reovirus. Proc Natl Acad Sci USA 50:268–275, 1963

17. Spendlove RS: Unique reovirus characteristics. Prog Med Virol 12:161–191, 1970
18. Gonatas NK, Margolis G, Kilham L: Reovirus type III encephalitis: Observation of virus-cell interactions in neural tissues. II. Electron microscopic studies. Lab Invest 24:101–109, 1971
19. Raine CS, Fields BN: Reovirus tpe III encephalitis: a virologic and ultrastructural study. J Neuropathol Exp Neurol 32:19–33, 1973
20. Fields BN, Laskov R, Scharff MD: Temperature-sensitive mutants of reovirus type 3: studies on the synthesis of viral peptides. Virology 50:209–215, 1972
21. Cross RK, Fields BN: Temperature-sensitive mutants of reovirus type 3: studies on the synthesis of viral RNA. Virology 50:799–809, 1972
22. Fields BN, Raine CS, Baum SG: Temperature-sensitive mutants of reovirus type 3: defects in viral maturation as studied by immunofluorescence and electron microscopy. Virology 43:569–578, 1971
23. Fields BN: Genetic manipulation of reovirus: a model for modification of disease. N Engl J Med 28:1026–1033, 1972
24. Raine CS: Differential effects of viruses and their mutants on central nervous system development. The Neurosciences, Vol 3. Edited by FO Schmitt, F Worden. Cambridge, Mass, M.I.T. Press, 1974, pp 1043–1047
25. Raine CS, Feldman LA, Sheppard RD, Bornstein MB: Ultrastructure of measles virus in cultures of hamster cerebellum. J Virol 4:169–181, 1969
26. Feldman LA, Raine CS, Sheppard RD, Bornstein MB: Virus-host cell relationships in measles-infected cultures of central nervous tissue. J Neuropathol Exp Neurol 31:624–638, 1972
27. Bornstein MB, Murray MR: Serial observations on patterns of growth, myelin formation, maintenance and degeneration in cultures of newborn rat and kitten cerebellum. J Biophys Biochem Cytol 4:499–504, 1958
28. Bornstein MB: Reconstituted rat-tail collagen as substrate for tissue culture on coverslips in Maximow slides and roller tubes. Lab Invest 7:134–137, 1958
29. Rapp F: Plaque differentiation and replication of virulent and attenuated strains of measles virus. J Bacteriol 88:1448–1458, 1964
30. Byington, DP, Castro AE, Burnstein T: Adaptation to hamsters of neurotropic measles virus from subacute sclerosing panencephalitis. Nature 225: 554–555, 1970
31. Dales S, Gomatos PJ, Hsu KC: The uptake and development of reovirus in strain L cells followed with labeled viral ribonucleic acid and ferritin-antibody conjugates. Virology 25:193–211, 1965
32. Nakai M, Imagawa DT: Electron microscopy of measles virus replication. J Virol 3:187–197, 1969
33. Waterson AP: Measles virus: seminar on the epidemiology and prevention of measles and rubella, Paris, 15–17 June 1964. Arch Gesamte Virusforsch 16:57–80, 1965
34. Walters MN-I, Joske RA, Leak PJ, Stanley NF: Murine infection with reovirus. I. Pathology of the acute phase. Br J Exp Pathol 44:427–436, 1963
35. Margolis G, Kilham L, Gonatas NK: Reovirus type 3 encephalitis: Observations of virus-cell interactions in neural tissues. I. Light microscopy studies. Lab Invest 24:91–100, 1971
36. Jenson AB, Rabin ER, Phillips CA, Melnick JL: Reovirus encephalitis in

newborn mice: an electron microscopic and virus assay study. Am J Pathol 47:223–239, 1965

37. Kilham L, Margolis G: Hydrocephalus in hamsters, ferrets, rats, and mice following inoculations with reovirus type I. I. Virologic studies. Lab Invest 21:183–188, 1969
38. Margolis G, Kilham L: Hydrocephalus in hamsters, ferrets, rats, and mice following inoculation with reovirus type I. II. Pathologic studies. Lab Invest 21:189–198, 1969
39. Nielsen SL, Baringer JR: Reovirus-induced aqueductal stenosis in hamsters: phase contrast and electron microscopic studies. Lab Invest 27:531–537, 1972
40. Fields BN: Temperature-sensitive mutants of reovirus type 3: features of genetic recombination. Virology 46:142–148, 1971
41. Connolly JH, Allen IV, Hurwitz LJ, Miller JHD: Measles-virus antibody and antigen in subacute sclerosing panencephalitis. Lancet I:542–544, 1967
42. Oyanagi S, ter Meulen V, Mueller D, Katz M, Koprowski H: Comparison of subacute sclerosing panencephalitis and measles viruses: an electron microscope study. J Virol 7:176–187, 1971
43. Johnson KP, Byington DP: Subacute sclerosing panencephalitis (SSPE) agent in hamsters. I. Acute giant cell encephalitis in newborn animals. Exp Mol Pathol 15:373–379, 1971
44. Byington DP, Johnson KP: Experimental subacute sclerosing panencephalitis in the hamster: correlation of age with chronic inclusion-cell encephalitis. J Infect Dis 126:18–26, 1972
45. Johnson RT: Mumps virus encephalitis in the hamster: studies of the inflammatory response and noncytopathic infection of neurons. J Neuropathol Exp Neurol 27:80–95, 1968
46. Johnson RT, Johnson KP: Hydrocephalus following viral infection: the pathology of aqueductal stenosis developing after experimental mumps virus infection. J Neuropathol Exp Neurol 27:591–606, 1968
47. Johnson RT, Johnson KP: Hydrocephalus as a sequela of experimental myxovirus infections. Exp Mol Pathol 10:68–80, 1969
48. Johnson KP, Klasnja R, Johnson RT: Neural tube defects of chick embryos: an indirect result of influenza A virus infection. J Neuropathol Exp Neurol 30: 68–74, 1971
49. Raine CS: Viral infections of nervous tissue and their relevance to multiple sclerosis, Multiple Sclerosis-Immunology, Virology and Ultrastructure. Edited by F Wolfgram, GW Ellison, JG Stevens, JM Andrews. New York, Academic Press, 1972, pp 91–118
50. Wiśniewski H, Raine CS, Kay WJ: Observations on viral demyelinating encephalomyelitis: canine distemper. Lab Invest 26:589–599, 1972
51. Albrecht P, Schumacher HP: Neurotropic properties of measles virus in hamsters and mice. J Infect Dis 124:86–93, 1971
52. Adams JM, Imagawa DT: Measles antibodies in multiple sclerosis. Proc Soc Exp Biol Med 111:562–566, 1964
53. Albrecht P, Shabo AL, Burns GR, Tauraso NM: Experimental measles encephalitis in normal and cyclophosphamide-treated rhesus monkeys. J Infect Dis 126:154–161, 1972
54. Kim CS, Kriewaldt FH, Hagino N, Kalter SS: Subacute sclerosing panen-

cephalitis-like syndrome in the adult baboon (*Papio* sp.). J Am Vet Med Assoc 157(suppl):730–735, 1970

55. Prineas JW: Paramyxovirus-like particles associated with acute demyelination in chronic relapsing multiple sclerosis. Science 178:760–763, 1972
56. Ratanabe I, Okazaki H: Virus-like structure in multiple sclerosis. Lancet II:569–570, 1973
57. Raine CS, Powers JM, Suzuki K: Acute multiple sclerosis: confirmation of "paramyxovirus-like" intranuclear inclusions. AMA Arch Neurol 30:39–46, 1974

Acknowledgments

The authors thank Dr. Robert D. Terry for his valuable advice and constructive criticism throughout this study. The myxovirus work is the result of collaborative work with Rachel D. Sheppard, Dr. Lawrence A. Feldman and Dr. Murray B. Bornstein. Drs. Luiz Horto-Barbosa (NIH), Kenneth P. Johnson and Donald J. Bvington (Cleveland) supplied the SSPE virus material, for which we are extremely grateful. The excellent technical assistance of Earl Swanson, Marlene Halili, Howard Finch and Miriam Pakingan is gratefully acknowledged.

[Illustrations follow]

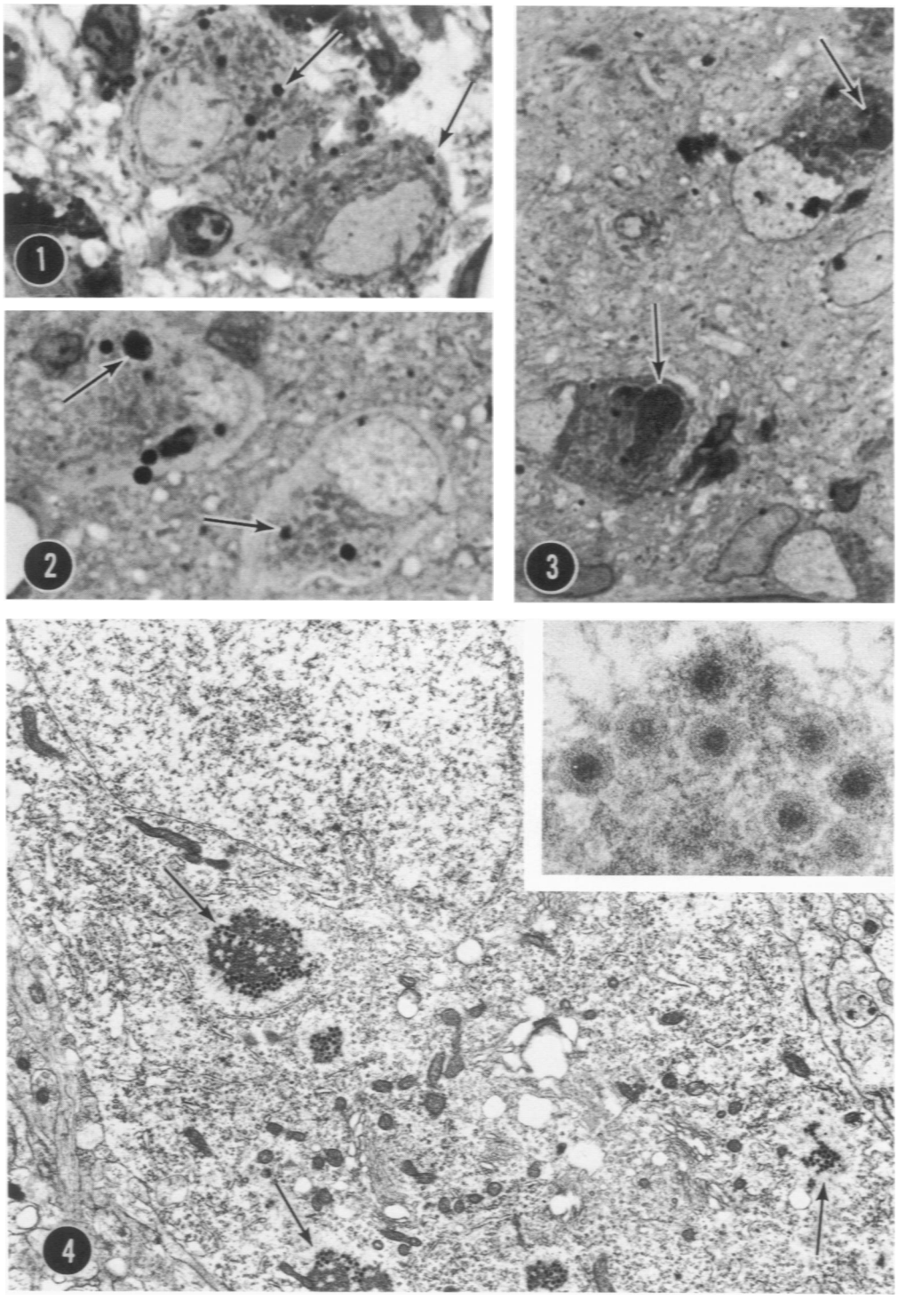
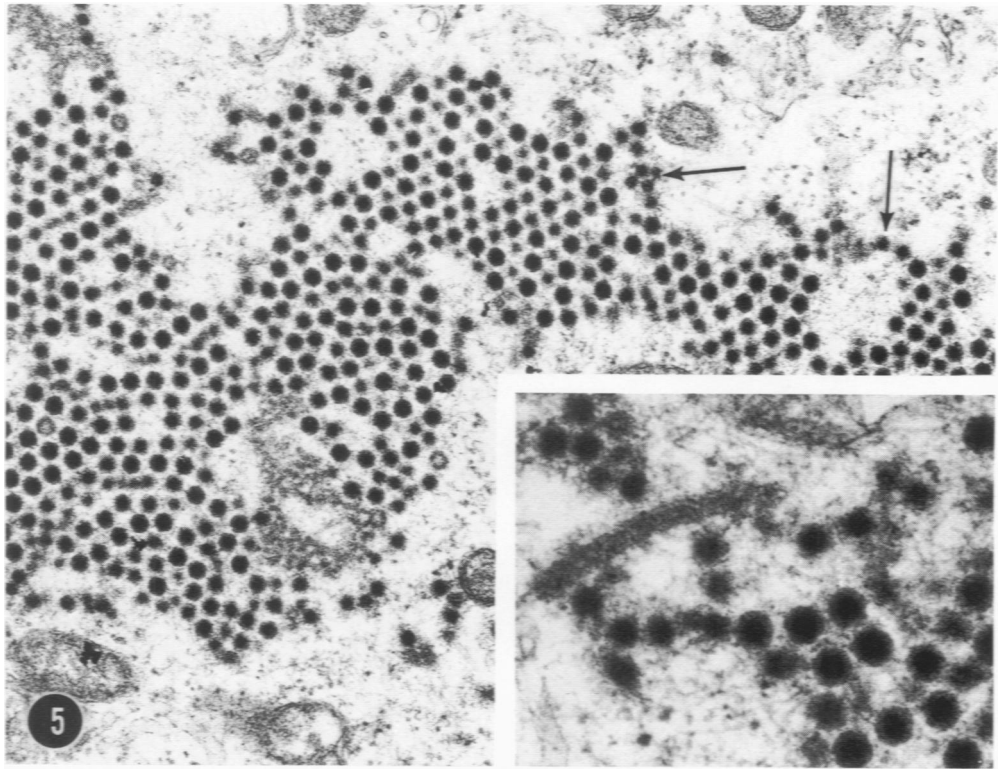


Fig 1—Wild reovirus III; 8 days postinoculation (PI). Two neurons from the hippocampal cortex show small densely staining cytoplasmic viral factories (*arrows*) (1-μ Epon section, toluidine blue stain, × 1200). **Fig 2**—B mutant reovirus; 8 days PI. Two cortical neurons contain large viral factories within their cytoplasm (*arrows*) (1-μ Epon section, toluidine blue stain, × 1200). **Fig 3**—C mutant reovirus; 8 days PI. Two cortical neurons show pale collections of viral material within their perikarya (*arrows*) (1-μ Epon section, toluidine blue stain, × 1200). **Fig 4**—An infected neuron (nucleus, *N*) from the cerebral cortex contains several small wild type viral factories (*arrows*), each of which is embedded in a fibrillar matrix. **Inset**—Detail of a group of 75-nm virions. Note double nature of the viral capsid which surrounds the RNA core. Kinky filaments surround the particles (× 10,000; **Inset,** × 100,000).



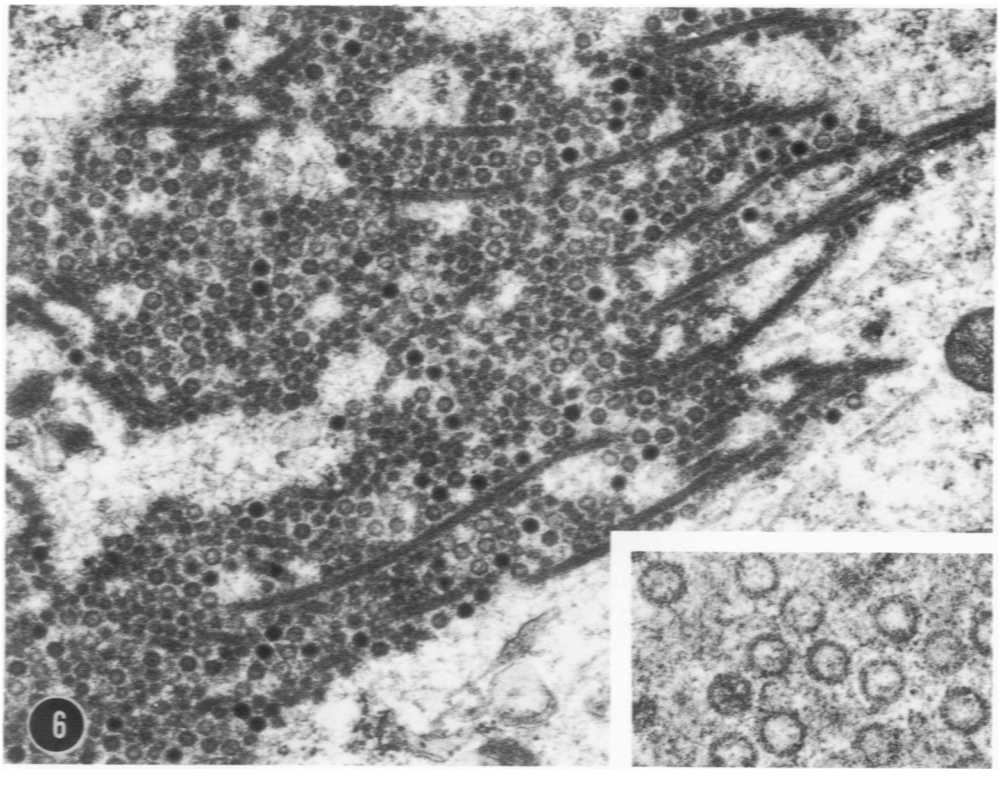
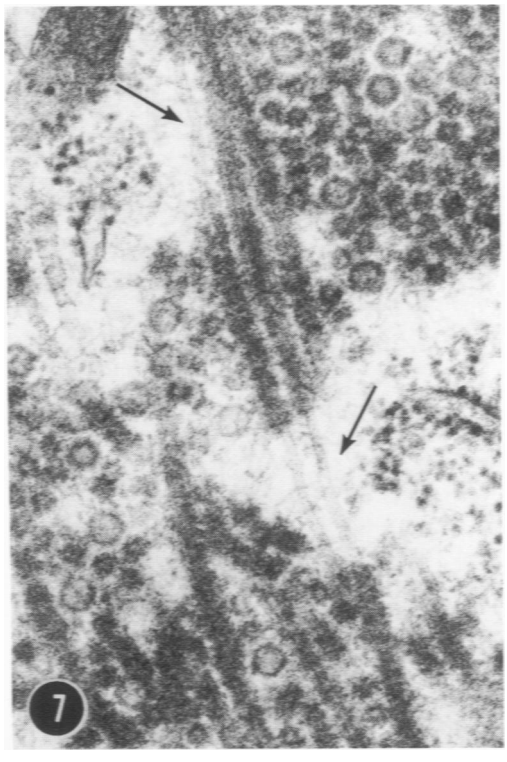
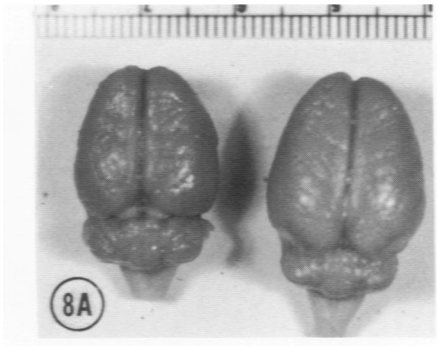
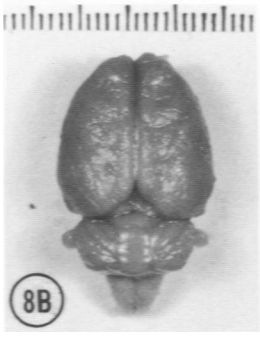
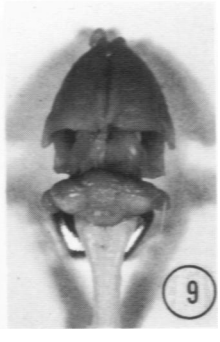


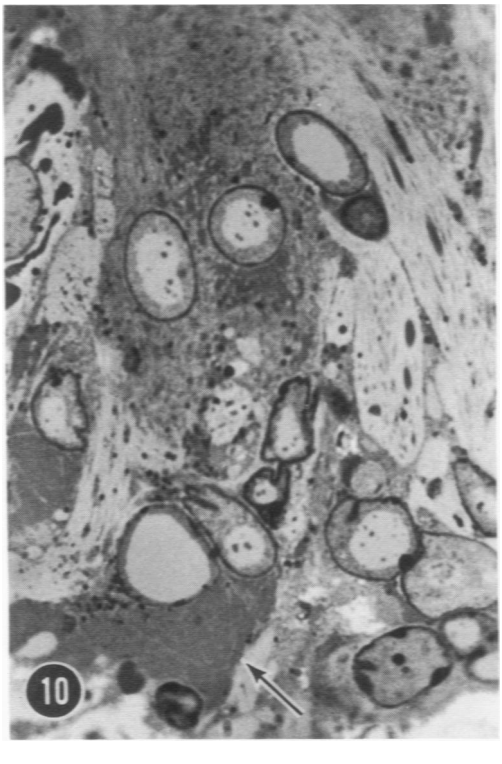
Fig 5—A B mutant factory from an infected neuron shows a mixture of complete and incomplete (mainly core material) particles (*arrows*). **Inset**—Detail of a similar inclusion which contains a coated microtubule, several particles containing only core material, and some apparently complete virions with a diameter some 5 nm less than wild reovirus type III (× 28,000; **Inset,** × 80,000). **Fig 6**—A C mutant factory within a cortical neuron 8 days postinoculation contains abundant coated microtubules between which "empty" C mutant virions can be seen. Core-containing virions are also present. **Inset**—Detail of the 75-nm mutant virions (× 40,000; **Inset,** × 100,000).











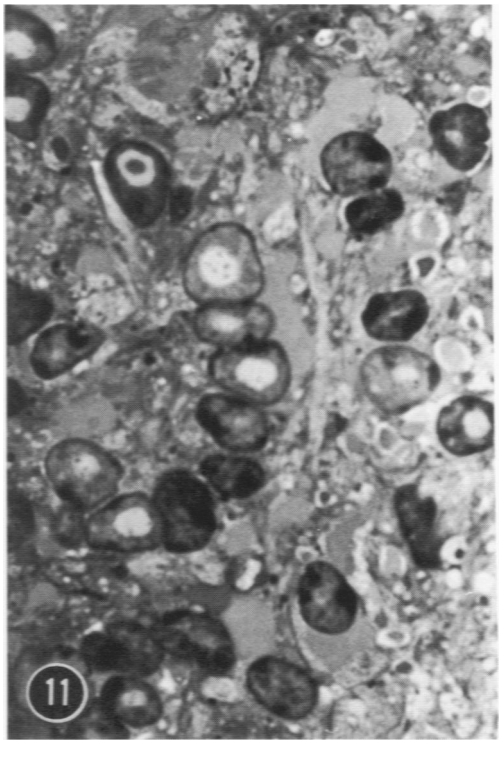
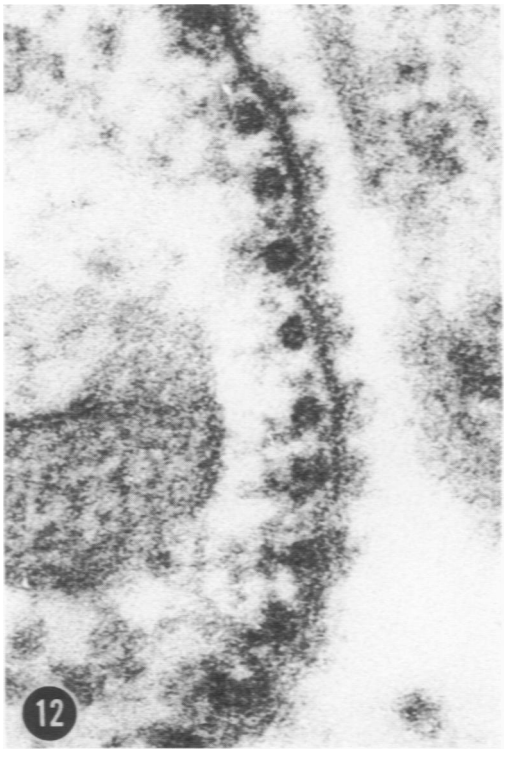
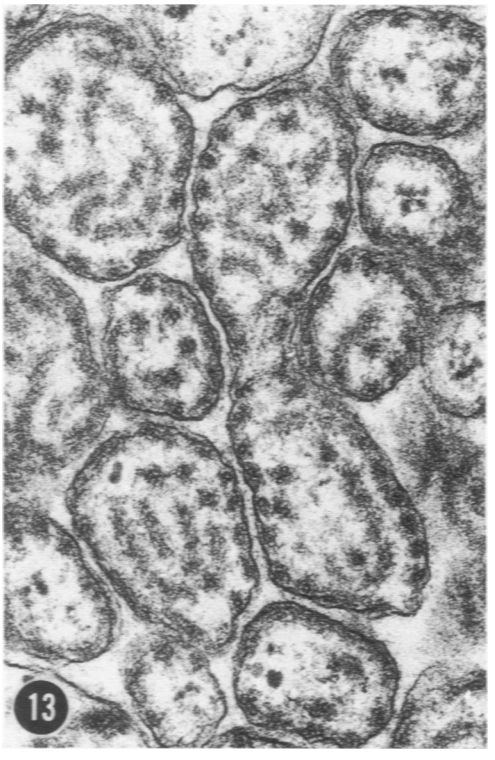
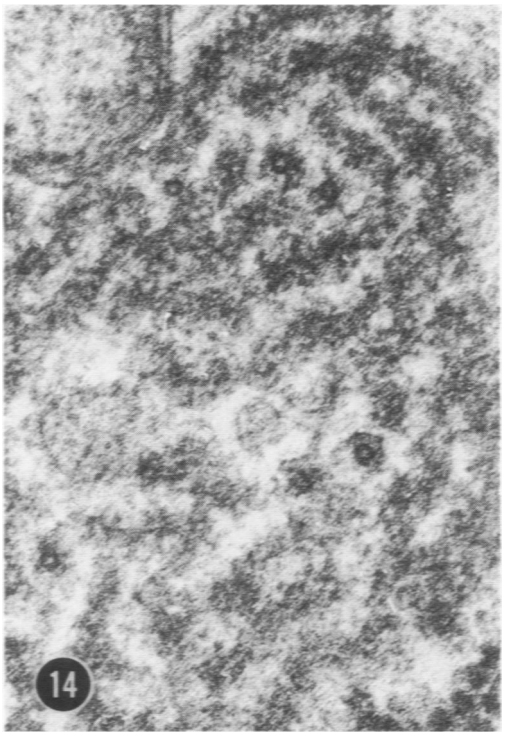


Fig 7—A portion of an axon hillock from a C mutant infected neuron shows coating of neurotubules in the vicinity of empty virions. Uncoated segments of the tubules are also seen (*arrows*) (× 70,000). **Fig 8A**—Brains of 2 rats 3 months after inoculation with B mutant. Both show swelling of the cortical mantle, the one on the right is more severely affected. **B**—A normal rat brain is shown for comparison with **A** (Glutaraldehyde perfusion, scale in millimeters). **Fig 9**—Brain of a rat 8 months after inoculation with B mutant. The cortical mantle has been eroded away from the occipital poles, exposing the diencephalon (Glutaraldehyde perfusion). **Fig 10**—Hamster cerebellar culture 60 days after infection with Edmonston strain measles virus. The culture has been reduced to a mat of fibrous astrocytes and synctia formed from them. This giant cell shows numerous Cowdry type A intranuclear inclusions, large collections of cytoplasmic nucleocapsid (*arrow*) and thick bundles of astroglial filaments (*center, right*) (1-μ Epon section, toluidine blue stain, × 1200). **Fig 11**—Hamster cerebellar culture 55 days after infection with hamster brain-adapted Mantooth stain of SSPE virus. The densely staining, spherical nuclei (some of which contain viral inclusions), belong to neurons (granule cells) which still persist even after this long period of infection. Homogenous, grey material seen in background represents cytoplasmic SSPE nucleocapsid (× 1200).







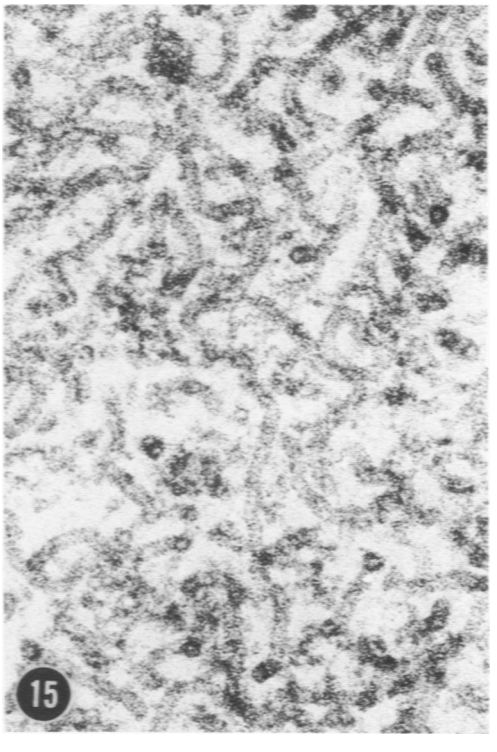
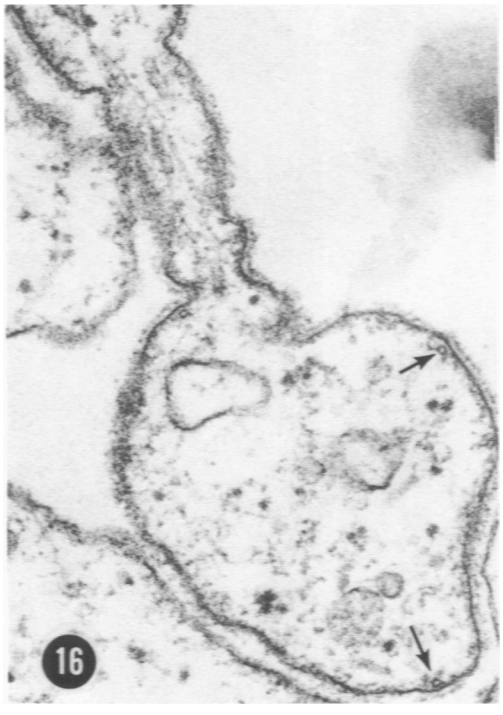
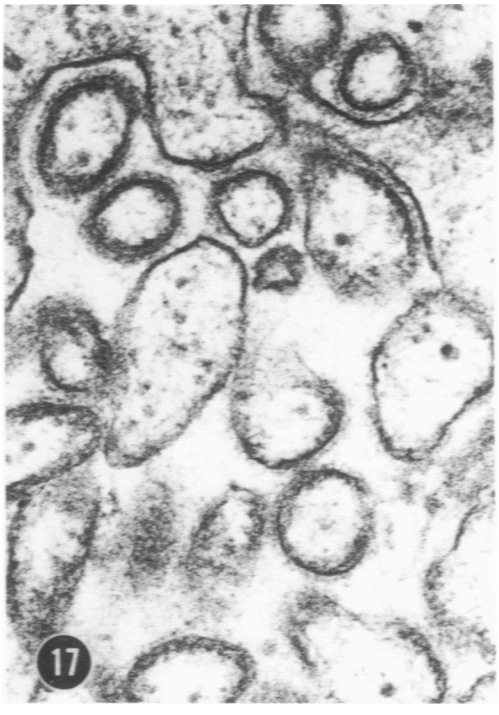


Fig 12—A portion of an astroglial cell membrane 10 days after infection with measles. Viral nucleocapsid is aligned beneath the membrane, and ridges of granular material exist on the outer surface, opposing each 18-nm nucleocapsid (× 180,000). **Fig 13**—Budded measles virions lie within the extracellular space, 38 days PI. Note regular alignment of viral nucleocapsids beneath the envelope (× 66,000). **Fig 14**—Detail of a cytoplasmic measles inclusion. Each nucleocapsid is tubular in cross section, 18 nm in diameter, and surrounded by a granular coating (× 120,000). **Fig 15**—Detail of an intranuclear measles inclusion. Each tubular nucleocapsid is tubular and 18 nm in diameter, of indeterminate length and shows cross-striations, suggestive of a helical form (× 100,000).





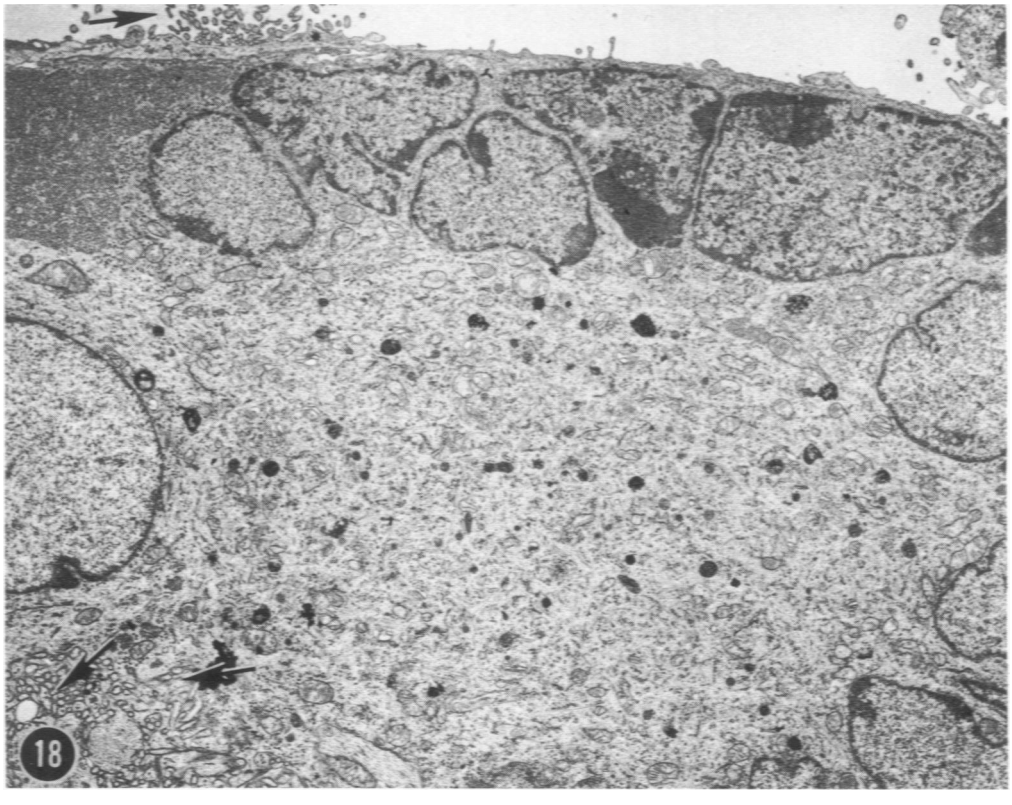
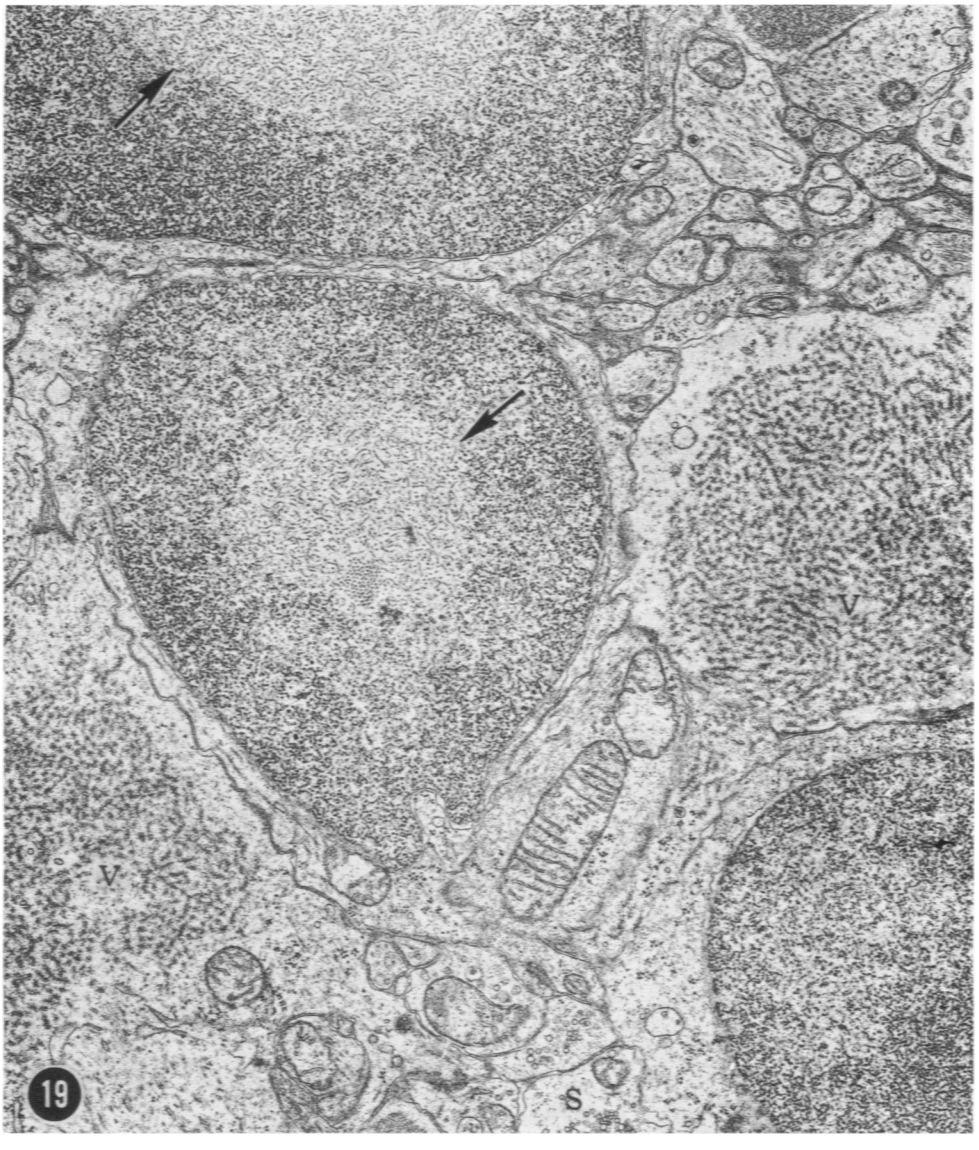


Fig 16—A budding SSPE virion in a hamster cerebellar culture, 5 days PI. An outer coating is seen to surround the particle but subplasmalemmal nucleocapsids are few (*arrows*) in comparison to measles (Figure 13) (× 80,000). **Fig 17**—Budded SSPE virions show absence of nucleocapsid beneath the viral envelope (× 80,000). **Fig 18**—A giant cell from a cerebellar culture 11 days PI with SSPE virus. Budding virions are seen (*arrow*) and a large cytoplasmic inclusion is situated at the upper left (× 5700).



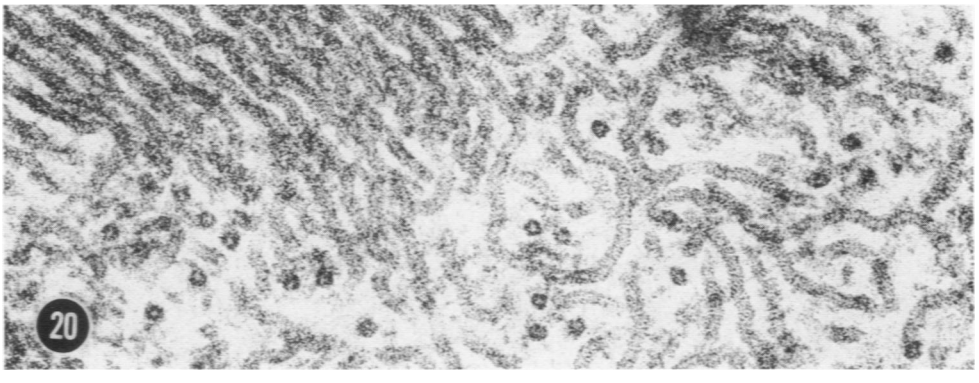


Fig 19—A group of granule cells, 55 days after infection with SSPE virus. Note the intranuclear, Cowdry type A inclusions (*arrows*), the cytoplasmic viral material (*V*) and synaptic vesicles (*S*). Neurons rarely survived in measles cultures after 21 days PI (× 15,000).
Fig 20—Detail of an SSPE intranuclear inclusion. The tubular, helical nucleocapsids (note cross-striations) are identical to those associated with measles infection (× 100,000).