ty of plaque formation was further demonstrated in several experiments by incubating normal spleen cells with RNA from donors immunized to sheep cells and then by plating of the cells in agar containing either sheep or chicken red blood cells. Plaques were observed only in the plates containing the sheep erythrocytes.

The increase in the number of plaque-forming cells in cultures of normal spleen cells treated in vitro with RNA extracts from immune donor mice suggests that the ability to synthesize sheep hemolysin had been actively induced. The continued increase during incubation in tissue culture medium may have occurred either by an increase in the total number of cells responding to the RNA, or by proliferation of the initial number of cells which were "activated" by RNA. Removal of RNA from the cultures by several washings with buffer had no detectable effect on plaque formation.

The results presented here do not offer a definitive explanation concerning the mechanism of the acquisition of antibody plaque-forming activity by normal spleen cells treated in vitro with RNA from immune donors. They do, however, extend previous observations on acquisition of immunologic capabilities by nonimmune individuals (*8*) and by normal lymphoid cells after treatment with RNA or subcellular fractions derived from immunized donors (*3, 4, 9*). Whether RNA extracts contain an antibody "coding" mechanism, an immunogenic antigen–nucleic acid complex, or an antibody precursor which may induce immunologic activity in nonimmune cells is not known. However, experiments with gradient centrifugation and chromatography techniques suggest that the RNA in the extracts used in this study has properties consistent with low molecular weight RNA (*10*).

These experiments, together with those reported by others (*1, 2*) demonstrate that localized antibody plaque formation in gel is a valuable procedure for investigation of antibody production by cells cultured in vitro as well as by cells obtained directly from animals immunized with foreign erythrocytes.

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Enzootic Sendai Virus Infections in Mouse Breeder Colonies within the United States

Abstract. *Naturally occurring enzootic infections of Sendai virus have been detected by serologic monitoring and virus isolation in mouse breeder colonies from five states in the United States of America. Six of 21 colonies tested were found to be infected with Sendai virus, and the mean incidence of infection within infected colonies was 76 percent. Forty-one of 120 attempts to isolate the virus were successful.*

Sendai virus belongs to *Myxovirus parainfluenzae* type 1 and is identical with newborn pneumonitis virus, hemagglutinating virus of Japan (HVJ), and influenza D virus (*1*). Although the related human pathogen, hemadsorption virus 2, is worldwide in distribution, naturally occurring infections with Sendai virus have been reported only from Japan, China, and the U.S.S.R. (*2*). The infection was reported to be distributed widely among laboratory mice in Japan (*1*), and the virus has been isolated from mice, swine, and hamsters (*3*). Sendai virus is the only member of the parainfluenza virus group known to naturally infect mice. The question of human infection by Sendai virus has been obscured by technical problems in virus isolation and by serological cross reactivity with other parainfluenza viruses, although one apparently valid virus isolation has been made (*4*).

During studies to determine the cause of high mortality rates in suckling mice obtained from various breeders and held in our experimental animal-holding facility, it was discovered that serums from many of the mice inhibited hemagglutination by Sendai virus. Concurrently, during routine serologic monitoring of mouse breeder colonies (*5*), a mouse breeding colony was found to have a high incidence of hemagglutination-inhibition (HI) antibody for Sendai virus. Since naturally occurring Sendai virus infection heretofore had not been reported in the United States, studies were undertaken to identify further the infecting agent and to determine to what extent the virus was present in other mouse breeder colonies within the United States.

The Sendai/52 strain of Sendai virus was obtained from R. M. Chanock, and two stock pools of virus were prepared for use in serologic testing, the virus for one pool being cultured in kidney tissue of the Rhesus monkey, and the virus for the other, in chick allantoic fluid. Mice used in this study were obtained from various commercial and institutional mouse breeder colonies. A minimum of 25 mice, and in most cases more than 50 mice, were tested from each colony. Mice tested were of both sexes and generally were more than 5 months of age. Individual mouse serums were prepared as previously reported (*6*). All serums were heated at 56°C for 30 minutes prior to testing. For screening the mouse serums we conducted hemagglutination-inhibition and complement fixation tests, utilizing Microtiter (*7*) serological plates. Hemagglutination-inhibition tests were performed at room temperature, four hemagglutination units of antigen and human type O RBC being used, and the results were read by the pattern method (*6*).

Virus isolations were performed in duplicate in both embryonated hens' eggs and tissue culture. For each isolation, 0.05 ml of a 10-percent homogenate of mouse lung was inoculated amniotically into 8-day-old embryonated hens' eggs and the amniotic fluids were tested for hemagglutinins 4 days later. Positive hemagglutinating fluids were tested by hemagglutination-inhibition with antiserum specific for the Sendai virus prepared in germfree mice. Test tubes of primary cultures

of Rhesus monkey kidney tissue maintained in the presence of 0.2 percent antiserum to parainfluenza 2 (SV_5) were also inoculated with 0.05 ml of the same lung homogenate and held and observed for cytopathic effects for 8 to 12 days; fluids were tested for hemagglutination each 4th day. Positive hemagglutinating fluids were checked for inhibition by the antiserum specific for the Sendai virus.

Colonies of mice tested were from the states of New York, Pennsylvania, New Jersey, Maryland, Tennessee, Kansas, Texas, Wisconsin, and California. Twenty-one breeder colonies, comprising a total of 2126 mice, were screened for Sendai hemagglutination-inhibition antibody. Six colonies, coming from New York, Pennsylvania, Maryland, Texas, and Wisconsin, were positive. The distribution of Sendai antibody within infected colonies is shown in Table 1. Seventy-six percent of the mice in positive colonies were positive for hemagglutination-inhibition antibody. The amounts of complement-fixing antibody were similar to the amounts of hemagglutination-inhibition antibody, and both procedures were of comparable sensitivity for detecting the antibody. Twenty positive serums from colony No. 1 were tested by the complement fixation test for cross reactivity to prototype strains of parainfluenza 1 (HA-2 and Sendai), 2 (CA and SV_5), and 3(HA-1) and mumps virus antigens at a 1 : 10 serum dilution (*8*). Reactions were observed only with Sendai and HA-2 antigens; however, the reactions were less frequent and of lower titer against HA-2 than Sendai virus. An additional five serums were tested by the hemagglutination-inhibition test against the aforementioned parainfluenza and mumps virus antigens. With the exception of Sendai virus, no reactivity was noted at a 1 : 10 serum dilution. Titers against Sendai virus ranged from 1 : 80 to 1 : 160. This pattern of cross reactivity is comparable to that reported for Sendai virus by Cook *et al.* (*9*). When selected serums that gave positive reactions in the hemagglutination-inhibition test were treated with receptor-destroying enzyme (*Vibrio cholerae* filtrate), their titer of Sendai hemagglutination-inhibition antibody was not affected.

All attempts to isolate the virus were made in duplicate, in eggs and tissue culture, to minimize accidental contamination in our laboratory. Isolations from mouse lung were made successfully on numerous occasions from mice in colony No. 1 and in our animal rooms. Of 120 attempts to isolate the virus, 41 were successful, and except in two instances the virus was isolated in both tissue culture and eggs. The virus could be readily isolated again from positive lung suspensions. All of the isolated agents were characterized by serological reactivity (hemagglutination-inhibition and complement fixation tests) with standard mouse antiserum to Sendai virus, and ten were cross-typed by complement fixation with eight units of standard antiserums to HA-2, Sendai, CA, SV_5, HA-1, and mumps viruses. Reactivity was observed with Sendai and HA-2 antiserum; again the titers were 8 to 16 times greater against the Sendai antiserum.

Table 1. Incidence of Sendai virus antibody within infected mouse breeder colonies.

Mouse colony	No. tested	Age (mo)	Hemagglutination inhibition			Complement fixation		
			No. positive (%)	Mean titer of positives* (reciprocal)	Titer range of positives (reciprocal)	No. positive (%)	Mean titer of positives* (reciprocal)	Titer range of positives (reciprocal)
1	69	9	94	35	10– 160	91	20	10– 80
2	25	5	92	61	20– 160	96	58	10– 160
3	48	10	79	22	10–≧80	85	21	10– 80
4	74	3–10	88	28	10– 640	89	27	10– 160
5	50	9	88	18	10– 80	92	27	10– 160
6	61	8–11	20	12	10– 20	26	15	10– 20
Total	327		76	27		77	24	

* Geometric mean titer.

An additional six isolates were tested by another laboratory for complement fixation reaction against guinea pig antiserum (4 units) to the HA-2, Sendai, CA, SV_5, HA-1 and mumps viruses (*8*). Strong reactivity was observed against Sendai antiserum, while only trace or negative fixation was observed against the other specific antiserums. In addition, the identity of these six strains was confirmed by hemadsorption inhibition tests conducted by Chanock.

Fukumi (*1*) observed that epizootic infections of Sendai virus in mouse colonies in Japan was sporadic, lasting for 3 to 4 months and then disappearing spontaneously with no special measures for elimination having been taken. For long periods of time thereafter no infection was detected either by the presence of antibodies or by virus isolation procedures. In contrast, infected colonies of mice reported here do not appear to be undergoing a similar type of epizoology. The infection apparently persists within these colonies, since antibody has been detected consistently in colony No. 1 in repeated sampling over the past 2 years, including monthly testing for the past 8 months. Likewise, antibody has been detected consistently for the past 8 months in serums from the mice in colony No. 3. Virus isolates from colony No. 1 have been obtained monthly for the past 5 months. Mice of various ages have been tested, but virus isolations have been made only from those 21 to 38 days of age. Virus isolations were made from clinically healthy mice, and no overt disease manifestations or increased mortality appears to be associated with the virus infection in its natural state.

To obtain information on the duration of Sendai virus infection in our animal-holding facility, stored serums obtained over the past 3 years were tested for hemagglutination-inhibition antibody. Serum pools taken 2 to 3 years ago were negative, whereas the great majority of serums obtained in the past 18 months were positive. Thus, the infection is known to have existed prior to the receipt or use of Sendai virus in our laboratory, and it seems probable that it originated from mice received before this period from one of the infected colonies. The source of infection in the other positive colonies is not known. In at least three of the positive colonies, it is known that the mice have not been exposed to experimental procedures in which viruses have been used.

Some pertinent observations have been made on the epizoology of the infection in our animal-holding facility. At the time of discovery of the infection in our animals, a systematic check was made to determine the ex-

tent of the infection. Of 29 mice, 9 rats, 10 guinea pigs, 5 chickens, and 2 rabbits held in the rooms for not less than 20 days, 23 mice, 1 guinea pig, and all 9 rats tested were positive for HI and CF antibody to Sendai virus. The chickens and rabbits were negative. The infection is apparently being maintained by the introduction of uninfected mice from mouse breeder colonies which in turn become infected at a high rate within 1 to 4 weeks. Only mice held in filter-topped jars (*10*) were provided with a barrier sufficient to prevent infection.

In a study reported elsewhere (*5*), many of the serums of mice from the breeder colonies reported here were also tested for antibody to reovirus type 3, pneumonia virus of mice, K virus, polyoma, Theiler's encephalomyelitis (GDVII), mouse adenovirus, and mouse hepatitis. The Sendai virus was the fifth most prevalent indigenous mouse virus of the eight viruses for which we tested, with 6 of 21 colonies examined being found positive. Sendai infection differed from other indigenous murine virus infections in that the mean incidence of infection within infected colonies was 76 percent (Table 1) whereas the mean incidence of each of the other examined murine virus infections was less than 50 percent.

The results reported here indicate that Sendai virus exists within mouse breeder colonies in the United States as a widespread enzootic infection. The disease is highly prevalent within infected colonies and appears to be continuous rather than cyclic.

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Control of Synthesis of RNA and Protein in Diapausing and Injured Cecropia Pupae

Abstract. *Injury to diapausing pupae of the cecropia silkworm moth stimulates the synthesis of RNA in all pupal tissues and the synthesis of several blood proteins; such injury also induces the precocious synthesis of a protein, termed "injury protein," which normally appears in the blood during adult development. Actinomycin D, injected in concentrations of 2 micrograms per gram of body weight, blocks the injury-stimulated increase in blood protein synthesis and the injury-induced synthesis of injury protein. However, at concentrations of 0.5 micrograms per gram it prevents the induction of injury-protein synthesis but does not prevent the increased synthesis of other blood proteins. These results suggest that low concentrations of actinomycin may inhibit the synthesis of new kinds of messenger RNA but still permit the continued synthesis of messenger RNA's already in production at the time the actinomycin is injected.*

Injury to diapausing cecropia pupae induces the synthesis of a new blood protein and increases the rates of synthesis of preexisting blood proteins. In this report we show that threshold concentrations of actinomycin D prevent the synthesis of the new blood protein but do not prevent the injury-induced stimulation of synthesis of proteins already in production.

Previous experiments have established that within a week of pupation the giant silkworm moth *Hyalophora cecropia* enters a period of pupal diapause which commonly lasts for 6 to 10 months. Except for the hemocytes and gonads, its cells neither divide (*1*) nor engage in DNA synthesis (*2*) during this period. Even extensive injury does not stimulate cell division (*1*) or DNA synthesis in tissues not directly involved in closing the wound (*3*). Because the metabolic processes associated with DNA synthesis and cell division are absent, other metabolic changes stand out in sharp contrast.

For example, the diapausing pupa is capable of considerable synthesis of RNA and protein, and the degree of this synthetic activity can be altered by several means. If the pupa is wounded by removing a portion of the integument, its rate of respiration increases manyfold (*4*), and this is accompanied by a generalized increase in the rates of synthesis of RNA (*5*) and protein (*6*, *7*). Blood proteins, among others, are synthesized rapidly in injured pupae (*6*).

In the experiments described here, cecropia pupae were injured by excising the legs. To determine which tissues showed an increased synthesis of RNA in response to injury, conventional autoradiographic methods were employed with the liquid emulsion technique (*8*). Tritiated uridine (10 μc/g wet weight) was injected at various times after injury. Autoradiographs revealed that RNA synthesis increased in almost all pupal tissues within 24 hours of injury. Similar results were obtained with diapausing pupae of a number of species. The results of a careful analysis of autoradiographs of 20 diapausing and injured pupae of the closely related cynthia moth, *Samia cynthia*, are summarized in Table 1. Some humoral agent released at the site of injury (*9*) stimulated an increased synthetic activity throughout the pupa. Similar studies with tritiated thymidine revealed that injury caused no general stimulation of DNA synthesis in any tissue but the hemocytes.

Increased synthesis of certain blood proteins by cecropia pupae was demonstrated by electrophoresis on horizontal starch gels (*10*). Blood (10 μl) was taken for electrophoresis from a small slit in the wings at the time of the injury and at intervals thereafter. The changes in protein concentration were so great as to be immediately evident in visual comparisons of stained gels.