Summary. The results of the comparative sero-epidemiology of herpes-like viruses (HLV) and herpes simplex virus (HSV) infections in man are presented. Sera from 573 subjects of various age groups were examined for the presence of complement-fixing antibodies to these viruses. Antibodies to HLV appeared at an earlier age than HSV antibodies and reached a frequency of 65% in the 3–5 year age group. Both antibodies were present in 85–90% of the adult population and were distributed on a worldwide basis. Both antibodies persisted for at least 10 years in sera of 26 normal individuals. Fifty percent of sera obtained from 99 rhesus monkeys within 1–4 days after time of capture contained antibodies reacting with HLV antigen.

Cultures of Raji and P_3 cells for antigen production were prepared by Dr. E. Jensen, Chas. Pfizer and Co., Inc., Maywood, N. J., who also checked these cells frequently for the presence of mycoplasma and other microorganisms. Mr. I. Toplin, from the same laboratory, prepared the partially purified antigens used in the present study. Production of cells and antigens was carried out under contract PH 43-66-98, Special Virus Leukemia Program, Etiology Area, National Cancer Institute.

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Characteristics of a Murine Hemagglutinin Induced by the M-P Virus (33062)

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The M-P virus (MPV) originally isolated from association with Ehrlich carcinoma cells in 1963 produces a marked lymphopenia of the lymph organs, bone marrow, and peripheral blood in all strains of mice accompanied by the development of peritoneal and pleural fluid in Swiss mice at high infection doses. Lymphopenia occurs also in guinea pigs, rats, and dogs. Some physical and chemical properties of the virus are: labile at 56°C for 30 min, unstable in 0.145 *M* NaCl, ether sensitive, and pH 3.0 sensitive, stable at −196°C in Hanks' essential basal medium with 5% fetal calf serum for

12 months thus far tested (1, 2). Recent EM studies (unpublished) reveal a spherical virus 80 mμ in diameter having a double limiting membrane with a corona of attached particles 10 mμ in diameter.

The marked lymphopenia produced by injection of the virus directed our attention to a study of its effect on antibody formation. During the acute viremia a sheep red blood cell hemagglutinin (SRBC) was induced preceding the development of antiviral antibody.

An (SRBC) hemagglutinin, normally present in mice, was described by Stern and Davidsohn for 13 inbred mouse strains among which the highest incidence occurred in C_{57}B1/6 mice. The agglutinin was not of the Forssman type and was independent of an additional agglutinin for chicken red blood cells (3).

The M-P virus increased the titer of the existing SRBC hemagglutinin. A preliminary report of this study was included in the summary of the properties of the M-P virus (2). This virus-induced hemagglutinin (HA) is described herein.

Materials and Methods. The M-P virus was serially cultivated in HeLa cell spinner culture on Hanks' essential basal medium supplemented with 5% fetal calf serum, containing 100 units of penicillin and 1 μg of streptomycin/ml, and by intraperitoneal passage in Balb/C and C_{57}B1/6 mice. Spinner cultures harvested 6 days postinoculation, centrifuged free of cell debris, sterile filtered, and aliquoted in 1-ml ampuls, were stored at —196°C in liquid nitrogen. Mouse passage virus started from HeLa seed virus was harvested 9–10 days postvirus infection by exsanguination from the jugular vein. Viremic serums obtained following centrifugation were sterile filtered, aliquoted in 1-ml ampuls and stored at —196°C.

HeLa cell cultures were free of PPLO contamination as determined periodically by broth culture agar plate assay. The HeLa cell culture virus has been assayed for and found to be free of lactic dehydrogenase virus (LDV).

Strains of inbred mice (C_{57}Bl/6, Balb/C, C_3H, and A/He) were derived from a nucleus obtained in 1958 from the NIH inbred colony. Swiss mice were random bred in the Waldemar colony. All mice were housed in stainless steel cages, with water *ad libitum*.

The C_{57}Bl/6 mice, 6–9 weeks of age, were grouped equally by sex and weight, and housed no more than six to a cage. Blood was obtained by bleeding from the suborbital sinus with heparinized hematocrit capillary tubes, 75 × 0.2 mm. Hematocrit tubes were centrifuged and the plasma was pooled within each group.

Eight groups of mice, each group composed of 12 mice, were injected intraperitoneally with a $10^4 \times ID_{50}$ dose of M-P virus. Two groups were injected with PBS and one group with an equivalent dose of heat-inactivated M-P virus. One group made tolerant to the M-P virus, following the procedure used for the LCM virus (4) was injected intraperitoneally 24 hours after birth with 6×10^3 ID_{50} doses of virus. In all groups, blood samples were tested prior to virus administration to obtain the natural HA titer. Blood samples were obtained at varying intervals up to 60-days postinjection. In each instance, an average HA titer was obtained on pooled plasma for a given test period. The increase in HA titer is the logarithmic difference between the natural HA titer and the HA titer developed for each testing period during the post viremia.

Biophysical and chemical properties of the HA were studied on viremic and convalescent serums harvested from groups of 200 C_{57}Bl/6 mice, 10 and 60 days postvirus injection. These serums were centrifuged at 5°C, sterile filtered through a 450 mμ membrane under positive pressure of 20 psi N_2, and stored in 5-ml aliquots at —20° C. Convalescent serums, virus-free but containing virus neutralizing antibody, were prepared and stored in a similar manner.

Virus neutralization tests were made by serially diluting heat-inactivated sera with $10^3 \times ID_{50}$ dose of virus for 2 hours at 37°C, followed by 18 hours at 4°C prior to administration and assay in Swiss mice. The production of pleural or peritoneal fluid in 7–10 days postvirus inoculation was the criterion for determining 50% infectivity end

TABLE I. The SRBC Hemagglutinin Titer in C57Bl/6 Mice Following M-P Virus Infection.

Postinfection (days)	Increase in hemagglutinin titer of virus treated animals	SD
0	0	± 2.35
7	17.5	± 7.51
10	320	± 9.77
12	298	± 64.1
14	160	± 31.6
17	96	± 11.3
24	52	± 5.78
35	36	± 4.08
60	32	± 2.35

points (ID_{50}) according to the method of Reed and Muench (5).

Hemagglutinin assay was performed following the microtechnique of Sever (6) employing fresh PBS washed SRBC. After incubation at 37°C for 1 hour, plates were read, and a Xerox copy of the HA test plate was taken for permanent record. All titers were expressed as a reciprocal of the dilution end point. Balb/C, C_3H, A/He, and Swiss mice were also tested for virus induced HA.

Red cells from other mammals and fowl, such as guinea pig, goat, calf, ox, horse, human O, mouse, swine, chicken, and duck, screened for possible cross agglutination, were prepared in a manner similar to the sheep red blood cells.

Serums were differentially filtered through gradocol membranes of varying porosity. Filtrates were assayed for virus activity, neutralizing antibody (NA), and HA activity as described above.

One-ml aliquots of HA serums were incubated overnight at 4°C with 0.25 ml of packed SRBC, then centrifuged free of SRBC, prior to retesting for absorption of HA activity. Heat stability of the HA was tested by incubating sera at 56, 60, and 70°C for 30 min.

Results. The red blood cells from various animals and fowl tested with post M-P viremic sera gave HA activity only for sheep and goat red blood cells. No HA activity was obtained for RBC from other species. Sheep red blood cells were chosen for further studies on the HA.

The HA titers of virus treated $C_{57}Bl/6$ mice are given in Table I. Live virus treated mice produced an HA titer which peaked between the seventh to tenth days postinjection, gradually decreasing thereafter to a level of 32.

An HA was not induced in adult $C_{57}Bl/6$ mice injected intraperitoneally with heat-inactivated M-P virus, or in adult $C_{57}Bl/6$ mice with a persistent tolerant infection produced by neonatal injection of the M-P virus. No HA was induced in control animals injected with PBS.

The peak HA titer was associated with a peak virus population in the circulating blood. A drop in hematocrit and rise in total WBC appeared approximately 1 week following the drop in absolute lymphocyte count and coincided with the elevation in HA titer (Fig. 1).

The HA was stable at 60°C but was inactivated following incubation at 70°C for thirty minutes.

Pooled M-P viremic serums 10-days postinjection, pooled postviremic serums 60-days postinjection, filtered through gradocol membranes and assayed, indicated that the HA

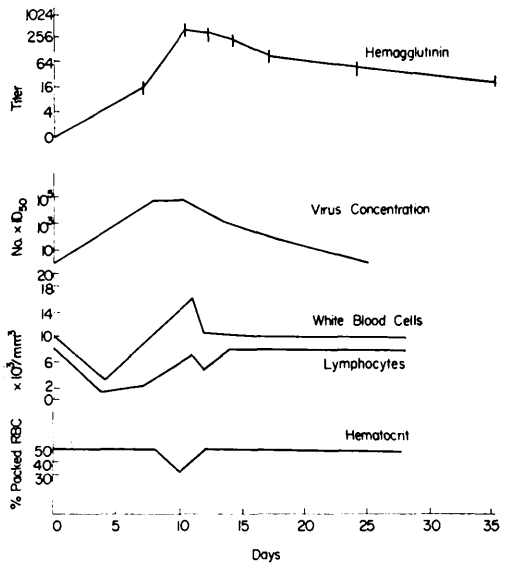

FIG. 1. The variation in time of the induced hemagglutinin, virus concentration, white blood cell and absolute lymphocyte count, and hematocrit value, in M-P virus infected $C_{57}Bl/6$ mice.

TABLE II. Infectivity, Hemagglutinin and Neutralizing Antibody Titers of Differentially Filtered C56Bl/6 MPV 10-day and 60-day Sera.

Post-infection (days)	Gradacol filter pore size (mμ)	Titer		
		HA	Virus	NA
10	450	128	$10^4 ID_{50}$	
	220	128	$10^4 ID_{50}$	
	150	128	0	0
	110	32	0	0
	88	16		
	52	2		
	29	0		
60	450	16	0	8
	220	16	0	8
	150	16		8
	110	8		4
	88	8		
	52	2		
	29	0		

was separable by filtration from the infective virus. The virus does not pass through a 150 mμ filter while the HA factor was stopped by a 52 mμ filter. The virus free 150 mμ filtrate containing the maximum titer of induced HA does not exhibit any virus neutralizing activity (Table II), and therefore is also different from virus neutralizing antibody.

When the HA was removed from 10- and 60-day postinjection serums by absorption with SRBC, the absorbed 10-day serum had not decreased in infectivity, nor did the absorbed 60-day serum lose any virus neutralizing antibody activity (Tables III and IV). This confirmed the findings by filtration that the hemagglutinin differs from the virus and the virus neutralizing antibody.

TABLE III. Neutralization Test of Viremic Sera after Absorption of Hemagglutinin with SRBC.

Serum dilution	Mice treated with	
	HA absorbed sera	Complete viremic sera
1:2	5/5	5/5
1:4	5/5	5/5
1:8	5/5	5/5
1:16	5/5	5/5

Fraction is no. of mice with viremia/no. of mice.

The M-P virus infected HeLa cells did not absorb sheep red blood cells nor did the supernatant media demonstrate any HA when free of cells and containing 4×10^6 ID_{50} of virus/ml. The M-P virus injected intraperitoneally into C_3H, A/He, Balb/C, or Swiss mice did not induce a SRBC hemagglutinin or increase the natural low SRBC hemagglutinin titer found for these mouse strains.

Discussion. The M-P virus induction of an HA in $C_{57}Bl/6$ mice occurred 7-days postvirus injection and reached a maximum titer 10-days postinjection. The HA was not produced following the injection of heat killed virus or in $C_{57}Bl/6$ mice with a persistent virus infection resulting from tolerance to the M-P virus by neonatal injection. These data suggest that the HA is host produced during the immunologic reaction to the MPV. The observation that HeLa cell cultivated virus does not produce an HA *in vitro*, but does so on introduction into adult $C_{57}Bl/6$ mice supports the conclusion that the HA is host produced. Present data is insufficient to determine whether the active component is under control of the virus genome as is the case with vaccinia virus, where host factors also play a role in HA production (7).

TABLE IV. Neutralizing Activity of Convalescent Sera after Absorption of Hemagglutinin with SRBC.

Serum dilution	Mice treated with	
	HA absorbed sera	Complete convalescent sera
1:2	0/5	0/5
1:4	0/5	0/5
1:8	3/5	2/5
1:16	5/5	5/5

The M-P virus induced HA appeared to have the character of a primary antibody response to an antigen stimulation. This conclusion was reached because the rate of increase of the induced HA in mice with or without a prior natural HA was the same. This suggests also that a different antigen is responsible for the natural HA and the HA resulting from the viral infection.

It is noted that while the M-P virus was infective in all mouse strains tested (Swiss and inbred strains) the HA for SRBC is stimulated only in $C_{57}Bl/6$ mice. A parallel was noted in that vaccinia virus grows in some hosts unaccompanied by a hemagglutinin (7).

Summary. The M-P virus (MPV) infection is accompanied by a sheep red blood cell hemagglutinin (HA) in $C_{57}Bl/6$ mice. The hemagglutinin titer is increased beyond the natural HA achieving a peak titer between the seventh and tenth day. The hemagglutinin, separable from the virus, occurred as an *in vivo* reaction of $C_{57}Bl/6$ mice against the infective virus and had the characteristics of an antibody different and separate from the antiviral antibody. The hemagglutinin was not produced in *in vitro* culture.

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The Entrance of Exogenous RNA into the Mouse Ascites Cell* (33063)

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During the past few years, a number of papers have appeared on the biological functions of exogenous RNA (1,2,5–7,11–16, 18–20,22, and others). It is of prime importance to make sure that the input RNA actually enters the recipient cell as an intact macromolecule, i.e., without apparent degradation. The aim of this paper is to provide experimental evidence for the entrance of RNA into the ascites cells as undegraded molecules.

Materials and Methods. Rats weighing about 200 gm were starved 15–24 hours. Each of 10–12 rats in a set of experiments received an intraperitoneal injection of 1 ml of saline containing 25 μC of adenine-^{14}C plus 100 μC of uridine-^{3}H, and immediately were allowed to feed. They were killed the next morning by a blow on the head. The liver was immediately removed and washed with ice-cold saline. The RNA was isolated from the pooled livers following the modified cold phenol procedure as reported by Hillman and Niu (7). Some of its properties are shown in Table I.

TABLE I. Characterization of the Double Labeled Rat Liver RNA.

1.	OD (mμ) 260/280 = 0.497
2.	OD (mμ) 260/230 = 2.43
3.	E(P) value (8) = 8000
4.	Protein contamination (10) = 1–2%
5.	DNA contamination (3) = nil
6.	Specific activity of orotic-5-^{3}H acid = 85,879 cpm /mg of RNA
7.	Specific activity of adenine-8-^{14}C = 14,688 cpm/ mg of RNA
8.	Proportion of ^{3}H and ^{14}C = 5.85

The Nelson mouse ascites tumor cells used in the present study were maintained in

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