Mouse Hepatitis Virus Infection as a Highly Contagious, Prevalent, Enteric Infection of Mice. (27980)

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Of the previously reported strains of mouse hepatitis viruses, none has been recovered from uninoculated, normal mice; the great majority have appeared as extraneous agents in studies of human infectious hepatitis or mouse leukemia, or were "activated" by inoculation of *Eperythrozoon coccoides*. One strain, the JHM virus(1), was isolated from an uninoculated animal, being recovered from the central nervous system of a spontaneously paralyzed mouse.

Knowledge of the natural history of mouse hepatitis virus infection has consequently been lacking; even results of serologic testing for antibody have given little information. On the basis of the ability of *E. coccoides* to activate the agent, Gledhill(2) has advanced the theory that the virus is present in the liver in a latent state, perhaps resembling lysogeny.

This report presents evidence that mouse hepatitis virus infection is a prevalent and highly contagious enteric infection of laboratory mice.

Materials and methods. Mice (CD-1) from a colony (Charles River Farms, Brookline, Mass.) free of the virus were used for virus isolation studies, production of virus and antigen stocks, and production of antisera. The freedom of the CD-1 mice from infection is indicated by: a) absence of virus in tests of pooled feces of weanling mice, as described below; b) absence of neutralizing antibody for MHV-1, JHM, or the S strain of mouse hepatitis viruses in pooled sera of retired breeders; c) absence of complement-fixing (CF) antibody for the S strain in several hundred retired breeder and weanling mice tested individually; d) failure to induce hepatitis by 12 serial passages of *E. coccoides* in the form of 10% liver-spleen suspensions passed weekly in weanling mice; e) failure to elicit hepatitis in any of the more than 200 weanling mice used for weekly passage of *E. coccoides* in the form of a 10^{-5} dilution of blood; and f) the high degree of susceptibility of infant mice to spontaneous infection when exposed to the virus. This colony is Caesarian-derived, and reared in strict isolation.

Mice from various conventional, or in one case, Caesarian-derived, stocks were used as source of experimental material, for virus neutralization tests, and for some tests of pathogenicity.

Experimental mice were housed in 3 ways–a) in conventional jars or pans with perforated lids, b) in battery jars with a tightly fitting pad of ⅜″ glass fiber filter material inserted in the lid, to reduce airborne contamination, and c) in Trexler-type plastic isolators, using filtered air, sterilized food, bedding, and water, but without the use of air-locks for passing materials in or out.

Feces suspensions were prepared as 10% suspensions in Eagle's basal medium with 20% veal infusion broth, containing penicillin (100 u/ml) streptomycin (100 μg/ml) achromycin (10 μg/ml) and mycostatin (25 u/ml); the suspension was clarified by 2 cycles of centrifugation, first at 2500 rpm for 15 minutes, and then at 8000 rpm for 5 minutes, at 2-4°C. Suspensions were inoculated immediately or after one cycle of freezing (–65°C) and thawing.

Virus isolations and passages were carried out by intracerebral (i.c.) (0.02 ml) and intraperitoneal (i.p.) (0.03 ml) inoculation of infant CD-1 mice kept in plastic isolators. Materials were kept at 2°C for handling, and were stored at –65°C.

The mouse antibody production (MAP) test, as used for polyoma virus(3), was also used for virus detection. Weanling CD-1 mice were inoculated intraperitoneally with 0.2 ml of test material, held in filter top jars, and exsanguinated at 19-21 days. The jar was opened only once during this period–at

12 to 14 days for cleaning. The sera were tested individually at a 1:10 dilution for development of CF antibody to the S strain. Fifteen to 30 uninoculated or saline-inoculated mice were included in each test.

Complement fixation (CF) tests were carried out with either crude or acetone-ether extracted suckling mouse brain, or brain plus liver antigens, or with supernatant fluids from infected tissue cultures of the mouse liver cell line, Clone NCTC 1469(4,5); a micro plate modification of the Bengtson procedure was used. Details of serologic procedures will be reported later.

Results. Isolation of viral strains. Our attention was drawn to mouse hepatitis viruses by two observations. First, in attempts to isolate Theiler's viruses from feces of mice from colonies negative for HI antibody to GDVII virus, i.c. or intraspinal (i.s.) inoculation of feces extracts into weanling mice resulted in paralytic disease in about 20% of animals, after incubation periods of 5-25 days. In contrast to Theiler's viruses, the agents recovered from the brains were ether-sensitive; did not pass in weanling mice by the i.c. or i.s. route, although lethal for infant mice; and the brain suspensions of moribund infant mice were negative for hemagglutinin for human erythrocytes and did not react in CF tests with hyperimmune mouse antiserum to GDVII virus.

The second observation was that newborn mice of the CD-1 strain had an extremely high mortality rate when held in a large experimental animal room which housed mice from a variety of stocks. Mortality in such mice was 50 to 75% occurring mostly at 4 to 10 days of age, while mortality in comparable mice held in an adjacent laboratory was less than 10%. Clinical signs in these mice were either non-specific, with weakness and inanition, or were suggestive of encephalitis, with spasticity and incoordination; the only gross finding was occasional animals having small yellow or whitish spots on the liver. Microscopic examination was occasionally negative, or demonstrated focal hepatic necroses or focal areas of edema and degeneration of the tips of intestinal villi, similar in some respects to those described by Kraft(6) as produced by infection with the "lethal intestinal virus of infant mice (LIVIM)." From a pool of viscera, excluding intestines, of 8 sick CD-1 mice, a filterable agent was established in serial passage in infant mice, using combined i.c.-i.p. inoculation in early passages, and i.c. inoculation in later passages. The characteristics of the agent, designated the S strain, were determined with virus stocks consisting of brain-liver suspensions of the sixth suckling mouse passage; the agent passed through Selas 03 filters; was partially or completely inactivated by 2 hours exposure to diethyl ether; was pathogenic for suckling mice by i.c. or i.p. inoculation, generally titering 10^3 to 10^4 LD50 per 0.03ml of suspension in mice 1 to 10 days of age, producing signs of encephalitis and hepatitis; produced demyelinating encephalomyelitis when inoculated i.c. into 19-21-day-old mice, closely resembling that produced by the JHM virus; induced occasional focal, or more rarely, generalized hepatitis after i.p. inoculation of weanling Swiss or CDF_1 mice pretreated with *E. coccoides,* but no disease in mice without *E. coccoides*; and a giant-cell cytopathic effect in the NCTC-1469 cell line identical to that produced by the MHV-1(7), MHV-3(8), A-59(5), H747(9), and JHM strains of mouse hepatitis viruses. Definitive identification of the S strain as belonging to the mouse hepatitis group of viruses was provided by serological tests, using antiserums prepared in CD-1 weanling mice held in plastic isolators. Reciprocal CF tests and quantitative cross-neutralization tests in suckling mice and by the plaque reduction technic (Hartley, J.W., and Rowe, W.P., unpublished) revealed extensive sharing of both CF and neutralizing antigens with the MHV-1, JHM and A-59 strains.

In view of these two findings, it appeared probable that certain normal mice were excreting mouse hepatitis viruses in the feces, and that these viruses induced fatal disease in nearby infant mice of an uninfected strain. The first conclusion was verified by virus isolation tests on pools of feces obtained from about 25 weanling mice of various colonies; the specimens were collected immediately on arrival of the mice in our laboratory, and

TABLE I. Detection of Mouse Hepatitis Viruses in Pooled Feces of Uninoculated Mice.

	8-30-61					
	9-13-61					
	10-26-61	+				
	1-24-62	+			+	
	3-14-62			—		
	4-11-62		+	+	+	+
	6-06-62				+	
	8-20-62					+
					+	+
	9-13-61	+				
"	1-24-62	+				(—)*
	8-27-62	—			+	—
"	2-15-62	+			+	
	8-27-62	+			+	—
	3-20-62	+			+	
	4-23-62	+			+	(—)*
					—	—
	11-20-61	—				
	3-06-62	—			—	
	4-18-62	—			—	
	8-27-62	—			—	—
	4-17-62				—	

* Tested after being frozen and thawed several times.

were tested in various virus detection systems, with results shown in Table I. The recovered strains were identified as mouse hepatitis strains by neutralization tests in suckling mice and tissue culture, presence of specific CF antigen in brain-liver suspensions, ether sensitivity, failure of the infected suckling mouse brain suspensions to hemagglutinate human O erythrocytes, and by the histologic picture of focal hepatitis and encephalomyelitis in infected suckling mice. From Table I it is seen that hepatitis viruses were recovered with regularity from feces of the strains other than CD-1 and germ-free mice; mice of the M strain were consistently positive in shipments received over a one-year period.

Sera from retired breeder mice of the M, H, N, and GP strains were frequently positive for low titer neutralizing and/or CF antibodies to the S strain, while the CD-1 and germ-free mice have been consistently negative. *Contagiousness of mouse hepatitis virus infection.* The high degree of contagiousness of mouse hepatitis viruses was demonstrated by serologic testing of CD-1 mice held under different environmental conditions. The occurrence of CF antibody to the S strain in sera of uninoculated or saline-inoculated control CD-1 mice bled after 3 to 5 weeks residence in our laboratory is shown in Fig. 1. With infrequent exceptions, CD-1 mice held in plastic isolators or in jars with filter material in the lids remained negative, while the great majority of mice housed conventionally in the experimental animal room acquired infection. The latter mice were exposed to both normal and experimental mice, so the source of infection is not clear; how-

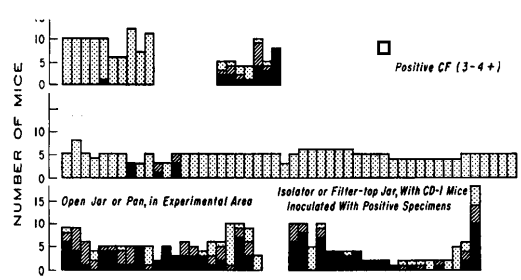

FIG. 1. CF antibody response to MHV-S of saline inoculated or uninoculated weanling CD-1 mice held for 3 to 5 wk under various caging conditions.

ever, the portion of Fig. 1 showing responses of CD-1 mice held in isolation with uninoculated strain M mice shows that the normal mice of this colony are highly infectious. The figure also shows that experimentally infected mice, the great majority of which were inoculated parenterally, were likewise highly infectious for contacts. The data in Fig. 1 can also be interpreted as providing strong support for the specificity of the CF test, even when the degree of reaction is partial; in this connection, it should be pointed out that the one positive serum of a non-contact isolator mouse was also positive for neutralizing antibody for the S strain.

The lethality of hepatitis viruses for exposed CD-1 infant mice was demonstrated by contact experiments. Litters of newborn CD-1 mice were placed in cages with 5 normal weanling mice of other strains caged above each litter, separated by ¼″ hardware cloth so that the excreta of the weanlings fell into the cages with the newborns; these cages were placed in plastic isolators. The mortality in the CD-1 infants between the 3rd and 9th or 10th day is shown in Table II. The exposure to excreta of carrier stocks or of CD-1 mice inoculated with the mouse passage S strain virus resulted in extremely high mortality rates, strongly indicating that hepatitis viruses were the cause of the neonatal mortality described above. In this connection it should be noted that hepatitis virus was recovered from viscera of a sick 3-day-old mouse under the N strain mice. This thesis is somewhat weakened by the lack of mortality in mice exposed to weanlings infected with tissue culture grown virus (8th passage); however, only a small proportion of the exposed infant mice developed antibody, and there is evidence that the tissue culture virus is partially attenuated in its pathogenicity for infant mice. It is possible that the infant mortality was due to another prevalent virus to which CD-1 mice are also highly susceptible and which may contaminate the mouse passage virus but not the tissue culture line. The LIVIM agent is particularly suspected in this regard.

TABLE II. Mortality in CD-1 Infant Mice Exposed to Excreta of Various Mice.

	Deaths in exposed CD-1 newborns	
Mice in upper compartment	Deaths/ total	% dying
M – Normal weanlings	47/62	76
GP – " "	16/26	62
N – " "	31/38	82
CD-1 – Inoculated with mouse passaged MHV-S 4 days previously	28/30	93
CD-1 – Inoculated with tissue culture passaged MHV-S 4 days previously	1/86	1
CD-1 – Normal weanlings	11/80	14

Discussion. The similarity of the epidemiologic pattern implied by the present findings to that of the mouse encephalomyelitis (Theiler's) viruses is evident, that is, a superficial enteric infection acquired early in life, giving rise to limited serologic response and low grade immunity. The duration of intestinal virus excretion remains to be determined; preliminary evidence suggests that it is not longer than several weeks. Preliminary evidence also indicates that virus is infrequently present in the liver of enteric carriers; the occasional activation of hepatitis by *E. coccoides* or mouse leukemia may represent virus gaining access to the liver by transient portal viremia or, less probably, by fecal contamination of the inoculating needle or skin. The present findings do not rule out the possibility of latent carriage in the liver, but do provide a new frame of reference and more sensitive technics for study of this question.

In view of the high pathogenicity for exposed CD-1 mice and the limited resistance against inoculation of hepatitis virus of infant mice born of infected mothers, it would seem probable that the endemic infections are responsible for some infant mortality in carrier stocks. During the present work, uninoculated litters of conventional mice held in the experimental animal room showed higher mortality than at times when work on these agents was not in progress. However, the causal role of MHV infection has not been established.

It is apparent that precise animal experimentation with these viruses cannot be done

without strict isolation facilities and known virus-free mice.

Summary. Mouse hepatitis viruses were readily isolated, by a variety of technics, from pooled feces of weanling mice of the majority of colonies tested. The infection was highly contagious to contacts from a non-infected mouse stock. Evidence is presented that contact infection with hepatitis viruses produced high mortality in infant mice of the non-infected stock.

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Antagonistic Relationship Between *myo*-Inositol and 2-*O*,*C*-Methylene-*myo*-Inositol in Animals.* (27981)

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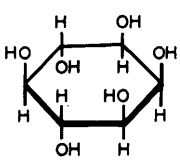
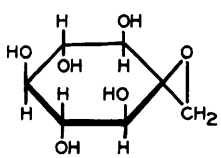
Recent researches have provided information about the reactions by which *myo*-inositol (I) is incorporated into phospholipids in animal tissues(1), or is converted to well-known intermediates of carbohydrate metabolism(2), but the physiological significance of these processes is not clear. Since investigations of this kind, and the discovery of additional metabolic pathways, are facilitated by the availability of metabolic antagonists, it seemed of interest to search for an anti-inositol active in animals. On testing a number of cyclitols, some of which had been shown to be antagonists of *myo*-inositol in certain microorganisms(3), we found that 2-*O*,*C*-methylene-*myo*-inositol (II) was toxic to mice and rats. Our observations on the effects of this compound, and on the suppression of these effects by *myo*-inositol, are reported here.



myo-INOSITOL
I



2-O,C-METHYLENE-
myo-INOSITOL
II

Materials and methods. 2-*O*,*C*-Methylene-*myo*-inositol, which hereafter will be designated as *myo* methylene oxide, was prepared from *myo*-inosose-2(4) essentially as described by Posternak(5).† Compounds were administered as aqueous solutions by intraperitoneal injection.

Male weanling mice (Taconic strain, Rolfsmeyer Farms) and male weanling rats (Holtz-

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† Posternak referred to the compound as "oxyde de méthylène-penta-oxy-cyclohexane." The name 2-*O*,*C*-methylene-*myo*-inositol describes the stereochemical configuration, and is consistent with the cyclitol nomenclature currently used in the U. S.