Pathogenicity of Minute Virus of Mice (MVM) for Rats, Mice, and Hamsters[1] (34710)

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This report concerns the pathogenicity of the minute virus of mice (MVM) for 3 common laboratory animals, of which two, the rat and mouse, are both natural hosts. MVM was originally discovered by Crawford (1) in preparation of mouse adenovirus. As a potential contaminant of any virus propagated in mouse or rat tissues, *in vivo* or *in vitro* the behavior of MVM as a pathogenic agent should be of concern as its effects in combination with other viruses may be difficult to assess. The physical, chemical, and *in vitro* biologic properties of MVM have been well described by Crawford (1) and by Crawford and associates (2).

Materials and Methods. Viruses. Our main source of MVM in present experiments was the commercial antigen supplied by Microbiological Associates of Bethesda, Maryland. A lesser number of experiments were carried out with a virus preparation obtained from Dr. L. V. Crawford (1). The H-1 virus employed was the KA-strain isolated from the lung of a rat given immunosuppressive doses of cortisone (3) and, in a lesser number of experiments, Toolan's original strain (4). The source of H-1 virus was infected RETC. MVM was prepared in infected mouse embryo tissue culture (METC).

Animals. Mice were purchased from the Charles River Breeding Laboratories, Inc. of North Wilmington, Massachusetts and both rats and mice from the National Laboratory Animal Company of St. Louis, Missouri; animals from these sources being designated by the prefixes CR and NL, respectively. Hamsters were obtained from the Lakeview Hamster Colony of Newfield, New Jersey. All of the above were purchased as pregnant animals, timed to deliver within 3 to 4 days of arrival at the laboratory. NL rats and mice used were free of Hi antibodies to MVM. In our experience NL mice have been the only ones naturally free of these antibodies. A proportion of NL rats were also free but a majority of those tested had serological evidence of infection. Animals used were maintained in isocages purchased from the Carworth Division of Becton, Dickinson, and Company.

Inoculation and harvesting. Neonatal rats, mice, and hamsters were inoculated by a combination of intracranial (ic) and intraperitoneal (ip) routes, using volumes of approximately 0.03 and 0.06 ml. Mice and rats were inoculated within 24 hr of birth and hamsters at 2 days of age. Tissues harvested were usually from 2 to 3 animals. These were killed with ether anesthesia, exsanguinated, and the tissues were frozen prior to grinding and preparation into 1:10 suspensions in TC fluid. Continuous passages were made with pools of infected livers, kidneys, spleens, and brains.

Tissue cultures. Mouse (METC) and rat embryo (RETC) tissue cultures were prepared from fetuses in their fourteenth to sixteenth days of gestation by methods described elsewhere (5). Both types of tissue culture were maintained in Eagle's basic medium (BME) containing 2% calf serum from which the gamma globulin had been removed, in addition to penicillin and streptomycin.

Serology. Neutralization tests in hamsters

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were performed by combined ic and ip inoculations of 2-day-old suckling hamsters with 1:1 mixtures of undiluted serum, inactivated by heating at 56° for 30 min, and various dilutions of virus. Deaths from infections were used in calculations of 50% end points for these tests as well as for infectivity titrations. Neutralization tests in day-old suckling mice were performed in a similar manner except that end points were determined by histologic examination for presence or absence of characteristic intranuclear inclusions, cytolytic effects (CPE) or hypoplastic changes in cell germinal centers and other rapidly replicating tissues in the central nervous system, particularly in the cerebellum (6). Extraneural tissues were also surveyed for these effects.

All animals were counted 2 days after inoculation, deaths prior to this time being regarded as nonspecific.

Inhibition of hemagglutination (Hi) tests were performed by Microbiological Associates. Procedures used in histologic studies were those described in a preceding report (3); sucklings were exsanguinated and the brain perfusion was fixed by the method of Malm (7) and other structures were immersion-fixed in Bouin's.

Results. Passage of MVM in hamsters. MVM was most pathogenic for hamsters. Thus 10 neonates inoculated at 2 days of age with virus obtained from Dr. L.V. Crawford were dead or moribound 6 days later. These results were repeated and served to indicate that MVM does not require prior adaptation to kill hamsters. A pool of livers, spleens, kidneys, and brains, harvested from these first passage animals had an LD_{50} of $10^{-6.5}$ when tested in a second passage. Similar material from a third passage in neonatal hamsters was used as the inoculum in neutralization tests. The purpose of these tests (see Table I) was 2-fold: (i) to demonstrate that the virus involved was actually MVM and not RV or H-1 and (ii) to show that the hamster-passaged virus was still capable of inducing characteristic MVM effects in mice.

Suckling hamsters became ill within 5 to 8 days. At this time an anal exudate was usually present and afflicted animals were small in comparison with uninoculated littermate controls. Similar pathogenic effects were obtained on first passage of the commercial preparation of MVM which had an HA titer of 1:256. Occasional hamsters surviving the effects of a low dose of MVM have had features of the mongolism described elsewhere for RV (8) and H-1 viruses (4), as well as peridontal disease (9) which is being made a subject of continuing study in cooperation with Dr. Paul Baer of the National Institute of Dental Research.

Passages of MVM in mice. MVM was carried through 9 continuous passages in neonatal NL mice. None of these mice showed signs of illness when killed for harvesting of infected tissues 5 or 6 days after inoculation,

TABLE I. Results of Neutralization Tests Performed in Newborn NL Mice Against MVM Previously Carried 3 Passages in Hamsters.

	Clinical		Wt (av 2 animals)			Histology	
Parvovirus used in preparation of rabbit antisera	Illness	Death	Inoculated (g)	Uninoc. controls (g)	Day postinoc.	No. with cerebell. lesions/no. inoc.	Days postinoc. examined
RV	Dwarf; weak	2	5	8	7	2/2	7
H-1	Dwarf; weak	2	3.75	7	7	5/5	7–22
MVM	None	None	7	7.13	7	0/5	7–22
Normal control ser.	Dwarf	None	ND	ND	—	6/6	6–22

but within the next 3 to 4 days many had failed to grow and were small in comparison with uninoculated littermate controls. This growth retardation was further illustrated in a litter of eighth passage mice in which 2 inoculated individuals weighed 10 g each and an uninoculated littermate 17 g when all were weighed at 3.5 weeks of age.

The cerebellum was a special target of MVM in infected mice, all examined having had mild or moderate lesions in the external germinal layer (EGL). The limited degree of involvement was compatible with a finding that none developed clinical ataxia. Attraction of virus to the cerebellum was further exemplified by the finding of cerebellar lesions in 2 out of 3 uninoculated controls in a litter inoculated 9 days previously. The lesions in these animals were at an earlier phase of development than those in inoculated littermates harvested at the same time, an indication that spontaneous transmission from infected to control mice had probably occurred.

The extent to which MVM proliferated in NL mice inoculated at birth and killed 7 days later is shown in Fig. 1. Points of note are that MVM (i) proliferated to highest titer in brains; (ii) was present in relatively high titers in urine as well as in gut, and (iii) that the level of MVM in blood was lower than in most of the organs tested. Each of the specimens were pools from 3 mice.

Hi tests performed on convalescent sera confirmed that mice inoculated were infected with MVM. Thus pooled serum from third passage mice, 62 days after inoculation, had a titer of 1:160 against MVM and another pool taken 39 days after inoculation from eight passage animals, of 1:80. Both sera were more negative for antibodies to RV and H-1 viruses. Sera from 6 uninoculated NL mice used as controls were negative.

There was no demonstrable increase in the virulence of MVM in 9 passages in mice and there were no deaths attributable to virus. On the other hand 3 passages in hamsters appeared to increase mouse virulence as indicated by deaths induced (see Table I).

Comparative study of H-1 virus in mice.

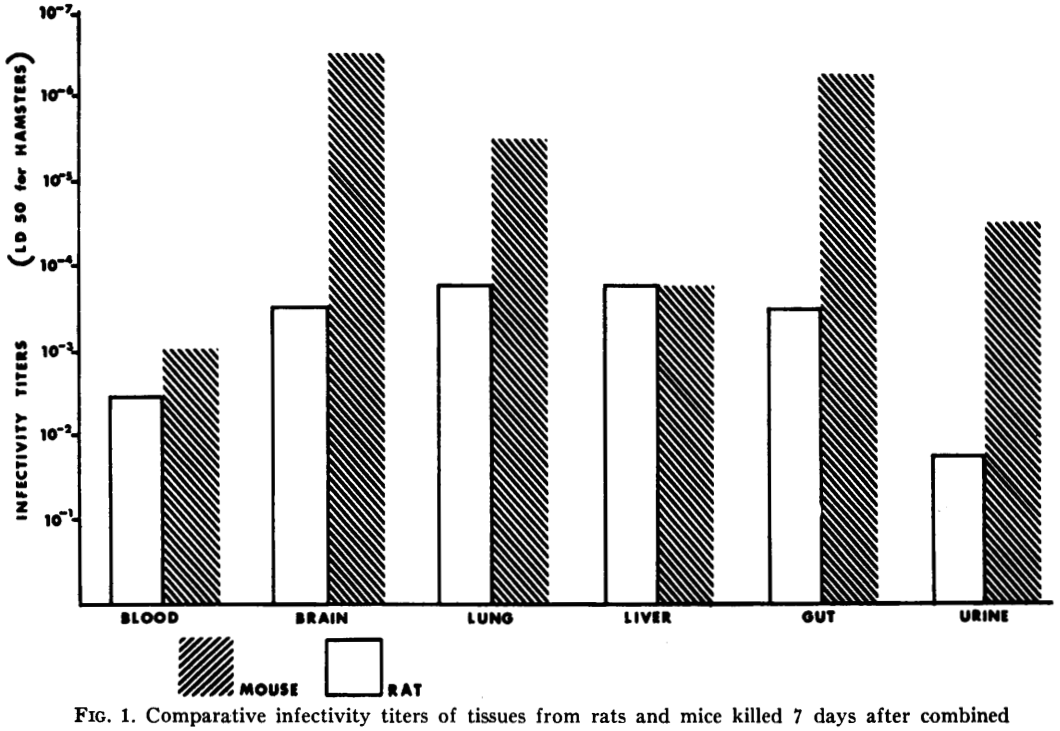

FIG. 1. Comparative infectivity titers of tissues from rats and mice killed 7 days after combined intracranial (ic) and intraperitoneal (ip) inoculations with MVM.

TABLE II. Results of Neutralization Tests Performed by ic Inoculations of Serum–Virus Mixtures, Containing 10 Cerebellum Infecting Doses of H-1 Virus into Newborn Mice.

Results (11–12 days postinoc.)	Rabbit antiserum used		
	vs. MVM	vs. H-1	Normal rabbit
No. pos*/no. inoc.	2/2	0/2	2/2

* Positive = viral attack on EGL of cerebellum, as seen on histological section.

Both NL and CR mice were susceptible to infections with H-1 virus. As with MVM the EGL of the developing cerebellum was the main target of attack. H-1 virus, however, was considerably more pathogenic not only as reflected in greater degrees of cell destruction seen on histologic sections of the cerebellum but also in the clinical finding that animals inoculated with H-1 developed ataxia within 2.5 to 3 weeks of inoculation. Results of neutralization tests with specific antiserums (Table II) indicated that the mice were infected with H-1 virus.

MVM infections in rats. NL rats in contrast to mice showed no runting or other signs of illness following inoculation of MVM within 24 hr of birth. The virus did, however, induce subclinical infections. This was shown when rats tested over successive days had LD_{50} titers of less than 10^{-1} on Day 1 and of approximately 10^{-2} in brains and livers on Day 4, there being no detectable viremia on either occasion. Peak titers of virus were attained by Day 7 when, as shown in Fig. 1, there was a blood level of $10^{-2.5}$, somewhat higher levels in brain and liver, and presence of virus in specimens of both urine and gut. It was apparent here that the clinically milder infections in the rats as compared to mice were accompanied by lower levels of recoverable virus. MVM was pantropic in both species but proliferated relatively less well in rat than in mouse brain. MVM was not recovered from urine or feces of suckling rats 21 days after inoculation.

The pathological effects of MVM in suckling rats were confined to the ependyma and choroid plexus in which focal areas with typical intranuclear inclusions and cytolytic effects were observed. The cerebellar external germinal layer showed no cytolytic effects and extraneural tissues were also unaffected. We have noted this pattern of disease previously in extremely mild examples of parvovirus infections.

MVM in populations of wild rats. Table III gives results of Hi tests on the sera of wild rats trapped on 3 town refuse dumps in the vicinity of Hanover, New Hampshire. Features of note are (i) that rats from 2 of the dumps had seriological evidence of infection with all 3 parvoviruses, namely RV, H-1, and MVM; and (ii) that while H-1 was the most prevalent virus at White River, MVM was the most prevalent virus among the total of 110 wild rats tested; and finally (iii) that RV was the least prevalent parvovirus in all 3 localities. Another feature was the varying levels of Hi titers. Here the majority of Hi titers for RV and H-1 viruses were 1:40 and above, while 70% of those for MVM were only 1:20. One might wonder whether these low serum titers of MVM antibodies were nonspecific reactions or might be a reflection of immunological relationships between the agents. Twelve rats in the total series, for example, had sera positive for all 3 parvoviruses, and a higher proportion of the MVM titers in this series, namely 7 of

TABLE III. Distribution of Positives in Hi Tests Run Against 3 Parvoviruses in Sera of Wild Rats.

Wild rats		Percentages positive in Hi tests		
Trapped in	No. tested	MVM	RV	H-1
Lyme	23	70	48	None
White River	82	65	32	80
Enfield	5	100	20	100
Percentages positive out of total tested for each virus		69.5	34	61

12, were 1:40 or above. On the other hand another 12 rats were positive for MVM alone and negative for both RV and H-1. These tests indicate that MVM is widely prevalent parasite of wild rats, a finding consistent with the observations of Parker and associates (10) that it is prevalent in colonies of domestic rats as well.

Collins and Parker (11) found MVM to be widely distributed in breeding colonies of laboratory mice. We tested 6 sera from wild house mice (*Mus musculus*) and found that all were negative.

Discussion. In comparison with other parvoviruses, MVM resembles H-1 and RV (3, 6) and panleukopenia (PVL) (12) in abilities to induce transplacental infections as well as to destroy cells of the external germinal layer of the cerebellum. On the other hand its behavior differs from that of H-1 and RV in inducing infections in both rats (10) and mice (11); in failing to induce CPE when proliferating in METC and RETC and third, in being a relatively poor producer of hemagglutinins as well as of intranuclear inclusions. All of these attributes may be associated, in some yet undetermined way, with the mildness of MVM-induced infections in its natural hosts. In this regard one might arrange observations on suckling animals an ascending scale of virulence in which MVM induced no overt disease in rats; a runting which may be transitory and unaccompanied by other signs of illness in mice, and finally lethal infections, even at high dilutions, in hamsters. If mild infections are indicative of a long-term, mutual adaptation of host and parasite, then one might consider MVM as being primarily a virus of rats. We still do not know whether MVM is a natural parasite of populations of wild house mice. It is worthy of note here that RV and H-1 virus are more virulent for mice than MVM. As reported by Matsuo and Spencer (13), RV behaves much like MVM in mice in inducing dwarfism and in proliferating to highest titers in brain.

A further point of similarity between RV, H-1, and MVM is that all 3 are pantropic. It is shown in present experiments, that MVM appears to proliferate in a wide variety of organs including the brain and to be excreted in urine and feces (as indicated by high titers in gut) which could both serve as natural routes of transmission. MVM should be considered as a potential contaminant of stock preparations of any virus prepared in tissues of rats or mice, *in vivo* or *in vitro*, in the manner, for example, that RV was found to contaminate preparations of the Moloney leukemia virus (14).

In summary, overall aims of present studies were (i) to gain understanding of the behavior of MVM in its natural as contrasted with an artificial host, and (ii) to enlarge concepts of the behavior of parvoviruses in general. These problems may well have relevance to important infections of man for, as discussed in previous reports, rat parvoviruses offer comparisons to human conditions such as Down's disease (9, 15) cerebellar hypoplasia (6), and hepatitis (16) among others.

One might speculate that if the 2 types of human hepatitis virus, I.H. and S.H. should be found, eventually, to be parvoviruses, then their interrelations might not be found to be too different from the model of the rat, which may harbor 3 types of parvoviruses, H-1, RV, and MVM simultaneously.

Summary. In studies made of its general behavior in laboratory animals MVM was found to induce lethal infections in suckling rats, and runting accompanied by proliferations of virus to high titers in many organs in mice. MVM attacked the cerebellum of infant mice, but without sufficient damage to induce clinical ataxia. When H-1 virus was studied in a comparable manner, however, it was found to induce marked ataxia accompanied by an extensive cerebellar hypoplasia. A survey of wild rats revealed that Hi antibodies to MVM (69.5%) were more prevalent than those to either H-1 (61%) or to rat virus (34%).

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