those caused by complexes in antibody excess, and those with antibody alone as the sensitizing agent.

A preliminary test with a polysaccharide antigen (Streptococcus C) and rabbit antibody indicates that the phenomenon of sensitization by antigen-antibody complexes may be accomplished with this system as well.

Discussion. The specific combination of antigen with antibody has been described by physico-chemical laws(6). The results of this combination are evident *in vitro* in the precipitin test and its derivative reactions, and *in vivo* in specific immune or allergic phenomena(6). In the present study the ability of specific precipitates and of antigen-antibody mixtures in great antigen excess to sensitize tissues to subsequent anaphylaxis suggests that optimal biological reactivity of antibody persists, despite apparent neutralization of antibody in *in vitro* tests. As little as 0.01 and 0.03 μg AbN, amounts approximating that required for threshold sensitivity, remain capable of sensitization despite the initial presence of excess amounts of antigen. When amounts of this order are utilized, it is improbable that multiple antigen-antibody systems account for the biological reactivity noted. In addition, the skin irritating activity of the complex(4) is considerably diminished so that control injections with dye alone do not result in dye localization. Apparently the antibody continues to react with antigen until extreme antigen excess is present, an indication of greater combining ratios of antigen with antibody than is ordinarily appreciated from *in vitro* tests.

Alternatively, the dissociation *in vivo* of antigen from antibody with conservation of the reactive properties of the antibody may be postulated. The small amount of antibody used suggests that antibody may be reutilized almost quantitatively in some processes of immunity and allergy. The present data do not permit the exclusion of either alternative.

Summary. Small amounts of antibody and excess antigen sensitize guinea pig skin so that challenge with intravenous antigen and dye elicits passive cutaneous anaphylaxis. The significance of the biological reactivity of antigen-antibody complexes is briefly discussed.

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Sendai Virus Antibody in Acute Respiratory Infections and Infectious Mononucleosis. (24074)

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Japanese investigators have recently studied a virus, variously referred to as newborn pneumonitis virus, hemagglutinating virus of mice, hemagglutinating virus of Japan, and Sendai virus, isolated from cases of human respiratory infection(1), from laboratory mice(2), and swine(3). First reports of virus isolations and of serologic evidence indicating human infection with this virus were limited to Ja-

* Data were submitted to Graduate School, University of Wisconsin, in partial fulfillment of requirements for degree Doctor of Philosophy.

pan, but recently Russian workers(4,5) reported isolation of the agent in Vladivostok from 5 patients with influenza-like illnesses. In 1955 Jensen *et al.*(6) presented serologic evidence suggesting that the population of United States has had contact with this or an antigenically closely related agent, but with the finding that infection with mumps virus results, in appearance of antibody reactive with Sendai virus†(7,8) the question remains as to whether Sendai virus itself is a cause of inapparent or overt human disease in the United States. In studies on respiratory infection in University of Wisconsin students a number of patients were found to develop serum reactivity with Sendai virus during illnesses that did not appear to be mumps infections. Our study of these illnesses is reported here.

Methods. The Sendai Strain of hemagglutinating virus of Japan, here referred to as Sendai virus, and Enders strain of mumps virus were used and cultivated in the allantoic sac of chicken embryos using standard methods previously described(7). *Serologic technics.* Hemagglutination and hemagglutination-inhibition tests were performed as outlined by Committee on Standard Serologic Procedures in Influenza Studies(9). Sera were treated with receptor-destroying-enzyme as previously described(7). Four hemagglutinating units of virus were used in hemagglutination-inhibition tests and titers are expressed as reciprocal of initial serum dilution. Complement-fixation tests were performed using allantoic fluid antigen and 2 full units of complement. Neutralization tests *in ovo* employed approximately 200 egg-infectivity doses of virus and serial dilutions of serum previously inactivated at 56°C for 30 minutes. Virus-serum mixtures were incubated 1 hour at 4°C and inoculated intra-allantoically. Inoculations were made into 11-day-old embryos, and incubated an additional 2 days before allantoic fluids were tested for presence of hemagglutinins. The 50% infectivity end points were calculated by the method of Reed and Muench(10). *Virus isolation procedures.* Penicillin and streptomycin were added to throat washings in concentrations of 1000 units and 1 mg/ml respectively. Six 13-day-old embryonated eggs were inoculated intra-amniotically with 0.2 ml of undiluted throat-washing and 6 three-week-old mice were inoculated intranasally with 0.05 ml while under light ether anesthesia. Embryonated eggs were incubated for 3 or 4 days at 35°C and amniotic fluids were then tested for presence of hemagglutinins using both chicken and guinea pig erythrocytes in 0.5% suspensions. At least 2 serial passages were made in eggs before specimens were considered negative. Mice were observed daily for 7 days and then sacrificed. Lungs were collected and emulsified in a glass tissue grinder to make a 10% suspension which was lightly centrifuged. Antibiotics were added to supernatant fluid and it was again inoculated intranasally into mice. Three serial passages in mice were made with each specimen.

Results. In view of reports by Japanese workers associating Sendai virus infection with influenza-like respiratory infections and pneumonitis, it seemed possible that this virus might play some part in the cause of non-bacterial, respiratory infections found in university student population. Paired sera were available from 85 cases variously diagnosed clinically as "grippe," bronchitis, or "influenza" which could be grouped together as non-bacterial respiratory infections and were generally characterized by fever, generalized muscular aches, headache, occasional sore throat or cough, paucity of abnormal findings on physical examination, and failure to develop a neutrophilia. There were also 50 cases that had been diagnosed as viral pneumonia or primary atypical pneumonia because of evidence of pneumonitis on physical examination or chest roentgenogram in addition to usual signs and symptoms of non-bacterial respiratory infection. Acute and convalescent serums from these 2 groups of patients were studied for antibody rises to Sendai virus.

† The designation "Sendai virus" is not one favored by Japanese workers and is not that previously used by us, but it has an advantage of succinctness and for that reason will be used here.

TABLE I. Antibody Titers against Various Antigens in Cases Diagnosed as "Grippe" and Primary Atypical Pneumonia and Developing Significant Antibody Rises to Sendai Virus.

Case No.	Antibody titers against: Sendai virus H.I. Acute serum	Sendai virus H.I. Conv. serum	Sendai virus Neut. *in ovo* Acute serum	Sendai virus Neut. *in ovo* Conv. serum	Mumps virus H.I. Acute serum	Mumps virus H.I. Conv. serum	Mumps virus C.F. Acute serum	Mumps virus C.F. Conv. serum	Heterophile antibody	Cold agglutinins	Streptococcus MG agglutinins Acute serum	Streptococcus MG agglutinins Conv. serum
1*	8	32	28	128	32	32	4	4	<20			
2	8	32	11	48	<8	<8	<2	<2	80†	<8	<8	
3	8	32	<4	32	"	16	4	4	<20	"	"	
4	<8	16	12	16	"	<8	<2	<2	"			
5	"	64	8	8	"	"	4	16	"			
6*	"	32			"	"	4	4	"		64	
7	16	128	<16	32	"	"	<2	<2			8	2
8	8	32			"	"	"	"				2
9	<8	16	12	23	"	"	16	16	"		32	4
10	"	16	8	>32	"	"	8	8			<8	4
11	16	64	<8	<8	"	"	8	8		"	"	4

* Cases 1 to 5 diagnosed as "grippe" and Cases 6 to 11 as primary atypical pneumonia. † For heterophile antibody and cold agglutinins 2 numbers indicate 2 or more serum specimens tested but not that they were acute and convalescent specimens.

Of 85 non-bacterial respiratory infections, 5 were found (Table I, Cases 1 to 5) to have 4-fold or greater increases in hemagglutination-inhibiting antibody against Sendai virus and 3 of these also had significant increase in neutralizing antibody during the illness. Serums of these cases had previously been tested and found negative for increases in hemagglutination-inhibiting antibody against influenza A, B, and C. Two patients had 4-fold increases in either hemagglutination-inhibiting or complement-fixing antibody against mumps virus, although they were not suspected of having mumps during their clinical illnesses.

Of 50 cases labelled viral pneumonia or primary atypical pneumonia, 6 (Table I, Cases 6 to 11) had 4-fold or greater increases in antibody against Sendai virus measured by hemagglutination-inhibition. When 4 of these were tested by neutralization *in ovo*, 2 had significant increases in neutralizing antibody. In none of the 6 cases was there any increase in hemagglutination-inhibiting or complement-fixing antibody against mumps virus. Sera from these patients had previously been studied by complement-fixation test for presence of antibodies against psittacosis, Q-fever, and adenoviruses and no serologic evidence of infection with these agents had been found. Five patients had definite roentgenographic evidence of pneumonitis and diagnosis of primary atypical pneumonia for the sixth patient (Case 9) was based on indication of pneumonitis on repeated physical examination and a cold agglutinin titer of 1:32. During hospital stay of 5 of the 6 patients, serum was tested for presence of cold-agglutinins on one or more occasions (Table I). Two of these had titers of 1:32 and 1:64 respectively. Repetition of cold agglutinin titrations at time of measurement of antibody against Sendai virus was not done because of limited supplies of serum and serious question that the paired serums had been properly collected for cold-agglutinin measurements. Measurements of Streptococcus MG agglutinins in acute and convalescent serums were made, however, and it was found (Table I) that in serum of 1 patient agglutinins appeared during the illness.

TABLE II. Antibody Titers and Physical Signs in Cases Diagnosed as Infectious Mononucleosis and Developing Significant Antibody Rises to Sendai Virus.

Case No.	Antibody titers to: Sendai virus H.I. Acute serum	H.I. Conv. serum	Neut. *in ovo* Acute serum	Neut. *in ovo* Conv. serum	Mumps virus H.I. Acute serum	H.I. Conv. serum	C.F. Acute serum	C.F. Conv. serum	Heterophile antibody	Physical signs: Exudative pharyngitis	Lymphadenopathy	Hematology—>50% lymphocytes	Abnormal liver function	Hepatomegaly	Splenomegaly
12	64	>256	213	1296	32	32	2	2	1024	++++	++++++++++	+	+		+
13	<8	32	<4	4	<8	16	<2	<2	>128						
14	64	>256	16	78	32	32	4	16	128				+		
15	8	32	<6	24	16	,,	<2	<2	256				+	+	+
16	<8	32			<8	<8	,,	,,	64						
17	32	128			32	32	2	8	256				+	++	+
18	64	256			,,	,,	8	8	<20						
19	8	32			<16	<16	,,	,,	,,						
20	<8	16			<8	<8	<2	<2	128	++++	++++++++++	+			+
21	16	64			,,	,,	,,	,,	<20						
22	<8	16			,,	,,	,,	,,	64				+		
23	,,	64			,,	,,	8	8	256						
24	,,	128			,,	,,	4	4	128	++	++++++++++	+			+

Both influenza-like illnesses and pneumonia groups occurred as sporadic cases during winter months for 2 years and were not associated with sharp outbreaks of infection.

Those patients who developed antibody reactive with Sendai virus frequently had moderate, generalized lymphadenopathy and sometimes had moderate lymphocytosis. In 1 patient lymphocytes were reported as "atypical" and in another heterophile antibody appeared in serum during the illness. This led to study of patients presenting syndrome of infectious mononucleosis. Cases that did not exhibit all usual features of infectious mononucleosis were initially selected for study in the belief that illnesses associated with Sendai virus might more likely be found among poorly defined illnesses resembling infectious mononucleosis in certain respects, but lacking a heterophile antibody response. Continued study, however, revealed cases in which all features usually identified with infectious mononucleosis were clearly present and in which there was a definite increase in antibody reactive with Sendai virus during the illness.

Paired serums from 110 patients hospitalized during 1954 and 1955 with diagnosis of infectious mononucleosis were examined. Thirteen patients in 2 years (Table II) had 4-fold or greater rises in hemagglutination-inhibiting antibody against Sendai virus. Titration of 4 of these by neutralization *in ovo* demonstrated (Table II) that increase in hemagglutination-inhibiting antibody was accompanied by increase in neutralizing titer. Two of the 13 patients also developed low levels of hemagglutination-inhibiting or complement-fixing antibody against mumps virus. Measurement of hemagglutination-inhibiting antibodies against Newcastle disease virus, against a 1954 strain of influenza A virus, a 1954 strain of influenza B virus, and influenza C virus were carried out with 9 pairs of serums and no increases in antibody reactive with these viruses could be detected.

Many of these cases had the cardinal features of infectious mononucleosis: exudative pharyngitis, lymphadenopathy, lymphocytosis with atypical cells, and heterophile antibody

in the serum (Table II). Several had splenomegaly and some had evidence of liver involvement.

Since the question of whether Sendai virus was the agent causing the 24 illnesses exhibiting anti-Sendai antibody rises would be considerably clarified by isolation of virus from these patients, efforts were made to recover a virus from throat washings. In connection with other studies throat washings from 7 patients previously had been used to inoculate cultures of HeLa cells and no isolation of a viral agent had been accomplished. During present study, throat washings were available from 17 of the 24 patients but these specimens had been stored at −20°C for 6 months to 2 years. Although this storage markedly diminished the likelihood of a successful isolation of Sendai virus, attempts at isolation of the virus were, nevertheless, made. Two successive passages in amniotic sac of chicken embryos and 3 serial passages in Swiss mice by intranasal route for each specimen failed, however, to yield evidence of a viral agent culturable by these methods.

During these studies paired serums from febrile illnesses of known etiology were examined on the possibility that the factor in serum reactive with Sendai virus might be a non-specific one arising in association with a febrile episode. Available paired serums from cases in which the possibility of infection with Sendai virus or mumps virus could clearly be excluded were few in number, but 41 cases consisting of patients with streptococcal pharyngitis (confirmed by culture), scarlet fever, cellulitis, poliomyelitis, varicella, sinusitis, and measles were examined and an increase in reactivity with Sendai virus failed to appear during these illnesses.

Discussion. The factor in serum reactive with Sendai virus appeared to be antibody since it was stable at 56° C for ½ hour, was not destroyed by crude *V. comma* culture filtrate, showed increase in titer during illness, and was demonstrable by both neutralization and hemagglutination-inhibition technics. In most cases there was no evidence, either clinical or serologic, that increase in antibody reactive with Sendai virus could be attributed to infection with mumps virus. Four patients, however, did have small increases in antibody reactive with mumps virus, although there was nothing about their clinical syndromes to suggest that they had mumps infections. It can be said that these latter patients may have been infected with mumps virus and therefore produced antibody reactive with Sendai virus, or it may be that they were infected with Sendai virus or an agent antigenically related to both mumps and Sendai viruses, and hence could have responded with antibody reactive with these 2 viruses. Our cases with influenza-like illness and pneumonia were similar in many respects to cases in Japan, Great Britain(8,11,12), and Russia(5) believed to be caused by Sendai virus. It is noteworthy that of 2 illnesses with antibody increases to Sendai virus previously reported from the United States(4), one had an influenza-like illness and one atypical pneumonia. A good possibility, however, is that these infections may have been due to another viral agent antigenically related to Sendai virus. The fact that infection with mumps virus may result in sharp increases in antibody reactive with Sendai virus and the recent isolation by Chanock *et al.*(13) of a myxovirus that stimulates production of antibodies reactive with Sendai virus serve to emphasize this possibility which seems particularly plausible in those cases with typical syndrome of infectious mononucleosis. Patients developing antibody against Sendai virus represented only 13 of 110 examined and were indistinguishable in other respects from other cases of infectious mononucleosis. The etiologic agent of infectious mononucleosis (at present unidentified), or at least some strains of it, may be antigenically related to Sendai virus and the right circumstances, or infection with certain strains could result in development of cross-reacting antibody. A factor is known to appear in serum of some patients with infectious mononucleosis that agglutinates erythrocytes previously treated with Newcastle disease virus (14, 15), and although the significance of this reaction is not yet clear, the fact that the reaction is concerned with another myxovirus lends some additional support to the possibility that a virus of this group, perhaps antigenically related to both Sendai virus and

Newcastle disease virus, may be involved in the etiology of infectious mononucleosis. A further suggestion that the serologic reactions reported here are not explained by Sendai virus infection *per se* lies in the fact that many studies have been carried out in this country in which attempts at isolation of virus from patients with non-bacterial respiratory infections, viral pneumonitis, and infectious mononucleosis have employed chicken embryos and mice in a manner that would be expected to result in isolation of Sendai virus if it were present. Sendai virus is also cultivated quite readily in several lines of tissue culture cells (16) frequently used in such studies in recent years. Failure to isolate this virus suggests that one, or perhaps several, much more fastidious agents may be responsible for the observed antigenic stimulation.

Summary. Measurement of antibodies in acute and convalescent serums from college students hospitalized because of acute, non-bacterial respiratory infections revealed 5 cases of influenza-like illness and 6 cases of viral pneumonia with 4-fold or greater increases in reactivity with Sendai virus measurable by hemagglutination-inhibition and neutralization *in ovo*. Thirteen of 110 patients hospitalized with the diagnosis of infectious mononucleosis were found to develop increases in serum reactivity with Sendai virus during their illnesses. Efforts to isolate a virus from throat washings of 17 of these patients were unsuccessful. The possible role of Sendai virus in the etiology of these illnesses and the significance of the antibody responses were discussed.

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Influence of Various Agents on Mast Cells Isolated from Rat Peritoneal Fluid.* (24075)

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There is considerable evidence that mast cells contain and are able to release acid mucopolysaccharides, histamine, and, in certain species, serotonin(1). Mast cell degranulation with loss of metachromatic material is effected by histamine-liberator substances *in*

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