

COMPARISON OF WILD-TYPE AND SUBACUTE SCLEROSING PANENCEPHALITIS STRAINS OF MEASLES VIRUS

Neurovirulence in Ferrets and Biological Properties in Cell Cultures

By HALLDOR THORMAR, PANKAJ D. MEHTA, AND HANNAH R. BROWN

*From The New York State Institute for Basic Research in Mental Retardation, Staten Island, New York
10314*

A strain of measles virus isolated in this laboratory from the brain of a patient with subacute sclerosing panencephalitis (SSPE)¹ (1) has remained cell-associated and nonproductive despite attempts to induce release of free virus from infected syncytia by cocultivation and serial passage in susceptible cell lines. This virus, designated strain D.R., has been found to be highly encephalitogenic in young adult ferrets when live or freeze-thawed syncytia on ferret cells are inoculated by the intracerebral (IC) route (1, 2). On the other hand, preliminary experiments in our laboratory indicated that wild-type (wt) measles virus, strain Edmonston, grown in monkey kidney cells was not virulent in adult ferrets. This raised the question of whether or not neurovirulence in adult ferrets is a characteristic of SSPE measles virus strains. To answer this question we grew a number of wt and SSPE strains of measles virus in Vero cell cultures and compared their neurovirulence in ferrets under identical conditions. We found that SSPE strains Mantooth, Halle, and LEC-S were like the wt strains Edmonston and Woodfolk in that they did not cause a detectable central nervous system infection in the animals, but elicited a strong serum antibody response. SSPE strains Biken, IP-3, LEC, and D.R. on the other hand, were clearly neurovirulent, although to a varying extent. In an attempt to determine whether the differences in neurovirulence of the various measles virus strains could be correlated with differences in their biological properties in cell culture, we compared a number of viral characteristics, such as cytopathic effect (CPE), virus production, and ultrastructural changes in Vero cells and in low passage monolayer cultures of ferret and human brain cells.

Materials and Methods

Cell Cultures and Media. Vero cells, a continuous line of African green monkey kidney cells (Flow Laboratories, Rockville, Md.) were grown in Eagle's basal medium (BME) supplemented with 10% inactivated fetal calf serum (FCS, Grand Island Biological Co., Grand Island, N. Y.), and maintained in BME with 2% FCS. Brain cultures from ferrets were prepared as previously described (1, 2). In brief, finely minced brain tissue was trypsinized and the dispersed cells washed and resuspended in growth medium consisting of BME with 20% FCS. The ferret brain (FB) cultures, in 250-ml plastic flasks (BioQuest, BBL & Falcon Products, Cockeysville, Md.), were changed at intervals until confluent cell layers had formed, and then subcultured by trypsinization. Human brain (HB) culture was obtained from a brain biopsy extracted for diagnostic purposes from the temporal lobe of a 12-yr old child. It was found to be free of any viral CPE. The original explant cultures were subcultured by trypsinization. The culture medium was BME with 20% inactivated FCS. The maintenance medium was BME with 2% FCS. Both FB and HB cultures were used after two to five passages by trypsinization. Both

¹ *Abbreviations used in this paper:* BME, Eagle's basal medium; CF, complement fixation; CPE, cytopathic effect; FB, ferret brain; FCS, fetal calf serum; HAI, hemagglutination inhibition; HB, human brain; IC, intracerebral; NINCDS, National Institute of Neurological and Communicative Disorders and Stroke; PI, postinoculation; RBC, erythrocytes; SFU, syncytia forming units; SSPE, subacute sclerosing panencephalitis; TCID₅₀, median tissue culture infective dose; wt, wild-type.

showed a mixed cell population with fibroblasts predominating, particularly in the FB cultures. Typical epithelial cells were not found in either culture.

Virus Strains. The following wt and SSPE measles virus strains were used: (a) The Edmonston strain, received from Dr. P. Albrecht of the Division of Biologics Standards, National Institutes of Health, Bethesda, Md. Since first isolated by Dr. J. Enders, this strain has been passed six times in human embryo kidney cells and five times in Vero cell cultures. (b) Strain Woodfolk, received from Doctors H. Koprowski and Y. Iwasaki, The Wistar Institute, Philadelphia, Pa. This strain was first isolated by Dr. E. H. Lennette from an acute case of measles and has undergone a number of passages in the CV-1 African green monkey kidney cell line. The virus was passed twice in Vero cell cultures in our laboratory. (c) SSPE strain Mantooh, received from Doctors J. Sever and L. Horta-Barbosa, National Institute of Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda, Md. This virus was isolated from the brain of an SSPE patient (3) and has undergone two passages in HeLa cells and two additional passages in Vero cells in our laboratory. (d) SSPE strain Halle, received from Doctors Sever and Horta-Barbosa. This strain was isolated from the lymph node of an SSPE patient (4) and has undergone three passages in HeLa cells and two additional passages in Vero cells in our laboratory. (e) SSPE strain LEC, received from Doctors Koprowski and Iwasaki. This strain has undergone 25 passages in CV-1 cells since it was isolated from a brain culture of an SSPE patient by fusion, using inactivated Sendai virus (5). It was passed three times in our laboratory by transfer of infected cells onto monolayers of Vero cells. (f) SSPE strain LEC-S, received from Doctors Koprowski and Iwasaki. This is a small plaque variant isolated from the LEC strain (6). It has undergone 14 passages in CV-1 cells and 2 additional passages in Vero cells in our laboratory. (g) SSPE strain Biken was isolated from the brain of an SSPE patient by co-cultivation with human embryonic lung cells (7). When received from Doctors Sever and Dubois-Dalq, NINCDS, the Biken strain was in its 44th passage in Vero cell cultures. It underwent 10–15 additional passages in our laboratory by trypsinization and addition of fresh Vero cells. (h) SSPE strain IP-3, received from Dr. Albrecht. This strain was isolated by Burnstein et al. (8) from the brain of an SSPE patient. It had undergone 17 passages in the BS-C-1 African green monkey kidney cell line, 5 passages in Vero cells, 1 brain passage in rhesus monkey, and 2 brain passages in suckling hamster when received by us. It was passed twice in Vero cells in our laboratory by transfer of live infected cells. (i) SSPE strain D.R. was isolated from the brain of an SSPE patient in our laboratory in 1970 (1). After 12 serial passages of the original brain culture it was cocultured with Vero cells and has undergone 25–30 additional passages by trypsinization and addition of fresh Vero cells.

Preparation of Virus for Ferret Inoculation. The different measles virus strains were grown in monolayer cultures of Vero cells in 250-ml Falcon plastic flasks. Wt strains Edmonston and Woodfolk and the virion-producing SSPE strains Mantooh, Halle, and LEC-S were inoculated into the Vero cultures at an input multiplicity of 0.02–0.05 median tissue culture infective doses (TCID₅₀) per cell. The cell-associated SSPE strains LEC, Biken, IP-3, and D.R. were similarly inoculated into Vero monolayers, using a suspension of infected Vero cells as inoculum. 3–4 days later the monolayers showed CPE consisting of syncytia and stellate cells covering the whole cell layer (Edmonston, Woodfolk, Mantooh, Halle, LEC-S) or of numerous distinct syncytia scattered over an otherwise normal looking cell layer (LEC, IP-3, Biken, D.R.). Each cell layer was rinsed once and then scraped off the plastic with a rubber policeman. The cells were suspended in 5 ml of maintenance medium and 0.5 ml of suspension was used for inoculation of each ferret. The remainder of the suspension was saved for infectivity assay in Vero cell cultures.

Infectivity Assays. Virus strains Edmonston, Woodfolk, Mantooh, Halle, and LEC-S were assayed by end-point titration in monolayer cultures of Vero cells by using five tubes per dilution. The CPE was read after 10 days and the titers expressed as TCID₅₀ per 0.1 ml. Syncytia-forming activities of SSPE virus strains D.R., IP-3, Biken, and LEC were determined by inoculation of 10-fold dilutions onto Vero cell monolayers in 35-mm plastic Petri dishes. After incubation at 37°C for 3–5 days the cell layers were fixed, stained with Giemsa, and the number of distinct syncytia counted. The infectivity is expressed as the number of syncytia-forming units (SFU) per 0.1 ml of cell suspension.

Inoculation of Ferrets. Young unimmunized male ferrets were purchased from a breeding

farm (Marshall Research Animals, Inc., North Rose, N. Y.) and used for inoculation at 3- to 4-mo of age. About 0.5 ml of infectious cell suspension was injected directly into the left cerebral hemisphere. The animals were observed for clinical signs for up to 8 mo and were bled at intervals by heart puncture. At the time of sacrifice the left half of the brain was frozen for immunological studies, whereas most of the right half was used for virological studies in tissue culture. Pieces of tissue from the right cerebral hemisphere and cerebellum were fixed in formalin for histologic examination.

Virus Isolation from FB. A portion of finely minced brain tissue was homogenized with Ten-Broeck tissue grinder (Corning Glass Works, Corning, N. Y.) and inoculated into Vero cell monolayers. The remainder was trypsinized as described before and the cell suspension either cocultured with freshly prepared Vero cell cultures or cultured without the addition of Vero cells. All the cultures were examined daily for CPE and passed by trypsinization after 2 and 4 wk. Some of the ferret brain cultures were cocultured with Vero cells in the first or second passage.

FB Extracts. The left half of each FB was washed with 0.15 M NaCl, sliced thinly, and homogenized with phosphate-buffered saline, pH 7.4, in an Omnimixer (Sorvall Inc., Newtown, Conn.) at 0°C. The homogenate was clarified by centrifugation in the Beckman model L2-65B ultracentrifuge for 1 h at 100,000 *g*. The supernate was concentrated by membrane filtration (Amicon Corp., Scientific Sys. Div., Lexington, Mass.) and stored frozen until used for immunological studies.

Quantitation of Protein and IgG in Brain Extracts. Protein determination was made by the method of Lowry et al. (9) using bovine serum albumin as standard. The quantitation of IgG was done by the radial immunodiffusion method (10) using agar plates containing specific rabbit anti-ferret IgG sera (2).

Neutralization Test. Serial twofold dilutions of sera and brain extracts were mixed with approximately 100 TCID₅₀ of measles virus, strain Edmonston, and incubated for 1 h at 20°C and overnight at 4°C. The mixtures were assayed by inoculation of 0.1-ml samples into tubes with Vero cells. The tubes were read for CPE after 10 days. Titers are expressed as the reciprocal of the dilution causing a complete neutralization of virus in 50% of the inoculated tubes.

Hemagglutination Inhibition (HAI) Test. Measles HAI test was done by the technique of Norrby (11) using 4 U of measles hemagglutination antigen (Microbiological Associates, Walkersville, Md.). The sera were treated with trypsin at 56°C and both sera and brain extracts were absorbed with monkey erythrocytes before use in the test.

Complement Fixation (CF) Test. Measles CF test was done by the microtiter technique of Sever (12) using commercial measles virus CF antigen (Microbiological Associates). The sera were inactivated at 56°C for 30 min but the brain extracts were tested without inactivation.

Inoculation of Cell Cultures. All studies of biological properties were done in monolayer cultures in 35-mm plastic Petri dishes (BioQuest, BBL & Falcon Products). For comparison of syncytiogenic activity and other CPE the virus strains, prepared as previously described for ferret inoculation, were inoculated in 10-fold dilutions into the Petri dishes. The cell layers were observed for CPE daily for 10 days. During this period cell layers showing characteristic CPE were fixed and stained with May-Grünwald-Giemsa. In studies of virus proliferation, monolayer cultures of FB and HB cells were inoculated with cell-free virus at an input multiplicity of 0.05 TCID₅₀ per cell. After an absorption period of 6 h the cell layers were thoroughly rinsed and incubated with 2 ml of maintenance medium. The whole cultures were harvested at intervals, freeze-thawed once, and clarified. The supernates were titrated by the end-point dilution method in tubes of Vero cells.

Hemadsorption. Infected monolayers were rinsed with Hanks' balanced salt solution and incubated with a 0.2% suspension of rhesus monkey erythrocytes (RBC) at 37°C for 30 min.

Electron Microscopy. Infected monolayers in 35-mm Petri dishes were fixed in situ in 2% glutaraldehyde in neutral phosphate buffer for 30 min at 4°C. After postfixation in 1% osmium tetroxide, the cell layers were dehydrated in alcohols and embedded in Epon 812 according to standard techniques. After polymerization, syncytia were located by light microscopy, cut out, and glued onto previously polymerized blocks to allow in situ examination. Sections were contrasted with uranyl acetate and Reynolds lead citrate and examined under a Hitachi HS-8-2 electron microscope.

Results

Wt Strains Edmonston and Woodfolk. 10 ferrets were inoculated with strain Edmonston, $10^{5.5}$ – $10^{6.5}$ TCID₅₀ of virus per inoculum. The ferrets were kept under observation for 8–22 wk and were bled 3 and 6 wk postinoculation (PI) and again at the time of sacrifice. None of the ferrets showed any signs of illness during this period and virus could not be recovered from their brains. No abnormalities were observed by histological examination of four brains sectioned at three different levels and stained with hematoxylin and eosin. Neutralizing antibodies against measles virus in titers of 16–128 were present in the ferret sera 3–6 wk PI. At the time of sacrifice the serum neutralizing titers varied from 16 to 256 (Table I) and had not increased significantly since 6 wk PI. HAI serum antibodies were present in two to eightfold lower titers and CF antibodies in at least 32-fold lower titers than the neutralizing antibodies. Brain extracts from the ferrets that showed the highest serum antibody titers were tested for IgG content and measles antibodies. The antibody titers were either very low or not detectable and there was no increase in the IgG content of the brains when compared with uninoculated ferrets (not shown in Table I) or with ferrets inoculated with uninfected Vero cells. Identical results were obtained from studies of four ferrets inoculated with wt strain Woodfolk.

SSPE Strains Halle, Mantooth, and LEC-S. Eight ferrets inoculated with strain Halle (10^7 TCID₅₀) showed no signs of illness during the 8–22 wk of observation and virus was not recovered from their brains at the time of sacrifice. Three of the brains were examined histologically after staining with hematoxylin and eosin. No lesions were found. The antibody responses in these animals are summarized in Table I. Neutralizing serum antibodies against measles virus reached titers of 64–256 at 3–6 wk PI and increased slightly after that in only one animal to a maximum of 512. HAI serum antibodies were present in about two to fourfold lower titers and CF antibodies in 8–64 fold lower titers than the neutralizing antibodies. Measles virus antibodies were either not detectable in brain extracts or were present in very low titers in extracts from ferrets showing the highest serum antibody titers. The brain IgG concentrations were not increased as compared with controls inoculated with uninfected Vero cells. Identical results were obtained from studies of eight ferrets inoculated with strains Mantooth and LEC-S, respectively.

SSPE Strain LEC. Six ferrets were inoculated with strain LEC, approximately 3.5×10^3 SFU per inoculum, as determined by inoculation onto Vero cell monolayers (1, 2). The virus caused an acute illness in two animals, 11–14 days PI, and a subacute illness in two animals, 4–7 wk PI. Thus, the incubation time was rather short in the subacute cases and the clinical signs were similar to those observed in acute cases, namely tremors, seizures, hyperexcitability, and finally coma leading to death in 1–3 days. Two animals had not shown any signs of illness at the time when they died from heart puncture 8 and 13 wk PI, respectively. Histologic lesions were not observed in five brains examined and viral synytia were only recovered from the brain of one animal with acute disease by cocultivation of the brain cells with Vero cells. No antibody response to measles virus was found in either the sera or brain extracts of ferrets with acute or subacute illness. However, the two animals that did not show clinical signs had slightly increased neutralizing and HAI antibody titers. The brain IgG content, however, was not increased.

SSPE Strain IP-3. Five of eight ferrets inoculated with 5×10^2 – 1.5×10^3 SFU of

TABLE I

Antibody Response in Ferrets Inoculated IC with Wt and SSPE Measles Virus Strains Grown on Vero Cells. Control Ferrets Were Inoculated with a Suspension of 10^6 Uninfected Vero Cells

Inoculum	Time of sacrifice		Serum antibody titer			Brain extracts		
	wk	PI	Neutralizing	HAI	CF	Tested	Neutralizing antibody titer	IgG, % of total protein
Wt strain Edmonston	8-22		16-256	<8-64	<4-16	2	<4	0.15
							4	0.31
SSPE strain Halle	8-22		64-512	32-256	<4-4	3	<4	0.15
							4	0.21
							8	0.22
Uninfected Vero cells	2		<4	<4	<4	3	<4	0.20
							<4	0.20
							<4	0.23

strain IP-3 developed an acute encephalitis within 2 wk. Viral syncytia were recovered from cell suspensions and cell-free homogenates of the FB by cocultivation or inoculation on Vero cells. Syncytia were also produced in monolayers of the FB cells, without Vero cells added. The syncytia showed the same characteristics as the IP-3 virus inoculated into the ferrets. Two ferrets were sacrificed at 14 wk PI without showing any signs of encephalitis. One ferret that died during heart puncture 8 wk PI had a significant serum antibody titer against measles virus, whereas a blood sample collected 4 wk earlier was negative. This animal, therefore, appeared to have a persistent infection. However, virus could not be isolated from his brain and histological and immunological studies of the brain were also negative.

SSPE Strain Biken. Seven ferrets were inoculated with approximately 3×10^3 SFU of strain Biken (Table II). Two ferrets came down with an acute neurological illness after 7 and 8 days, respectively, whereas four of the animals developed a subacute encephalitis from 10 to 32 wk PI. One ferret did not show any clinical signs when it was sacrificed 32 wk PI. Ferret 305 showed neurological signs reminiscent of the acute cases. Ferrets 302, 323, and 324, on the other hand, showed insidious signs of illness a few weeks before they became moribund, i.e. failure to thrive, listlessness, and in one case, an apparent blindness. The animals were sacrificed when they became either comatose or totally paralyzed. Histopathologic examination of the brains showed perivascular infiltrations and microgliosis that were particularly widespread in the ferrets with subacute encephalitis, whereas lesions were much less pronounced in the ferrets with acute disease. Viral syncytia were recovered from the brains of all ferrets showing clinical signs. Cocultivation of brain suspensions with Vero cells was the most effective method for recovery of virus, but inoculation of brain homogenate onto monolayers of Vero cells also yielded virus in all six cases. Monolayers of the FB cells, without Vero cells, produced syncytia in four of six cases and could be propagated by subcultivation, as described earlier for strain D.R. in FB cultures (1). In all cases, the virus isolated from infected FB showed the same characteristics as the virus used for

TABLE II
IC Inoculation of Ferrets with 3.5×10^3 SFU of SSPE Measles Virus Strain Biken. Neurovirulence and Antibody Response to Measles Virus

Ferret	Time of sacrifice PI	Clinical signs	Histologic lesions	Virus isolation from brain	Serum antibody titer			Brain extracts			IgG, % of total protein
					Neutralizing	HAI	CF	Antibody titer			
								Neutralizing	HAI	CF	
326	7 days	Acute	Slightly Positive	Positive	<4	<8	<4	<4	<8	—*	0.1
325	8 days		Positive	4	<8	<4	—	—	—	—	
305	10 wk	Subacute	Positive	Positive	16	32	<4	128	200	—	0.4
323	10 wk				1,024	128	16	4,096	200	8	1.5
324	22 wk				8,000	4,000	16	8,000	3,000	16	4.7
302	34 wk				1,024	128	16	4,096	200	16	2.4
304	34 wk	None	None	Negative	16	<8	—	32	<16	<4	0.4

* Not done.

TABLE III
Neutralizing Measles Virus Antibody Titers in Sera of Ferrets Inoculated IC with SSPE Strain Biken

Ferret	Antibody titers at wk PI					
	6	10	16	22	28	34
302	256	—*	512	256	256	1,024
304	256	—	64	32	32	16
305	8	16				
324	—	256	4,000	4,000		

* Not done.

inoculation. A piece of spinal cord was extracted from the lower thoracic region of one ferret (324) that showed a complete paralysis 22 wk PI. A large number of viral syncytia was isolated from the cord tissue which also showed inflammatory lesions by histologic examination. Virus could not be isolated from mesenteric and mediastinal lymph nodes or from the spleen of this animal. Three of the ferrets with subacute signs showed greatly increased neutralizing and HAI serum antibody titers and significant CF antibody titers to measles virus. High titers were observed in their brain extracts which also showed significantly increased IgG concentration (Table II). One ferret with subacute illness (305) and one without clinical signs (304) also showed significant, although much lower, antibody activities in the brain.

Blood samples from the ferrets were collected at intervals throughout the incubation period and the sera were tested in neutralization test against measles virus. The titers are shown in Table III. Ferrets 302 and 304 are of particular interest. In 302 a moderately high titer was maintained from 6 to 28 wk and then increased fourfold at the time of clinical signs, 34 wk PI. The serum antibody titer of 304, on the other hand, dropped from moderately high to low during this period and the animal had no signs of infection when sacrificed at 34 wk PI. However, it had a significant neutralizing antibody titer in its brain (Table II). Apparently, the initial encephalitis

TABLE IV

Wt and SSPE Measles Virus Strains: Syncytiogenic Activity of Cell-Associated and Cell-Free Virus in Vero and FB Cell Monolayers

Measles virus strain	Syncytiogenic activity				
	Vero cells				FB cells
	Live cell suspension	Freeze-thawed cell suspension	Supernate	Filtered supernate	Live cell suspension
Wt Edmonston	10 ^{5.5} *	10 ^{5.5}	10 ^{5.1}	10 ^{4.5}	<10 ¹
SSPE Mantooth	10 ^{7.5}	10 ^{7.5}	10 ^{7.5}	10 ^{6.9}	<10 ¹
SSPE Biken	4 × 10 ³ ‡	2.5 × 10 ³	2.5 × 10 ³	4 × 10 ²	6 × 10 ²
SSPE D. R.	4 × 10 ³	5 × 10 ¹	6 × 10 ¹	2 × 10 ⁰	7 × 10 ³

* TCID₅₀ per ml.

‡ SFU per ml.

was overcome in this animal, whereas it progressed to a clinically overt illness in ferret 302.

SSPE Strain D.R. Six ferrets inoculated with about 5 × 10² SFU of SSPE strain D.R. grown on Vero cells developed acute encephalitis 5–10 days later. Syncytia were isolated from all of the FB, but no antibodies against measles virus were found in sera or brain extracts.

Syncytiogenic Activity of Wt and SSPE Strains in Cell Monolayers. Vero cell cultures infected with the various strains of wt and SSPE measles virus were harvested at the time of maximal CPE. After scraping the cells into 5 ml of maintenance medium they were carefully suspended by pipetting and a sample taken. Another sample was freeze-thawed three times at –70°C and 37°C to disrupt the cells. The remainder of each suspension was freeze-thawed once and clarified by centrifugation in a PR-J refrigerated International Centrifuge (International Equipment Company, Boston, Mass.) at 2,000 *g* for 10 min. A sample of the supernate was used either directly or after passage through a 0.45-μm Swinnex-13 filter unit (Millipore Corp., Bedford, Mass.). Each sample was inoculated in 10-fold dilutions into 1-day old monolayer cultures of Vero and FB cells. The cultures were examined for the formation of syncytia and multinucleated stellate cells daily for 10 days. The results are shown in Table IV and Fig. 1. The strains fall into two groups. The first group, including wt strains Edmonston and Woodfolk, and SSPE strains Halle, Mantooth, and LEC-S showed high titers of cytopathic activity in Vero cells consisting of formation of both syncytia and stellate cells (Fig. 1 A and C). Syncytia were not formed in monolayers of FB cells, but patches of small refractile stellate cells with one or two nuclei (Fig. 1 B and D) appeared about 7–10 days after inoculation with the lowest viral dilutions and later showed a complete cell degeneration. The CPE, both syncytia and stellate cell

FIG. 1. CPE of wild-type and SSPE measles virus strains in monolayers of Vero and FB cells. A. Edmonston in Vero; B. Edmonston in FB; C. Mantooth in Vero; D. Mantooth in FB; E. Biken in Vero; F. Biken in FB; G. D.R. in Vero; H. D.R. in FB. All four strains are syncytiogenic in Vero cells, whereas only strains Biken and D.R. cause fusion of FB cells. May-Grünwald-Giemsa. Magnification: approximately × 80.

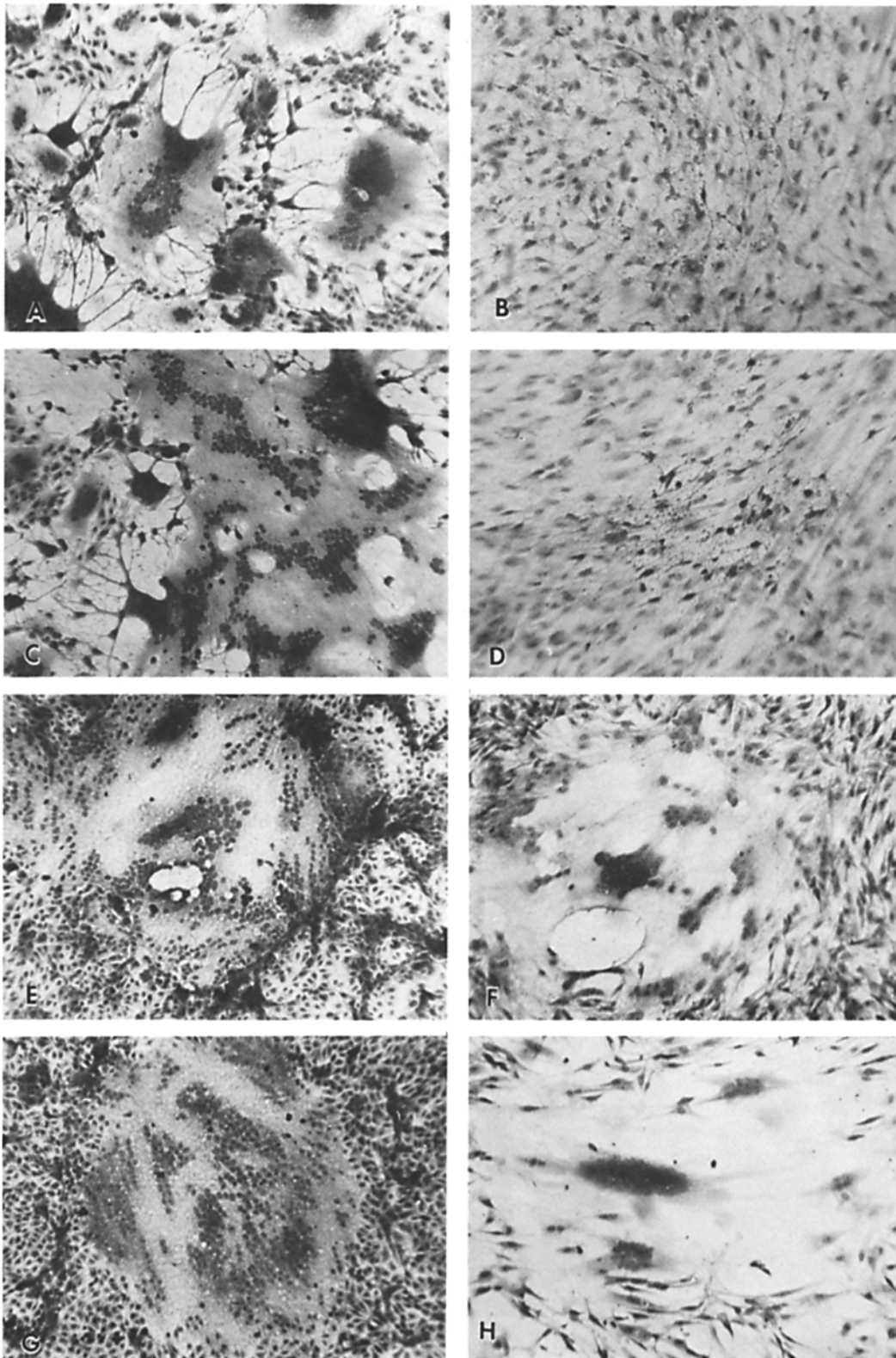


FIG. 1
681

formation, was caused by cell-free virus particles since it was produced by inoculation of high dilutions of supernates from freeze-thawed infected cells, both before and after filtration through a Millipore filter (Table IV).

SSPE strains LEC, Biken, IP-3, and D.R. on the other hand, were syncytiogenic both in Vero and FB cell layers (Fig. 1 E-H). The syncytia were well defined and surrounded by a normal cell layer. Stellate cells were not formed. It is noteworthy that strain Biken was much less syncytiogenic in FB than in Vero cells, as indicated by the number of syncytia formed in monolayers of these two cell types inoculated in the same way (Table IV). This was also true for strains IP-3 and LEC. Strain D.R., on the other hand, consistently showed stronger syncytiogenicity in FB than in Vero cells. It is also noteworthy that although the syncytiogenic activity of these SSPE strains was mostly associated with live infected cells, some activity was present in freeze-thawed suspensions and even in Millipore filter supernates. The number of filterable SFU varied from about 10% of the live cell SFU in strain Biken to about 0.05% in strain D.R. Strains LEC, Biken, and IP-3 showed no syncytiogenic activity in FB cells after freezing and thawing, whereas strain D.R. retained some of its activity in FB cells, even after filtration through Millipore filter (data not shown in Table I). It is noteworthy that syncytia isolated from brains of ferrets inoculated with strains Biken, IP-3, and D.R., and propagated in FB cells for a few passages, showed growth characteristics identical to their parent strains.

The wt and SSPE strains caused a similar type of CPE in HB cells as in FB cells (Fig. 2). Thus, SSPE strains D.R., IP-3, Biken, and LEC caused the formation of large syncytia with clusters of nuclei, whereas SSPE strains Mantooth and Halle and the wt strains caused the formation of smaller stellate cells with two to four nuclei, but did not form multinucleated syncytia.

Virus Production in FB Cultures. As described above, wt strains Edmonston and Woodfolk and SSPE strains Mantooth, Halle, and LEC-S were not syncytiogenic in FB cultures, but caused the formation of small stellate cells that later degenerated. To determine whether measles virus was produced by FB cells a number of monolayer FB cultures were inoculated with each of the above five virus strains at an input multiplicity of 0.05. 6 h later, the monolayers were rinsed three times and fresh maintenance medium added. The whole cultures were harvested at intervals, freeze-thawed once, clarified, and titrated. Both the wt and SSPE strains produced significant titers ($10^{2.6}$ – $10^{5.3}$ TCID₅₀ per ml) of free virus in the FB cell cultures during a period of 7–12 days, associated with various degrees of CPE. Cell-free supernates from Vero cultures infected with SSPE strains LEC, Biken, IP-3, and D.R. were also inoculated into FB cultures. They either did not cause any CPE in the FB monolayers for a period of 9 days or were slightly syncytiogenic (strain D.R.). Supernates from freeze-thawed infected FB cultures showed no infectivity when titrated in Vero cells. This indicates that no cell-free virus was produced by these SSPE strains in FB cell cultures. However, some cell-associated virus was maintained for 9 days in the FB cultures without the appearance of syncytia.

Hemadsorption. Hemadsorption of rhesus monkey RBC by the various measles virus strains was tested both in Vero and FB cell cultures. Strains Edmonston, Woodfolk, Halle, Mantooth, LEC-S, and LEC consistently showed positive hemadsorption. Hemadsorbing patches appeared in monolayers of FB cells well before the cytopathic changes. Areas showing CPE always hemadsorbed quite heavily. Syncytia of SSPE

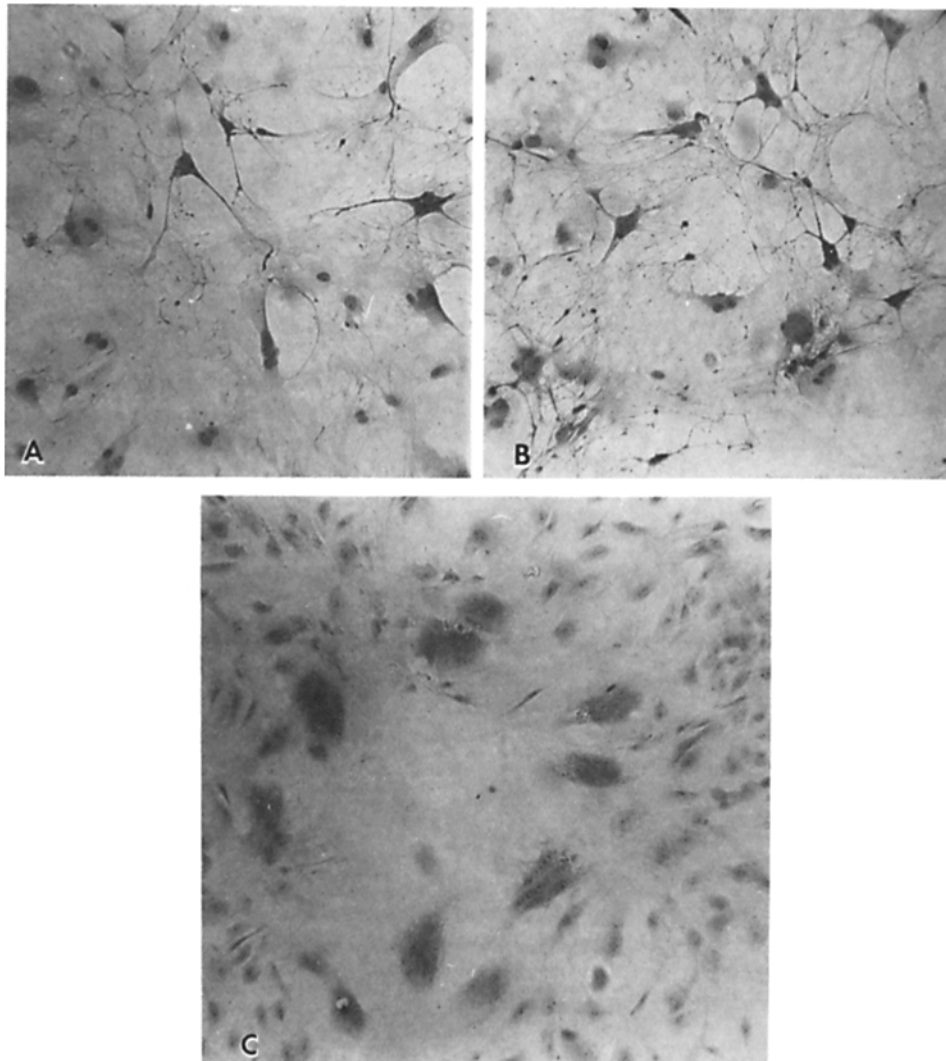


FIG. 2. CPE of wild-type and SSPE measles virus strains in monolayers of HB cells. A. Edmonston; B. Mantooth; C. D.R. The cells are fused by strain D.R., whereas strains Edmonston and Mantooth cause the formation of stellate cells without typical cell-fusion. May-Grünwald-Giemsa. Magnification: approximately $\times 80$.

strains Biken and D.R. did not show hemadsorption in either type of cell culture, whereas syncytia of strain IP-3 showed varying degrees of hemadsorption.

Electron Microscopy. Vero cell monolayers infected with the wt and SSPE measles virus strains were compared by electron microscopy. Cultures infected with wt strains Edmonston and Woodfolk, and SSPE strains Mantooth, Halle, and LEC-S all showed similar features, namely large numbers of fuzzy nucleocapsids in the cytoplasm, budding of the cell membranes with an underlying alignment of nucleocapsids, and an abundance of free measles virus particles surrounding the cells (Fig. 3 A and B). Syncytia infected with SSPE strains Biken and D.R. and most IP-3 syncytia, lacked

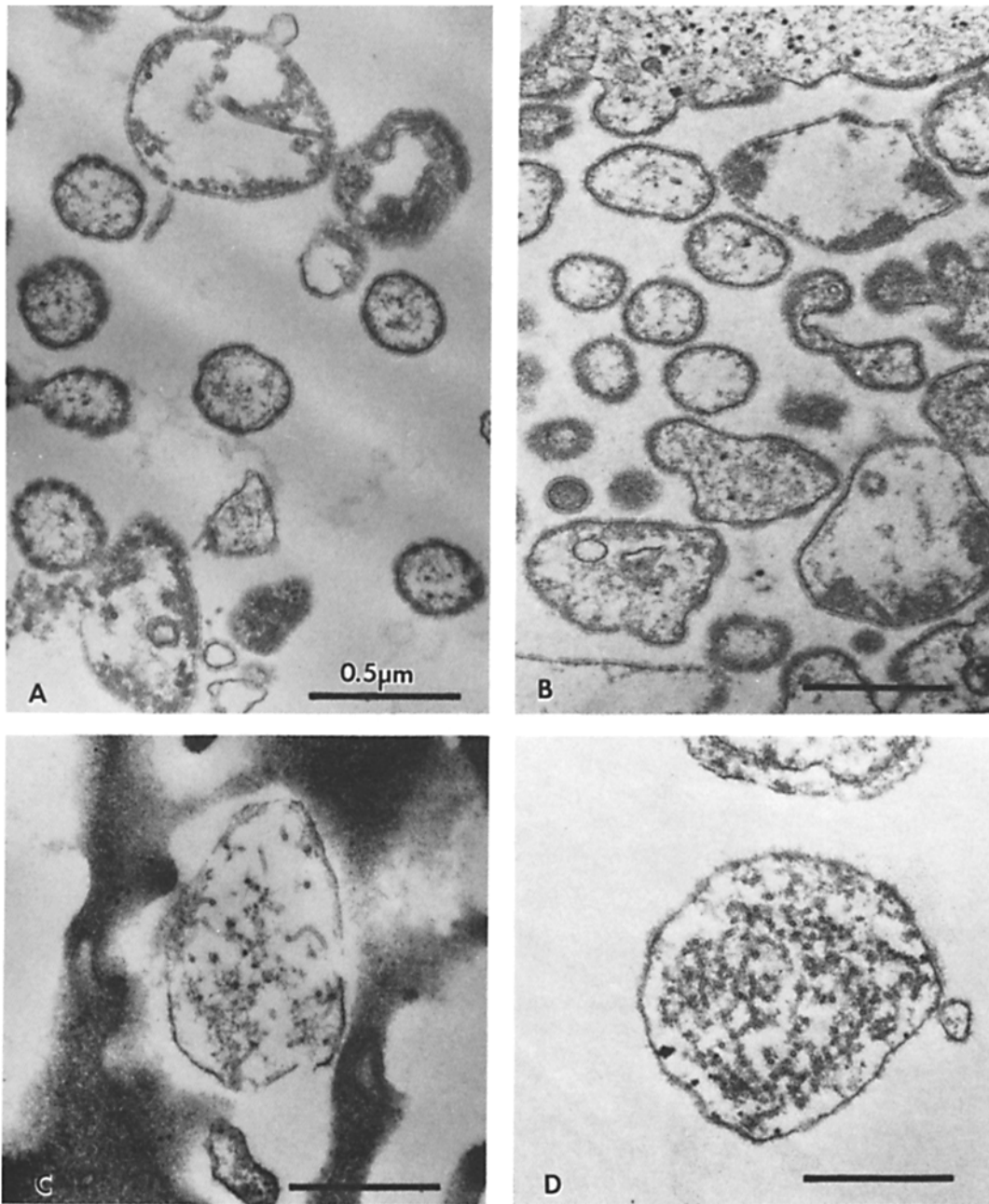


FIG. 3. Electron micrographs of particles of wild-type and SSPE measles virus strains in monolayers of Vero cells. A. Edmonston; B. Mantooh; C. Biken; D. IP-3. Note that both A and B contain oversized particles in addition to the typical measles virions. Particles shown in C and D were rare, and are atypical in terms of size, absence of spikes, lack of alignment of nucleocapsids, and presence of an unmodified enveloping membrane. Bars in A-D = 0.5 μ .

typical fuzzy nucleocapsids but contained varying amounts of smooth nucleocapsids, both intranuclear and cytoplasmic. Some of these cytoplasmic nucleocapsids were surrounded by a slightly fuzzy material. An occasional syncytium infected with strain IP-3 and most syncytia infected with strain LEC contained large amounts of fuzzy nucleocapsids. However, Vero cells infected with all four strains lacked alignment of nucleocapsids beneath the cell membrane and any other alteration of the membrane indicative of viral budding. Typical viral particles were also missing. However, a thorough search of a large number of syncytia from Biken and IP-3 infected cultures revealed a small number of cell free "particles" containing smooth nucleocapsids and covered by a single membrane without spikes (Fig. 3 C). The observed particles varied in size from approximately 400 to 1,000 m μ and differed significantly from the typical measles virions shown in Fig. 3 A. However, cultures infected with strain Edmonston were frequently found to contain similar atypical particles (Fig. 3 A). Cell-free particles were not observed in syncytia from Vero cultures infected with strain D.R.

Of all the wt and SSPE measles virus strains studied, only cells infected with strain LEC were found to harbor papova-like virus particles. These particles were present in the nucleus, often mixed with smooth nucleocapsids. It was noteworthy that the papova-like particles were observed in syncytia recovered from the brain of a ferret infected with strain LEC.

Discussion

This study confirms the previous observation (2) that measles virus strain Edmonston is not virulent in ferrets, even when virus-producing Vero cell cultures are inoculated in large quantities directly into their brains. No signs of illness were observed during periods of up to 22 wk after inoculation and there was no indication of a brain infection at the time of sacrifice. However, there was a very significant formation of serum antibodies against measles virus. Ferrets inoculated with wt measles virus strain Woodfolk and with SSPE strains Mantooth, Halle, and LEC-S responded in the same manner as ferrets inoculated with strain Edmonston.

In contrast to SSPE strains Mantooth and Halle, SSPE strain D.R. grown on Vero cell layers was found to be highly neurovirulent in ferrets. The virus used for ferret inoculation had been passed directly from the patient's (D.R.) brain cell culture into Vero cells. The strong neurovirulence of strain D.R. was, therefore, not due to previous adaptation to ferret cells, either *in vitro* or *in vivo*. Since the ferrets developed acute encephalitis within 2 wk of inoculation with strain D.R. no detectable antibody formation against the virus occurred, either in the serum or in the brain. However, previous immunization with live measles virus vaccine changed the course of the infection from acute to subacute in 50% of animals inoculated with strain D.R. grown on Vero cells (H. Thormar, unpublished observations). This confirms earlier work in ferrets inoculated with strain D.R. grown on ferret brain cell cultures (2).

SSPE strains LEC, IP-3 and Biken are all neurovirulent in ferrets, but markedly less than strain D.R. The majority of the ferrets inoculated with strain Biken developed a subacute disease that was remarkably similar to the subacute encephalitis produced in immunized ferrets inoculated with strain D.R. Particularly noteworthy was the active formation of measles virus antibodies within the brains of these animals. This, in conjunction with the widespread inflammation and presence of virus in the brain 10-34 wk PI, indicates that the ferrets developed a persistent SSPE virus infection in

their central nervous system, reminiscent of the SSPE infection in humans, and suggests that unimmunized ferrets inoculated with strain Biken may be a useful animal model of SSPE. It is of interest that in one ferret inoculated with strain Biken (Fe324) in which the spinal cord was examined, the cord tissue showed inflammatory lesions and considerable virus activity. Attempts to isolate virus from lymph nodes and the spleen were, on the other hand, negative. However, an extraneural distribution of the virus in ferrets with persistent brain infection is well possible and is worth further study.

SSPE strain LEC, although causing subacute illness in two of the inoculated ferrets, behaved somewhat differently from strain Biken. The LEC virus could not be recovered from the brains of the animals showing signs of encephalitis 4 and 7 wk PI. There was only a slight immune response to the virus and no increase in brain IgG. It is well known that the LEC strain contains a papova-like virus (13). This was also confirmed in our study and in the one case in which LEC virus was recovered in FB culture the cells were found to harbor the papova virions along with the paramyxovirus nucleocapsids. The papova-like agent was not found in any of the other SSPE virus strains included in this study and, therefore, does not play a role in the production of the SSPE-like subacute encephalitis observed in ferrets. However, it is unknown whether or not this agent interferes with or modifies the SSPE virus infection in the FB and whether it, therefore, might have caused the difference in virologic and immunologic response of LEC virus infected ferrets compared with the Biken virus infected animals.

Our results with the LEC strain are similar to those of Katz et al. (14) who were the first to use ferrets as experimental animals for studies of SSPE. In these early studies, SSPE virus was not recovered from the brains of infected animals and their serum antibody titers against measles virus remained quite low (15). A persistent brain infection similar to that produced in unimmunized ferrets by inoculation of strain Biken or in immunized ferrets after inoculation with strain D.R. was, therefore, not observed by these investigators in the LEC virus infected ferrets. On the other hand, a subacute encephalitis has been reported in an adult African green monkey inoculated subcutaneously with strain Biken (16). Albrecht and co-workers (17) produced subacute encephalitis in measles virus-immune adult rhesus monkeys by IC inoculation with strain IP-3, whereas unimmunized monkeys developed acute encephalitis. In our experiments, ferrets inoculated with strain IP-3 either died from acute encephalitis or showed no signs of illness. One animal had possibly developed a subacute infection at the time when he died from heart puncture 8 wk PI. However, the capability of strain IP-3 to produce subacute encephalitis in ferrets is questionable and needs further studies.

Strain Mantooh has been reported to show low neurovirulence in animals unless adapted to the brain by repeated IC passage (18). A hamster brain-adapted strain (HBS) causes an acute encephalitis in newborn hamsters (19) and an acute or chronic encephalitis in weanling hamsters (20). It does not, on the other hand, cause encephalitis in adult animals. Similarly, wild-type strain Edmonston has been adapted to newborn mice (21) and to newborn hamsters (22) but adult animals are not susceptible to the adapted Edmonston strain (23, 24). We have not studied the susceptibility of newborn or weanling ferrets to the various wild-type or SSPE measles virus strains. However, in view of the definite age dependent susceptibility of small

laboratory animals to measles virus and the correlation of SSPE in children to early measles infection (25) such a study might be worth doing.

It is not known whether the differences in neurovirulence demonstrated in this study are caused by the varied passage history of the SSPE virus strains or to innate differences already present at the time of their isolation from the patient's brain. Since the one SSPE strain that showed the most neurovirulence, i.e. D.R. and two of the strains that showed no neurovirulence, i.e. strains Mantooh and Halle, all had a short passage history in tissue culture, it seems that either the last possibility is the most likely or that changes in SSPE virus isolates can in some instances occur very rapidly during passage in measles virus susceptible cell lines. Given the variability in duration and course of the human SSPE infection, certain differences in the properties of the SSPE virus isolates can be expected. In any case, the question arose of whether the differences in neurovirulence of the various SSPE virus strains reflect differences in biological properties that can be demonstrated in cell culture. Certain biological properties of the various strains were therefore studied and compared in an attempt to find a correlation with their virulence in ferrets. It was found that the strains fall into two main groups. The first group, including wt strains Edmonston and Woodfolk and SSPE strains Mantooh, Halle, and LEC-S, produces large amounts of infectious virions by budding from the cellular membrane. This is in agreement with earlier studies by other workers (3, 4, 6, 26-32). In our study, it was noteworthy that significant titers of cell-free virus were produced by these strains in cultures of ferret brain cells without the formation of multinucleated syncytia by cell fusion.

The second group of measles virus strains studied, that included SSPE strains LEC, Biken, IP-3, and D.R. does not produce detectable amounts of typical virions by budding. This also agrees with previous studies (7, 8, 17, 30, 33). However, all of these strains contained a significant amount of filterable syncytiogenic activity in Vero cells (Table IV). Although particles in the filterable size range were not observed under the electron microscope, cell-free particles with a diameter of 500-1,000 m μ were observed in Vero syncytia infected with strains Biken and IP-3, but not in D.R. infected syncytia. These particles contained a single membrane without spikes and without an underlying nucleocapsid alignment but were filled with smooth nucleocapsids (Fig. 3). There was no indication that they were formed by budding. When the filtered particles were inoculated into Vero cell cultures their CPE and growth characteristics were indistinguishable from those of the original suspensions of live syncytia, indicating that they do not represent virion-producing variants of the SSPE strains. It was noteworthy, however, that the cell-free particles of strains Biken and IP-3 were not syncytiogenic in FB cultures and did not propagate in these cultures. The data indicate that the particles cannot be classified as measles virions, but resemble large atypical particles sometimes seen in Vero cells infected with strain Edmonston (Fig. 3A). They may be formed from infected cells in a manner less organized than the budding process.

In addition to the high degree of cell-association and the absence of typical measles virions in the above four SSPE virus strains they differ from the first group of wt measles and SSPE virus strains in being syncytiogenic in FB cultures. The two groups, therefore, differ in their ability to spread by fusion in cells of FB.

The biological properties of the various measles virus strains studied are summarized in Table V which also shows their neurovirulence in ferrets. There is a definite

TABLE V
Summary of Biological Properties in Tissue Cultures and Neurovirulence in Ferrets of Wild-Type and SSPE Strains of Measles Virus

Measles virus strain	Hemadsorption	Production of virions by budding	Cell-fusion in FB cultures	Neurovirulence in adult ferrets
Wt Edmonston				
Wt Woodfolk	+	+	-	-
SSPE Mantooth				
SSPE Halle	+	+	-	-
SSPE LEC-S				
SSPE LEC	+	-	+	+
SSPE IP-3	+/-	-	+	+
SSPE Biken	-	-	+	+
SSPE D.R.	-	-	2+	2+

correlation between the cell-association and cell-fusing activity of the various strains in cultures of FB cells and their neurovirulence in ferrets. This seems to be true to the extent that the neurovirulence in ferrets of a given SSPE strain can be predicted on the basis of these biological properties. It appears that the cell-association of the virus is necessary for the infection to persist since it would otherwise be cleared by the immune response. This probably occurs in ferrets inoculated with the wt and the productive SSPE measles virus strains. In these strains the production of virions by the ferret cells apparently leads to an early formation of antibodies that neutralize the virions and terminate the infection.

The ability of the cell-associated strains to spread by fusion in FB cells also seems to be of the greatest importance. It can be speculated that strain D.R., which shows the greatest cell-fusing activity in FB cultures, also spreads most rapidly by fusion in the FB and, therefore, causes an acute disease before the formation of antibodies. The course of the infection can be altered from acute to subacute by preimmunization with measles vaccine (2) and it can be eliminated altogether by a simultaneous intracerebral inoculation with wt strain Edmonston (H. Thormar, unpublished observations), which causes a significant formation of neutralizing serum antibodies within 2 wk.

Strain Biken spreads more slowly than strain D.R. in FB cultures and presumably also in the FB. Therefore, in some instances, the immune response has time to develop before clinical signs appear. This results either in a persistent or subacute encephalitis or in a complete clearing of the infection. It is possible that the large number of cell-free particles present in Vero cultures carrying the Biken strain (Table IV) contribute to the production of persistent infection in the ferrets, either by stimulation of the antibody response or by directly interfering with the spread of cell-associated virus by fusion.

It is of interest that all of the various measles virus strains tested behaved in an identical manner in cultures of ferret brain and human brain cells. Thus, the strains that were neurovirulent in ferrets also spread by cell-fusion in human as well as in FB cell cultures. It, therefore, seems possible that the correlation between cell-association,

cell-fusion, and neurovirulence holds true in humans like in ferrets. It can then be speculated that SSPE in children is caused by a modification of the wt measles virus resulting in a reduction of its virion producing activity and an increase in its cell-fusing activity. SSPE strains Mantooh, Halle, and LEC-S have apparently lost these SSPE characteristics by passage through tissue cultures after isolation from the brain. This raises the question of why some of the SSPE measles virus isolates acquire the properties of the wt strains so easily in tissue culture, whereas others, and apparently most of them (34), retain the properties prevalent at the time of isolation from the brain. The mechanisms whereby an SSPE virus reverts *in vitro* are unknown, but there are at least two possibilities. First, although the virus is present predominantly in the cell-associated, cell-fusing form in the SSPE brain, productive virus may also be present in some brains in amounts too small to be directly detectable. Passage in cell cultures, particularly in cocultures with measles virus-susceptible cell lines, would select for productive virus and the larger its concentration is in the brain, the sooner it would become detectable in the cell culture. Second, it is possible that the measles virus shows varying degrees of cell-association in different SSPE patients, possibly related to the clinical course of the disease. The isolates would therefore differ with respect to how easily they revert to the productive type in cell cultures.

The correlation between the biological properties of the different measles virus strains in FB cultures and their neurovirulence in ferrets is of interest because it not only helps predict the effect of a given strain when inoculated into the animal, but may also give some information on the pathogenesis of the brain infection. Further studies of this type, comparing viral properties in brain cell cultures and in the brain *in vivo*, seem worth doing, by using different animal species and virus strains with varying neuropathogenicity. Studies of viruses in human brain cell cultures might in some instances help us understand why they are virulent in the human brain.

Summary

The neurovirulence of two wild type (wt) and seven Subacute Sclerosing Panencephalitis (SSPE) measles virus strains was tested in young adult ferrets by intracerebral (IC) inoculation of infected Vero cell suspensions. Wt strains Edmonston and Woodfolk and SSPE strains Mantooh, Halle, and LEC-S did not produce a detectable encephalitis in the ferrets, but caused a significant formation of serum antibodies against measles virus. SSPE strains LEC, IP-3, Biken, and D.R., on the other hand, were all neurovirulent in ferrets, particularly strain D.R. which caused an acute encephalitis in all inoculated animals. Strain Biken was of particular interest since it caused a subacute encephalitis in four of seven ferrets. The subacute encephalitis was characterized by a long incubation time, persistence of virus in the brain for at least 8 mo, widespread inflammatory lesions, and production of measles virus specific IgG in the brain.

A study of the biological properties of the various measles virus strains showed that wt strains Edmonston and Woodfolk and SSPE strains Mantooh, Halle, and LEC-S produced free virus particles in significant titers both in Vero and ferret brain (FB) cultures. Cytopathic effect (CPE) with cell-fusion was marked in Vero cultures, whereas only minimal CPE and no cell-fusion were observed in the FB cultures. SSPE strains LEC, IP-3, Biken, and D.R., on the other hand, were mostly cell-associated in Vero and FB cultures, although atypical cell-free particles were produced by strains

Biken and IP-3. All four strains showed cell-fusing activity in FB cultures, particularly strain D.R., which was the only strain that spread more actively by fusion in FB than in Vero cultures. The results are discussed in relation to the neurovirulence of the various measles virus strains in adult ferrets. Pronounced cell-fusing activity in FB cells and cell-association with minimal or no production of cell-free virus seem to be essential to establish a brain infection in the animals.

We thank Dr. H. M. Wisniewski for critical review and discussion of this work, Dr. G. A. Jarvis and Dr. J. W. Shek for histological examination of the ferret brains, Mr. K. Arnesen, Mr. M. Barshatzky, Mr. A. Bove, and Mrs. A. Kane for technical assistance, Mr. L. Black and Mrs. E. M. Riedel for assistance in preparing the manuscript.

Received for publication 10 April 1978.

References

1. Thormar, H., G. A. Jarvis, S. C. Karl, and H. R. Brown. 1973. Passage in ferrets of encephalitogenic cell-associated measles virus isolated from brain of a patient with subacute sclerosing panencephalitis. *J. Infect. Dis.* **127**:678.
2. Thormar, H., K. Arnesen, and P. D. Mehta. 1977. Encephalitis in ferrets caused by a nonproductive strain of measles virus (D.R.) isolated from a patient with subacute sclerosing panencephalitis. *J. Infect. Dis.* **136**:229.
3. Horta-Barbosa, L., D. A. Fucillo, W. T. London, J. T. Jabbour, W. Zeman, and J. L. Sever. 1969. Isolation of measles virus from brain cell cultures of two patients with subacute sclerosing panencephalitis. *Proc. Soc. Exp. Biol. Med.* **132**:272.
4. Horta-Barbosa, L., R. Hamilton, B. Wittig, D. A. Fucillo, and J. L. Sever. 1971. Subacute sclerosing panencephalitis: isolation of suppressed measles virus from lymph node biopsies. *Science (Wash. D.C.)*. **173**:840.
5. Barbanti-Brodano, G., S. Oyanagi, M. Katz, and H. Koprowski. 1970. Presence of two different viral agents in brain cells of patients with subacute sclerosing panencephalitis. *Proc. Soc. Exp. Biol. Med.* **134**:230.
6. ter Meulen, V., M. Katz, and Y. M. Kackell. 1973. Properties of SSPE virus; tissue culture and animal studies. *Ann. Clin. Res.* **5**:293.
7. Ueda, S., Y. Okuno, Y. Okuno, Y. Hamamoto, and H. Ohya. 1975. Subacute sclerosing panencephalitis (SSPE): isolation of a defective variant of measles virus from brain obtained at autopsy. *Biken J.* **18**:113.
8. Burnstein, T., L. B. Jacobsen, W. Zeman, and T. T. Chen. 1974. Persistent infection of BSC-I cells by defective measles virus derived from subacute sclerosing panencephalitis. *Infect. Immun.* **10**:1378.
9. Lowry, D. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.
10. Fahey, J. L., and E. M. McKelvey. 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.* **94**:84.
11. Norrby, E. 1962. Hemagglutination by measles, part 4. A simple procedure for production of high potency antigens for hemagglutination-inhibition (HI) tests. *Proc. Soc. Exp. Biol. Med.* **111**:814.
12. Sever, J. L. 1962. Application of a microtechnique to viral serological investigations. *J. Immunol.* **88**:320.
13. Koprowski, H., G. Barbanti-Brodano, and M. Katz. 1970. Interaction between papova-like virus and paramyxovirus in human brain cells: a hypothesis. *Nature (Lond.)*. **225**:1045.
14. Katz, M., L. B. Rorke, W. S. Masland, G. B. Brodano, and H. Koprowski. 1970. Subacute sclerosing panencephalitis: isolation of a virus encephalitogenic for ferrets. *J. Infect. Dis.* **121**:188.

15. ter Meulen, V., M. Katz, and D. Müller. 1972. Subacute sclerosing panencephalitis: a review. *Curr. Top. Microbiol.* **257**:1.
16. Ueda, S., T. Otsuka, and Y. Okuno. 1975. Experimental subacute sclerosing panencephalitis (SSPE) in a monkey by subcutaneous inoculation with a defective SSPE virus. *Biken J.* **18**:179.
17. Albrecht, P., T. Burnstein, M. J. Klutch, J. T. Hicks, and F. A. Ennis. 1977. Subacute sclerosing panencephalitis: experimental infection in primates. *Science (Wash. D.C.)*. **195**:64.
18. Byington, D. P., A. E. Castro, and T. Burnstein. 1970. Adaptation to hamsters of neurotropic measles virus from subacute sclerosing panencephalitis. *Nature (Lond.)*. **225**:554.
19. Johnson, K. P., and D. P. Byington. 1971. Subacute sclerosing panencephalitis (SSPE) agent in hamsters. 1. Acute giant cell encephalitis in newborn animals. *Exp. Mol. Pathol.* **15**:373.
20. Byington, D. P., and K. P. Johnson. 1972. Experimental subacute sclerosing panencephalitis in the hamster: correlation of age with chronic inclusion cell encephalitis. *J. Infect. Dis.* **126**:18.
21. Imagawa, D. T., and J. M. Adams. 1958. Propagation of measles virus in suckling mice. *Proc. Soc. Exp. Biol. Med.* **98**:567.
22. Wear, D. J., and F. Rapp. 1970. Encephalitis in newborn hamsters after intracerebral injection of attenuated human measles virus. *Nature (Lond.)*. **227**:1347.
23. Janda, Z., E. Norrby, and H. Marusyk. 1971. Neurotropism of measles virus variants in hamsters. *J. Infect. Dis.* **124**:553.
24. Byington, D. P., and T. Burnstein. 1973. Measles encephalitis produced in suckling rats. *Exp. Mol. Pathol.* **19**:36.
25. Jabbour, J. T., D. A. Duenas, J. L. Sever, H. M. Krebs, and L. Horta-Barbosa. 1972. Epidemiology of subacute sclerosing panencephalitis (SSPE). A report of the SSPE registry. *J. Am. Med. Assoc.* **220**:959.
26. Nakai, M., and D. T. Imagawa. 1969. Electron microscopy of measles virus replication. *J. Virol.* **3**:187.
27. Nakai, T., F. L. Shand, and A. F. Howatson. 1969. Development of measles virus in vitro. *Virology* **38**:50.
28. Dubois-Dalcq, M., and L. H. Barbosa. 1973. Immunoperoxidase stain of measles antigen in tissue culture. *J. Virol.* **12**:909.
29. Dubois-Dalcq, M., L. H. Barbosa, R. Hamilton, and J. L. Sever. 1974. Comparison between productive and latent subacute sclerosing panencephalitis viral infection in vitro. *Lab. Invest.* **30**:241.
30. Brown, H. R., and H. Thormar. 1976. A non-productive subacute sclerosing panencephalitis (SSPE) virus of human and ferret: an ultrastructural study. *Acta Neuropathol. (Berlin)*. **34**:339.
31. ter Meulen, V., M. Katz, Y. M. Kackell, G. Barbanti-Brodano, H. Koprowski, and E. H. Lennette. 1972. Subacute sclerosing panencephalitis: in vitro characterization of viruses isolated from brain cells in culture. *J. Infect. Dis.* **126**:11.
32. Oyanagi, S., V. ter Meulen, M. Katz, and H. Koprowski. 1971. Comparison of subacute sclerosing panencephalitis and measles viruses: an electron microscope study. *J. Virol.* **7**:176.
33. Oyanagi, S., V. ter Meulen, D. Müller, M. Katz, and H. Koprowski. 1970. Electron microscopic observations in subacute sclerosing panencephalitis brain cell cultures: their correlation with cytochemical and immunocytological findings. *J. Virol.* **6**:370.
34. Katz, M., and H. Koprowski. 1973. The significance of failure to isolate infectious viruses in cases of subacute sclerosing panencephalitis. *Arch. Ges. Virusforsch.* **41**:390.