hands, the test has been no more time consuming or difficult than procedures now employed to detect myxovirus HI antibodies(8). Preliminary results suggest that the HI test can be utilized for purposes of diagnosis and population survey.

Summary. 1. A stable EMC hemagglutinating antigen was prepared by alternate freezing and thawing of virus-infected HeLa cell cultures. 2. Optimal hemagglutination titers were obtained at 4° and 25° C with human and guinea pig erythrocytes when diluents containing potassium and boron ions were used. 3. A hemagglutination-inhibition test employing kaolin treated serum and tissue culture antigen is described and its practical usefulness in detecting antibody in human and swine serum is demonstrated.

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Quantitative Aspects of Friend Leukemia Virus in Various Murine Hosts.*† (27081)

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The leukemia virus described by Friend (FV) is considered to have a narrow and specific adult murine host range(1,2,3), severely limiting the type of experiments which may be carried out. This virus has a relatively short latent period characterized by splenomegaly about one week after inoculation, with blood changes occurring later in the disease. Because of the obvious advantages of utilizing a virus producing early onset of overt disease, it seemed desirable to reinvestigate the host range with particular attention to the reliability of splenomegaly as an early indicator of infection and susceptibility. It was thought possible that inapparent infection might occur in some strains of mice without the usual early pathognomonic signs.

Materials and Methods. C57BL/Crgl, C3H/Crgl, C3H/Crgl/2, DBA/2NCrgl, A/Crgl mice were obtained from Cancer Research Genetics Laboratory, Univ. of California, Berkeley. BALB/c mice were either from our own colony or Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and are so indicated. The random bred Swiss mice were from the colony of the Hooper Foundation. All animals were 6-8 weeks old at time of inoculation.

The Friend virus used in these studies was received from Dr. C. F. T. Mattern of the Virus Laboratory, Univ. of California, Berkeley. He had received it from Dr. Charlotte Friend after an unstated number of passes in Swiss mice. The stock virus used was from a large pool of spleens prepared from the 11th pass in Swiss mice in this laboratory. Spleens were homogenized in a sucrose-phosphate buffer solution as a 20% suspension. This was centrifuged at 2,000 rpm for 10 minutes and the supernate distributed in sealed ampules

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and stored at −70°C. Before use the suspension was recentrifuged at 2,000 rpm for ten minutes.

To determine susceptibility, mice of each strain were injected intraperitoneally with 0.5 ml of the 20% suspension of virus. At 14, 21 and 28 days after inoculation 7 to 10 mice from each group were sacrificed, the spleens removed, weighed, and a piece of each taken for histologic examination. Those showing foci of proliferating reticulum cells were deemed infected. The remainder from each group was pooled and treated the same as the stock virus. All spleen suspensions were diluted decimally from 10^{-1} to 10^{-6} and each dilution inoculated into groups of 10 Swiss mice except as otherwise indicated. Deaths occurring prior to the 8th day after inoculation were considered to be non-specific. All animals dying after this time and those sacrificed at termination of the experiment on the 35th day were autopsied. Those with spleens weighing greater than 0.5 g were considered to be infected with FV and were not examined further on the assumption that histologic evidence of disease was also present. An ID_{50} was then calculated on the basis of overt disease. Those with spleens weighing less than 0.5 g were examined histologically and the ID_{50} was recalculated from the combined total of those showing overt disease or microscopic evidence of infection.

Spleen weights were also obtained from uninoculated mice of each strain.

During the latter stages of this work Dr. Friend kindly supplied us with 2 ampules of lyophilized virus passed in her laboratory. Our own BALB/c and Swiss mice were tested for susceptibility to this virus.

Results and Discussion. It is apparent from Table I that the strains of mice fell into 3 categories of susceptibility to FV. The A and C57BL showed neither splenomegaly nor histologic evidence of disease. Both groups of C3H represented an intermediate stage in that approximately 58% showed some evidence of infection. The DBA/2, BALB/c and Swiss mice appeared to be equally and maximally susceptible, 96 to 100% showing evidence of FV disease.

We have examined several thousand mice and have only rarely encountered a normal animal with a spleen weight of greater than 0.5 g, and in this respect splenomegaly was a reliable indicator of infection. However, it is obvious from Table I that a number of animals may be infected with FV without splenomegaly. This was particularly true among animals of intermediate susceptibility and in the early stages of disease.

These results appeared to be at variance with those reported by Friend concerning the limited mouse strain susceptibility. However, the inoculum given our mice was 5 times greater than she administered. Since this could have accounted for infection in the C3H and BALB/c mice, quantitative studies were carried out. From Table II it may be seen that the animals still fell into 3 well defined groups.

TABLE I. Relative Susceptibility of Various Strains of Mice to Infection with Friend Virus as Determined by Splenomegaly and Microscopic Examination.

Strain	Normal Spleen wt (avg)	Results at indicated time after intraperitoneal inoculation. 14 days			21 days			28 days		
		Spleen wt (avg)	No. Infected/Total Spleen wt*	Histo†	Spleen wt (avg)	No. Infected/Total Spleen wt*	Histo†	Spleen wt (avg)	No. Infected/Total Spleen wt*	Histo†
A/Crgl	.11	.24	0/10	0/10	.26	0/10	0/10	.28	0/10	0/10
C57BL/Crgl	.08	.21	0/9	0/9	.23	0/10	0/10	.29	0/10	0/10
C3H/Crgl	.12	.42	2/7	4/7	.71	4/7	4/7	.53	1/7	4/7
C3H/Crgl/2	.12	.34	1/10	4/10	.49	4/9	5/9	.49	4/10	8/10
DBA/2NCrgl	.10	.43	2/8	8/8	1.11	9/9	9/9	.95	10/10	10/10
BALB/cJax	.17	.55	4/8	7/8	1.99	9/9	9/9	2.80	9/9	9/9
BALB/c	.21	.64	5/9	8/9	1.66	9/9	9/9	2.07	10/10	9/10
Swiss	.22	1.19	8/10	10/10	2.02	9/10	9/10	1.08	9/10	10/10

* Spleens weighing >.5 g were considered infected.

† Spleens examined histologically and those showing foci of proliferating reticulum cells were considered infected.

TABLE II. Multiplication of Friend Virus in Various Strains of Mice as Determined in Swiss Mice.

Strain	Titer of virus from spleens harvested at indicated time after inoculation†					
	14 days		21 days		28 days	
	Gross*	Combined‡	Gross*	Combined‡	Gross*	Combined‡
A/Crgl	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
C57BL/Crgl	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
C3H/Crgl	<1.0	<1.0	2.8	3.1	2.5	2.8
C3H/Crgl/2	1.4	1.4	2.7	2.8	2.3	2.9
DBA/2NCrgl	3.0	3.8	3.7	4.4	3.7	4.4
BALB/cJax	3.5	4.4	3.9	4.3	3.9	4.5
BALB/c	3.5	4.6	4.4	4.8	4.2	4.6
Swiss	3.0	3.6	4.0	4.6	2.5	3.1

† $-\text{Log}_{10}$ of ID_{50} per .5 ml. * Spleens weighing >.5 g were considered infected.
‡ Combined titer calculated from total of spleens weighing >.5 g or showing histologic evidence of disease.

The virus apparently failed to multiply in the A and C57BL strains. Although the titer was less than 10^{-1} an occasional Swiss mouse inoculated with spleens from these animals showed evidence of disease. The virus multiplied slowly and at a low level in the C3H mice, reaching a peak by 21 days and maintaining this level through the 28th day after infection. The DBA/2 and BALB/c mice appeared to be maximally and slightly more susceptible than the Swiss mice. The virus titer reached its peak in the first 2 strains between 2 and 3 weeks and was maintained through the 4th week, while in the Swiss mice there appeared to be a decline in virus titer between the 3rd and 4th week. It is obvious, however, that at 21 days no significant differences could be observed in the susceptibility of these 3 strains of mice. The differences between the titer of virus as determined by gross examination and that determined after histological examination of the spleens weighing less than 0.5 g were not of statistical significance. Nonetheless, in the DBA/2, BALB/c and Swiss mice the titer was consistently higher after microscopic examination than when splenomegaly alone was depended upon as an index of infection. Animals which were positive only on histologic examination were found in all dilutions, indicating that this phenomenon was not entirely dependent upon dose of virus or incubation period. Since these titrations were carried out in Swiss mice and there exists an inherent resistance in 10 to 20% of these mice to overt disease(1,2), these results were not unexpected. The inbred C3H mice also exhibited a high degree of resistance to overt disease. Of the 33 C3H mice of both strains which were examined 3 and 4 weeks post inoculation, 30% had overt disease while 64% had histologic evidence of infection. In one group of these mice sacrificed 63 days after inoculation only 4 out of 10 had splenomegaly while 9 showed microscopic evidence of disease.

Since it was desirable to assay virus in mice of more uniform susceptibility, BALB/c mice were investigated further. Two separate lots of virus prepared from BALB/c spleens after 1 and 2 passes in these animals revealed gross titers of $10^{-3.6}$ and $10^{-4.8}$ respectively, as assayed in BALB/c mice. Among the animals without overt disease in these titrations only 2 mice were found to be positive microscopically, both of them in the 10^{-4} dilution of the first titration. Futhermore all 60 mice inoculated with dilutions 10^{-1} through 10^{-3} developed clinical infections which is in contrast with the irregular results attained in Swiss mice. Additional passage of BALB/c adapted virus in BALB/c mice failed to reveal resistant mice when these were inoculated with 0.2 ml of 10% mouse spleen suspension. At the 13th passage level all of 69 BALB/c mice showed overt signs of disease when sacrificed 20 days after inoculation of FV.

Since Friend had reported BALB and C3H mice to be resistant to infection with this virus (1,3), it seemed possible that our strain was either a variant or another virus. One of the ampules of virus received from Friend was diluted to 1 ml with water to make a 10% suspension and was inoculated intraperitoneally in 0.2 ml amounts into 5 Swiss mice. The

mice were sacrificed 20 days after inoculation and only one had splenomegaly. The spleens of these mice were made into 10% suspension and inoculated in 0.2 ml amounts into 30 Swiss and 10 BALB/c mice. They were sacrificed 17 days later and 90% of the Swiss and 70% of the BALB showed splenomegaly. Microscopic examination of the enlarged spleens from both Swiss and BALB/c mice revealed changes identical to those observed by us in Swiss, BALB/c, DBA/2 and C3H mice inoculated with virus previously passed in our laboratory.

Virus could not be isolated from the second ampule after 2 blind passes in BALB/c and Swiss mice.

Summary. The murine host range of Friend leukemia virus has been investigated quantitatively. Virus failed to multiply in C57BL and A strains. About 70% of C3H mice were susceptible, but overt disease developed in only 39%, the remainder being detectable by histologic examination only. Virus multiplied more slowly and to a lesser degree in C3H mice than in Swiss, DBA/2 and BALB/c, which were highly susceptible. Splenomegaly was more marked and more regular in BALB/c than in any of the other strains investigated; 100% of these mice being susceptible after initial adaptation.

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A Shortened Dental Caries Assay with the Cotton Rat.* (27082)

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One of the handicaps of dental caries research has been the lack of adequate means of evaluation of the process. Use of expermental animals in which actual initiation and progression of carious lesions are studied has been most helpful. Shaw *et al.*(1) devised an assay using the cotton rat. This animal has been used almost exclusively in this laboratory and has provided much data on dental caries in relation to the diet. However, for rapid progress required in the isolation studies, it was tedious and time-consuming. Shaw(2) has emphasized the importance of heredity in dental caries susceptibility. Constance *et al.*(3) found that withholding the feeding of a cariogenic diet until the teeth of the cotton rats were more mature, decreased their susceptibility to the caries process. Taketa *et al.*(4) demonstrated that the influence of the maternal diet was far less important than that of the diet fed the young cotton rat. With these observations at hand, it seemed that the calcification process was the fundamental point involved, that the early transfer of suckling young from mother to a cariogenic diet would enable us to reduce the time factor involved in the cotton rat assay method, and provide information less subject to individual variation caused by hereditary or other factors.

Experimental. Cotton rats, 16-19 days old were housed individually in wire mesh cages and given feed and distilled water *ad libitum*. The length of the experimental period was either 10, 30 days or 12 weeks. For the 10 and 30 day assay, animals were weighed only at the end of the experimental period and for the 12 week assay, they were weighed weekly. The animals were sacrificed and their teeth evaluated for caries according to the method of Shaw *et al.* For the purpose comparing the assay period, the 802 cariogenic diet and the 802 + 25% oat hulls non-cariogenic were used.

Results. A 10-day assay period was insufficient to develop and demonstrate carious le-

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