INFECTION AND IMMUNITY, Nov. 1981, p. 581-587
0019-9567/81/110581-07$02.00/0

Vol. 34, No. 2

Encephalitogenicity of Measles Virus in Marmosets

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Received 23 June 1981/Accepted 14 July 1981

Marmosets infected intracerebrally with the wild Edmonston strain of measles virus developed encephalitis, demonstrated histologically and by the fluorescent-antibody technique. The infection remained clinically silent over a 14-day observation period. Animals infected intracerebrally with the JM strain of wild measles virus had only mild encephalitic changes but died of the visceral form of measles infection. Marmosets inoculated with measles vaccine had no encephalitis and remained clinically well. Marmosets appear to be a sensitive indicator of the viscerotropic and neurotropic properties of measles virus.

In a previous study marmosets of the *Saguinus mystax* subspecies were shown to be highly susceptible to infection with measles virus (3, 10). When inoculated with wild strains of the virus, they responded with variable rates of mortality. On the other hand, inoculation of measles vaccine resulted in a nonlethal immunizing infection. None of the wild or attenuated strains was neuroinvasive after peripheral inoculation. The present study was undertaken to determine whether any of the virus strains tested previously are encephalitogenic when inoculated directly into the central nervous system (CNS). The results of these experiments show that measles virus strains differ in their neurotropic potential in marmosets and that the visceral and neutrotropic pathogenicity of a given strain do not necessarily coincide.

MATERIALS AND METHODS

Animals. Adult male and female moustached marmosets (*S. mystax*), captured in the wild in Peru, were used exclusively. They had been in captivity for at least 6 months before their use in these experiments. They weighed between 476 and 627 g.

Viruses. Two wild and one attenuated strain of measles virus were used. One of the wild viruses was strain Edmonston received through Hope Hopps, Bureau of Biologics, Food and Drug Administration, in its seventh passage in human embryonic kidney cells (5). It received one additional passage in African green monkey kidney cells and had a titer of 1.4×10^7 plaque-forming units per ml.

The second wild measles virus was strain JM isolated in this laboratory in 1977 from the throat swab of a patient with a typical measles rash. It was isolated and passaged eight times in Vero cell cultures and had a titer of 5×10^6 fluorescent focus-forming units per ml.

One commercial vaccine, strain Moraten, derived from the Edmonston strain of measles virus (8) and produced by Merck Sharp & Dohme (West Point, Pa.), was used without further passage in tissue cultures.

Cell cultures and media. Vero cells at passage levels 170 through 200 were used for virus titration, for virus isolation from marmoset tissues, and for antibody determinations. The cells were grown and maintained in Eagle minimum essential medium supplemented with 10% heat-inactivated fetal bovine serum, 0.03% glutamine, 50 μg of gentamicin per ml, and 0.4 μg of amphotericin B per ml. Overlay medium consisted of Eagle minimum essential medium containing 5% fetal bovine serum and a final concentration of 0.5% agarose (Seakem agarose, Microbiological Associates, Bethesda, Md.).

Experimental infection. The animals were infected intracerebrally into the right thalamic area with 0.2 ml of virus suspension. The virus preparations were diluted so as to yield approximately the same amount of virus per inoculum. Based on back titration, the animals received 2,800 plaque-forming units of wild Edmonston virus, 2,000 focus-forming units of JM virus, or 2,000 plaque-forming units of the vaccine virus. Two control animals were inoculated intracerebrally with diluent only. Six animals received 0.25 ml of human globulin (measles hemagglutination inhibition titer 1:256) per kg intramuscularly 24 h after virus infection (see Table 1). At given times after infection the animals were sacrificed by exsanguination under general anesthesia. The brain was removed aseptically and divided by coronal and longitudinal sections into 10 tissue blocks, which were used for virus isolation, fluorescent-antibody (FA) staining, and histology (Fig. 1).

Virus isolation. Parts of the brain, lung, lymph nodes, and spleen were minced, washed, and placed into Vero cell cultures. One week later the Vero monolayers were trypsinized, and the cells were subcultured. The virus cytopathic effect was read 1 week after subculture. Selected cytopathic cultures were stained by the FA technique to confirm the specificity of the isolates.

Antibody determination. The measles virus

plaque neutralization test has been described in detail (3). The antibody titer was expressed as the serum dilution reducing the number of virus plaques by 50%.

FA technique. Cryostat sections of brain tissue were fixed with acetone and stained by the indirect FA method using a hyperimmune serum (hemagglutination inhibition titer 1:2,048) in the first step and a fluorescein isothiocyanate-labeled antiglobulin in the second step. From each block of brain tissue, cryostat sections were cut at four levels approximately 250 μm apart, which encompassed about two-thirds of the depth of each tissue block.

Histology. Tissues were fixed in a solution composed of formaldehyde, acetic acid, and saturated solution of picric acid in water in proportions of 25:5:75 (Carnoy solution), embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin and eosin.

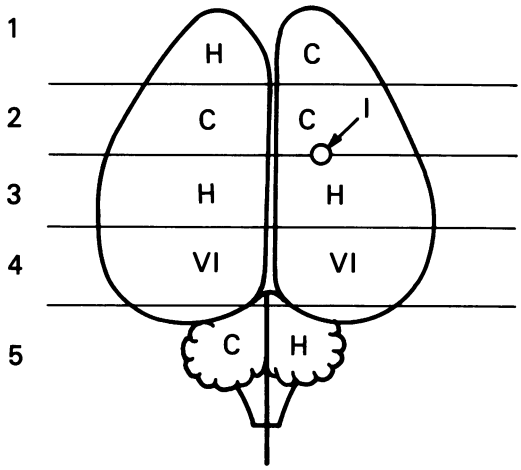

FIG. 1. *Dissection of the brain of marmosets for laboratory investigation. I, area of virus inoculation; C, tissue for cryostat processing and FA staining; H, tissue for histological examination; VI, tissue for virus isolation.*

RESULTS

Course of infection. Animals infected with the wild Edmonston virus or with the vaccine virus remained free of disease throughout the 14 days of observation (Table 1). The only exception was two animals infected with the Edmonston strain who did not receive measles-immune globulin and developed an atypical rash presenting as reddening of the face or chest and groin on day 13. Three animals inoculated with the JM strain of wild measles virus were severely sick for 2 to 3 days before being sacrificed. The most pronounced signs were general weakness and bloody stools. Animal no. 193 had a bright red skin on the neck, around the ears, and in the groin on day 10. Animal no. 192 had a bright red abdomen, groin, and vulva for 2 days before becoming moribund.

Antibody response. Control animals and Edmonston-infected animals immunized passively with human globulin developed transient low-level antibody titers which disappeared by day 13 (Table 2). Passive immunization interfered with the antibody response in both animals infected with the Edmonston virus. Animals which did not receive human globulin developed measurable antibody titers 14 or 30 days after infection. The antibody response in animals infected with the JM virus followed a different pattern and will be interpreted below.

Infection of the CNS. Table 3 shows that animals infected intracerebrally with the wild Edmonston virus had more severe encephalitic changes than animals infected with the JM strain. This was evident from the more frequent virus isolation, a wider spread of virus antigen in the brain tissue, and more pronounced histological changes in animals infected with the Ed-

TABLE 1. *Clinical response of marmosets to intracerebral infection with measles virus*

Animal no.	Virus[a]	Measles immune globulin	Sacrificed (days after infection)	Signs of disease at time of sacrifice		
				General	Rash	Wt change (g)
100	Edmonston	−	14	None	+	−78
187	Edmonston	−	14	None	+	−95
143	Edmonston	+	14	None		−50
158	Edmonston	+	14	None		−50
192	JM	−	13	Moribund, bloody stools	+	−176
193	JM	−	10	Moribund, bloody stools	+	−99
176	JM	+	14	Moribund, bloody stools		−105
177	JM	+	14	None		−47
162	Vaccine	−	14	None		−105
189	Vaccine	−	14	None		−48
144	Vaccine	−	30	None		+17
161	Vaccine	−	30	None		−16
85	Control	+	14	None		Not done
142	Control	+	14	None		+4

[a] The virus dose per animal was 2,800 plaque-forming units of Edmonston virus, 2,000 focus-forming units of JM virus, or 2,000 plaque-forming units of the vaccine virus.

monston virus. Viral antigen in the CNS was located in neurons and glia cells, as well as in what appeared to be mononuclear cells in perivascular spaces (Fig. 2). Neuronal damage, mild perivascular cuffs, and foci of glia proliferation were the hallmarks of histological changes (Fig. 3).

The attenuated measles virus caused no encephalitis, and virus was not isolated from the brain tissue. FA staining for viral antigen was also negative in animals inoculated with vaccine virus. These results from marmosets inoculated with vaccine were obtained in animals sacrificed both 14 and 30 days after infection.

TABLE 2. *Antibody response of marmosets to intracerebral inoculation with measles virus*

Animal no.	Virus	Measles immune globulin	Neutralizing antibody titer (reciprocal) at day after infection[a]: 0	7	10–14	30
100	Edmonston	–	<8	<8	400	NA
187	Edmonston	–	<8	<8	510	NA
143	Edmonston	+	<8	11.0	<8	NA
158	Edmonston	+	<8	9.0	<8	NA
192	JM	–	<8	<8	<8	NA
193	JM	–	<8	<8	<8	NA
176	JM	+	<8	<8	<8	NA
177	JM	+	<8	<8	31	NA
162	Vaccine	–	<8	<8	27	NA
189	Vaccine	–	<8	<8	123	NA
144	Vaccine	–	<8	<8	ND	800
161	Vaccine	–	<8	<8	ND	4,100
85	Control	+	<8	10	<8	NA
142	Control	+	<8	10	<8	NA

[a] NA, Not available; ND, Not done.

Infection of visceral tissues. Infectious virus and viral antigen were present in tissues of all animals 14 days after infection except control animals (Table 3). There was appreciably less viral antigen in tissues of animals infected with the vaccine virus compared with animals infected with the wild viruses. Histological changes in visceral tissues were similar to those described previously (3).

DISCUSSION

The most remarkable finding concerned the extent of CNS infection caused by the three viruses. The Edmonston virus appeared to be considerably more neurotropic on intracerebral inoculation than the highly viscerotropic strain JM. It was reassuring to find that the attenuated vaccine strain of measles virus was completely free of neurovirulence when inoculated intracerebrally into marmosets.

The CNS infection caused by the wild Edmonston virus remained clinically silent. This result may have been because of the early termination of the experiment, or because the de-

TABLE 3. *Virological and histological findings on marmosets infected intracerebrally with measles virus*

Animal no.	Virus	Brain: Virus isolation	Brain: Viral antigen[a]	Brain: Histology[b]	Visceral tissues[c]: Lung: Virus isolation	Lung: FA	Lymphatic tissue: Virus isolation	Lymphatic tissue: FA	Colon[d]
100	Edmonston	Positive	2+	1+	Positive	1+	Positive	2+	1+
187	Edmonston	Positive	3+	3+	Positive	2+	Positive	2+	0
143	Edmonston	Positive	3+	1+	Positive	1+	Positive	2+	2+
158	Edmonston	Positive	3+	2+	Positive	1+	Positive	2+	2+
192	JM	Negative	0	1+	Positive	1+	Positive	2+	3+
193	JM	Positive	0	0	Positive	0	NA	2+	3+
176	JM	Positive	2+	1+	Positive	2+	Positive	2+	1+
177	JM	Negative	0	0	Negative	2+	Positive	0	0
162	Vaccine	Negative	0	0	Positive	0	Positive	1+	1+
189	Vaccine	Negative	0	0	Positive	0	Positive	0	1+
144	Vaccine	Negative	0	0	Negative	0	Negative	0	0
161	Vaccine	Negative	0	0	Negative	0	Negative	0	0
85	Control	Negative	0	0	Negative	0	Negative	0	0
142	Control	Negative	0	0	Negative	0	Negative	0	0

[a] Extent of virus distribution in the CNS determined by FA staining of cryostat sections: 1+ = few measles-positive nerve cells in sections of one tissue block; 2+ = scattered positive cells in sections of two or more tissue blocks; 3+ = large foci of positive cells in one or two tissue blocks and scattered positive cells in other tissue blocks.

[b] Extent of inflammatory and degenerative lesions: 1+ = slight, mostly degenerative changes in the area of inoculation only; 2+ = moderate changes in sections of two or more tissue blocks; 3+ = pronounced changes in all tissue blocks.

[c] 1+ to 3+ = arbitrary scale of extent of antigen staining in visceral tissues. NA, Not available.

[d] FA staining results only; virus isolation not performed.

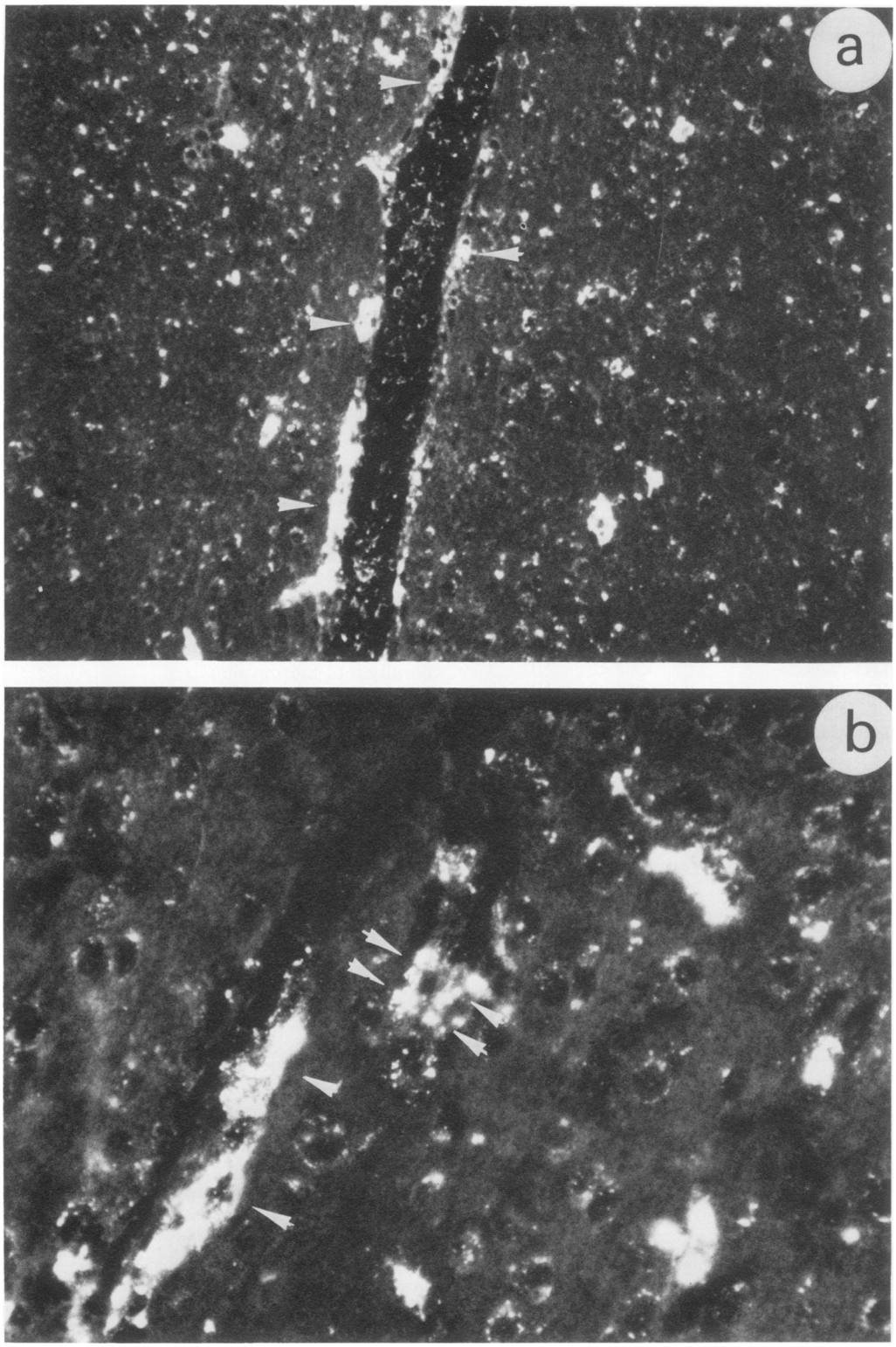

FIG. 2. *Brain of marmoset infected intracerebrally with the Edmonston strain of measles virus. (a) Measles antigen scattered in nervous tissue and in perivascular mononuclear cells (arrows). FA stain, ×600. (b) Measles antigen distribution at higher magnification. Arrows: antigen in perivascular space of small vessels. ×1,500.*

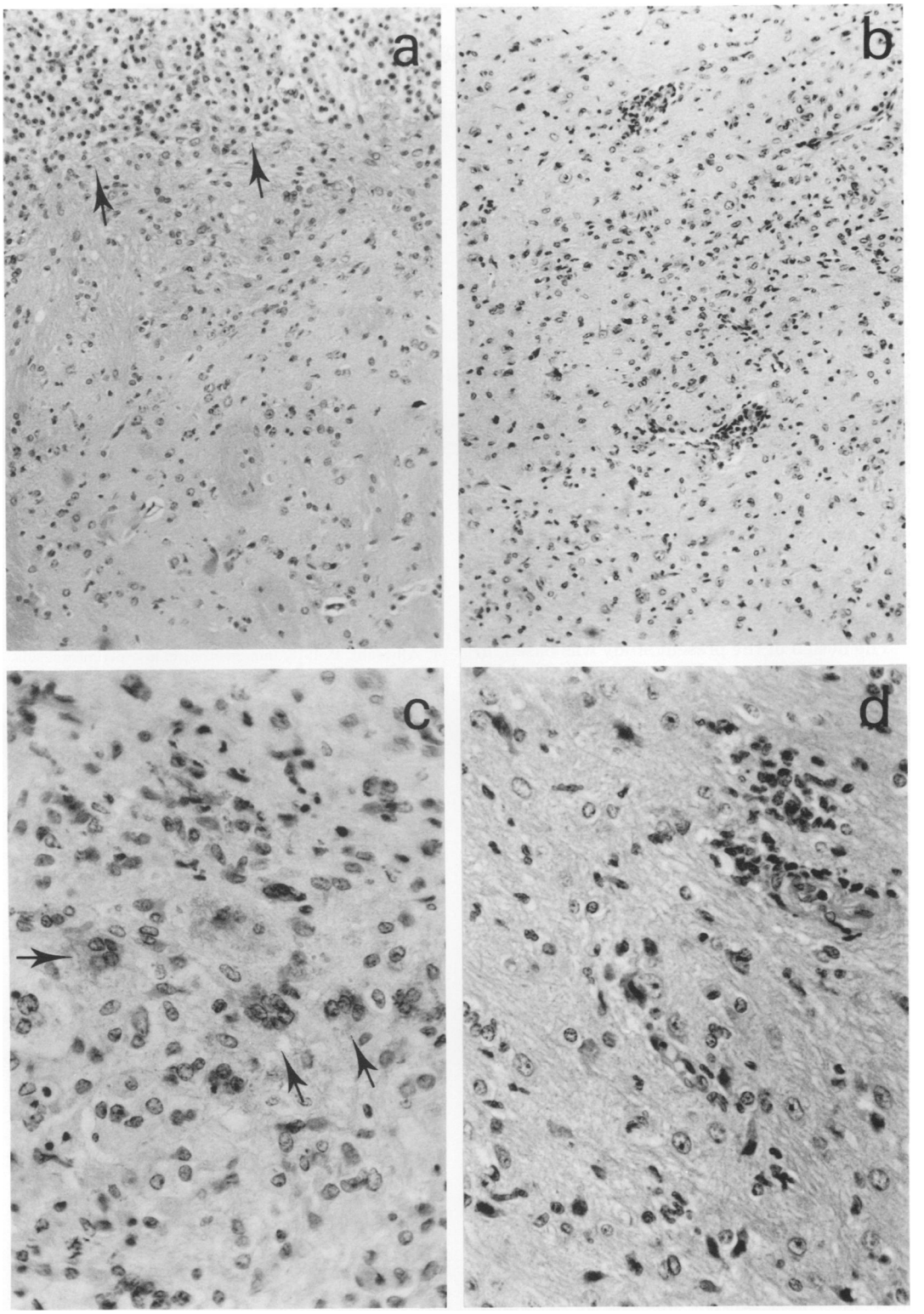

FIG. 3. *Brain of marmosets infected intracerebrally with measles virus. (a) Animal infected with measles vaccine. Normal brain tissue adjacent to inoculation track (arrows), ×240. (b) Animal infected with wild Edmonston virus. Perivascular cuffs and proliferation of glia cells in brain tissue, ×240. (c) Animal infected with wild Edmonston virus. Multinuclear giant cells in brain tissue (arrows), ×400. (d) Animal infected with Edmonston virus. Neuronal damage in area of inflammation, ×400. All sections stained with hematoxylin and eosin.*

gree of neurovirulence did not reach the threshold of clinical manifestation as is the case with subacute sclerosing panencephalitic measles isolates (2). A possibly similar situation was encountered in humans, whose electroencephalographic studies have shown that subclinical measles encephalitis in the course of natural measles was much more common than was thought on the basis of clinical symptomatology (6).

The present results suggest that marmosets are the most susceptible primate hosts of measles virus reported to date. Marmosets are considerably more vulnerable than rhesus monkeys. For instance, rhesus monkeys did not develop encephalitis on intracerebral inoculation of a number of wild measles virus strains, including strain Edmonston (1, 4, 5, 9, 13). Mortality in rhesus monkeys after natural measles infection is low (11, 12) and, after inoculation of laboratory strains, virtually unknown (1, 4, 7). It is fair to assume that marmosets may serve as sensitive indicators of attenuation for other human viruses currently under investigation for the development of live attenuated vaccines.

The design of the present study was based, in part, on previous experience, according to which both the JM and the Edmonston viruses caused generalized fatal infection when inoculated parenterally (3, 10). To avoid premature death from generalized measles infection, some of the animals infected with JM and Edmonston virus were given immune globulin 24 h after infection. It was expected that the dose of 0.25 ml/kg of weight recommended for measles prevention in humans (14) would not interfere with the replication of virus inoculated intracerebrally.

The results of the present experiments show that passive immunization mitigated, but did not prevent, visceral infection in animals infected intracerebrally with the wild measles virus strains. This mitigating effect was seen in the longer survival of passively immunized animals infected with the JM virus and in the absence of skin rash in animals infected with both JM and Edmonston virus. The effect of passive immunization on viral antigen distribution in visceral tissues was less obvious. Although reducing the extent of infection in the colon in animals infected with the JM virus, it apparently had an opposite effect on virus distribution in the colon of animals infected with the wild Edmonston virus. It is possible that these differences were in part due to a sampling error since only limited amounts of the colon were investigated. Most animals inoculated with measles virus had a weight loss of 3% (animal no. 161) to 30% (animal no. 192) on day 14 of the experiment, probably as a result of the infection.

Of particular significance was the antibody response in animals not protected by human serum globulin. As might be expected, the 14-day titers were higher in animals infected with the Edmonston virus than in animals who received the vaccine virus. Animals infected with the JM virus did not develop measurable antibody titers by the time they became moribund. This presumably was due to greater virulence for visceral tissues and the greater immunoparalytic effect of the JM virus as compared to the Edmonston virus (3). Passive immunization appeared to suppress or to delay seroconversion in animals infected with the Edmonston virus. Passive antibody could not be detected in sera of animals infected with the JM virus, probably having been absorbed by the more intensively replicating JM virus. It is possible that such early removal of passive antibody and the mitigating effect of passive antibody on the viral infection resulted in a measurable antibody response in animal no. 177 14 days after infection.

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