Parainfluenza Viruses in Southern Louisiana

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CERTAIN of the many viruses newly recognized during the past decade have been found to possess properties which justify their inclusion among the myxoviruses. Because their shared properties differ somewhat from those of the traditional myxoviruses, they have been placed together to comprise the parainfluenza group (*1*). Knowledge of their prevalence and association with human illness has emerged from Washington, D.C. (*2–5*), Cincinnati, Ohio (*6*), Toronto, Canada (*7*), California (*8*), and Louisiana (*9,10*).

In the course of attempting virus isolations from 343 infants and children hospitalized in New Orleans between May 1959 and April 1960, 13 hemadsorbing agents were isolated. In addition, serums from 380 other individuals were tested for neutralizing antibodies against four of the parainfluenza viruses.

Materials and Methods

Culture systems. Primary cell cultures were prepared from rhesus monkey kidneys (MK) and human amnion membranes (HAM). Continuous line cultures of HeLa (*11*) and FL human amnion (*12*) cells were also used. For monkey kidney cells, growth media included Hanks balanced salt solution (BSS), containing 0.5 percent lactalbumin hydrolysate and 3 percent calf serum. For the other cell types, growth media included Hanks BSS, 1 percent Eagle's basal medium stock concentrates, and 10 percent or 20 percent calf serum. Prior to use all cultures were washed with BSS and re-fed with a maintenance medium. Eagle's basal medium without serum was used for MK cells. For HAM, FL, and HeLa cells maintenance medium was the same with the addition of tryptose phosphate broth to 15 percent and heat inactivated normal rabbit serum to 5 percent. Penicillin, streptomycin, and mycostatin were in all solutions.

Ten- or eleven-day embryonated hen's eggs were employed, using standard virological techniques.

Viruses. Sendai virus strain D/Sendai/52 (*13*) was received from Dr. Keith Jensen in 1956. Allantoic fluid from two egg passages was used in this work. Strain C243 of hemadsorption virus type 1 (HA1) (*2*), strain C35 of hemadsorption virus type 2 (HA2) (*2*), and the Greer strain of croup associated virus (CA) (*6*) were received from Dr. Robert Chanock. Fluids from monkey kidney cell passages were employed as stock for HA1 and HA2. For CA virus stock, HAM cell passage fluid was used.

Detection of virus. Virus was detected in cultures by the hemadsorption technique of Vogel and Shelokov (*14*), as modified by Chanock and others (*2*).

Infectivity titrations were performed by inoculation of four cultures with each tenfold dilution followed by examination for hemadsorption 4 days later. Cell culture infective dose 50 percent (CID_{50}) was calculated by the method of Reed and Meunch.

Specimens. Specimens for isolation were

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collected, at the time of admission, from 322 of an approximate total of 570 patients hospitalized between June 9 and September 10, 1959, in the Tulane Medical School pediatric service of Charity Hospital. Pediatric house officers were urged to collect specimens from every patient admitted. Since it was generally known that these specimens were for virus isolation, it is likely that greatest effort was made to obtain specimens from those with clinical evidence of virus illness. Most of the 322 patients were admitted because of acute inflammatory disease. According to discharge diagnosis, principal localization of the inflammation was respiratory in 25 percent, enteric in 16 percent, central nervous system in 15 percent, and generalized in 5 percent. Three hundred throat swabs, 294 fecal swabs, and 71 cerebrospinal fluid specimens were tested. Specimens were stored at 4° C., and brought to the laboratory within 24 hours for immediate inoculation or further storage at −20° C.

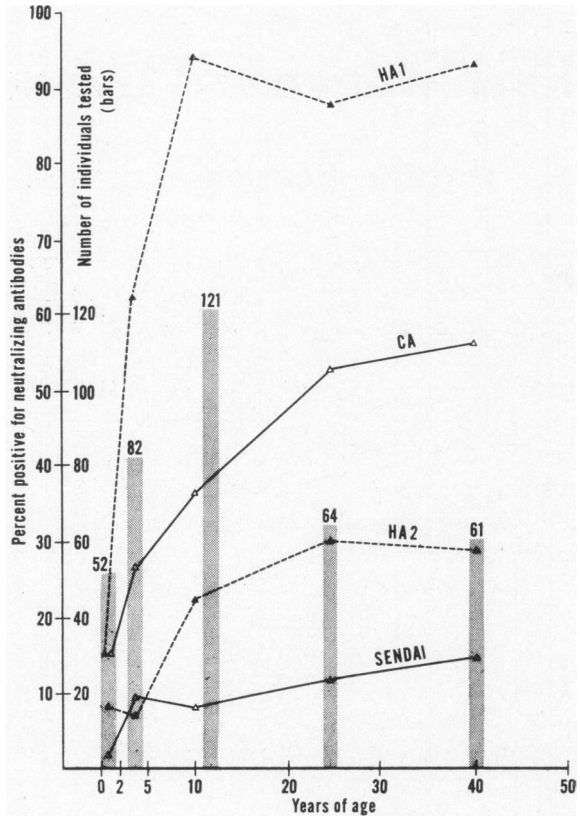
Additional specimens were collected within 24 hours of admission as follows: on May 22, 1959, throat, fecal, and cerebrospinal fluid specimens from a 16-year-old male patient with marked but transient encephalitis, hospitalized at Touro Infirmary in New Orleans; and between December 26 and March 21, 1960, throat swabs from 20 croup patients in Charity Hospital.

All specimens, including cerebrospinal fluid, were collected into 1-ml. aliquots of maintenance medium containing rabbit serum and tryptose phosphate broth.

Human serums were obtained in 1958 and 1959 from residents of New Orleans and two nearby semirural communities to the southwest, during viral ecologic studies directed by Dr. J. P. Fox and Dr. H. M. Gelfand. Serum contributors were members of relatively young, lower socioeconomic families selected because they had young children and were willing to participate. There was fairly even distribution according to sex, color, and residence in New Orleans or the two outlying communities. Serums were stored at −20° C.

Virus isolation. Except for specimens from the 20 croup patients, two cultures of each of the four cell types were inoculated with 0.1-ml. aliquots of a specimen. Incubation was at 37° C. and stationary. Cultures were examined at 7-day intervals for cytopathic effect and finally for hemadsorption at 21 days. When cultures were suspected of being positive for virus effect, second and third passages of the fluid were made to fresh cultures, the third passage providing stock material for the identification procedure. All isolates recognized by their cytopathic effect were tested for hemadsorption. Nearly all the throat specimens were tested in eggs after several months of storage at −20° C. and at least one thawing and refreezing. These attempts were negative and were considered unsatisfactory because of the circumstances of specimen storage.

Percentage of 380 human serums positive for neutralizing antibodies against four parainfluenza viruses, according to age



Specimens from the winter croup patients were inoculated amniotically into embryonated eggs and also into monkey kidney cell cultures. Amniotic fluids were tested for hemagglutination of guinea pig red blood cells after the second of two 3-day passages. Monkey kidney cultures were tested by hemadsorption at 6 days

and, if negative, two additional 6-day passages were made.

Uninoculated control cultures included with each group of primary isolation attempts manifested hemadsorption only in one instance involving FL cultures. There were no positives in the inoculated or other control tubes employed on that day.

Isolate identification. Identification of isolates was attempted by use of antiserums to the four parainfluenza strains studied. Antiserums were obtained from rabbits given repeated intravenous and subcutaneous injections of virus-containing fluid. In proper dilution these antiserums neutralized 100–1,000 CID_{50} of homotypic virus only. Isolates exhibiting hemadsorption were tested in aliquots containing 100–1,000 CID_{50} for neutralization by these four antiserums. Four isolates were typed for us elsewhere as indicated below.

Test for neutralizing antibody. Serums were heated at 56° C. for 30 minutes with the exception, where possible, of that aliquot tested against CA virus, because heat inactivation has been reported to markedly decrease titer of this antibody (*6*).

For screening, equal volumes of serum in 1:8 dilution and viral antigen containing 100–1,000 CID_{50} per 0.1 ml. were mixed and held at 4° C. for 30–60 minutes. Two-tenths milliliter of the mixture was added to each of two monkey kidney cultures, and after incubation at 37° C. for 4 days, the cultures were washed and tested for hemadsorption. Serums were thus screened simultaneously against the four viruses. Virus titrations accompanied each day's test.

Antibody titrations were similarly performed using serial twofold serum dilutions. Dilutions were considered positive for neutralizing antibody when hemadsorption was prevented in both cultures.

Results

Isolations. Data pertaining to the isolations are summarized in the table.

During July, August, and September 1959, specimens were collected from 322 patients.

Isolation of 13 hemadsorbing agents from 343 infants and children hospitalized in New Orleans

Specimen source and No.	Date collected	Culture system					Virus type	Clinical diagnosis	Source of negative specimens collected from same patient
		Cell culture				Egg			
		MK	FL	HeLa	HAM				
T77	May 1959	+	−	−	0	−	U	Nonbacterial meningoencephalitis.	F, CSF
F307	July 1959	+	0	0	0	−	HA1	Membranous tonsillitis.	T
T377	do	0	0	⊞	+	−	U	Mumps	F, CSF
T457	do	+	⊞	+	⊞	−	HA1	Sepsis, organism unknown.	F
F459	do	+	0	0	0	−	HA1	Lead encephalopathy.	CSF
T480	do	⊞	⊞	⊞	+	−	HA1	Congenital heart disease with left to right shunt ?Pneumonia.	F
T730	August 1959	+	0	0	0	−	U	Croup	F
T852	do	0	0	+	0	−	HA1	Dermatitis	F
T1066	Sept. 1959	+	0	0	0	−	U	Anemia unknown cause and "Leukemoid reaction"	F
T1191	March 1960	+	−	−	−	0	Flu A2	Croup	
T1194	do	+	−	−	−	⊞	Flu A2	Croup	
T1195	do	+	−	−	−	⊞	Flu A2	Croup	
T1196	do	+	−	−	−	0	HA1	Croup	

NOTE: T—throat swab; F—rectal swab; U—unidentified; CSF—cerebrospinal fluid; HA1—hemadsorption virus type 1; Flu A2—influenza type A2; −—not tested or not satisfactorily tested (see text under Virus isolation); 0—negative; +—positive; ⊞—typing not attempted.

Hemadsorbing agents were isolated from eight. There was no clustering of these positive patients with regard to age, sex, race, residence, or diagnosis. Nonhemadsorbing, cytopathogenic viruses were isolated from 27 percent of the patients (enterovirus 14 percent, adenovirus 4 percent, herpes simplex 2 percent, unidentified 7 percent), but these viruses were not found in patients yielding hemadsorbing agents which were isolated from rectal as well as throat specimens. Occasionally HeLa or HAM cells were more sensitive than monkey kidney cultures.

From December through March 1960 throat specimens were collected from 20 croup patients. Four yielded hemadsorbing and hemagglutinating agents which Dr. William Mogabgab in the department of medicine typed for us by hemagglutination inhibition. Although an etiological association between certain myxoviruses and croup has been observed by others (*5–7*), the lack of a control group for these 20 patients precludes similar interpretation in this instance.

Serology. The percentage of those individuals showing neutralizing antibodies to the four virus types was not markedly affected by race, sex, or residence. The effect of age is shown in the chart.

The percentage of persons positive to HA1 increased rapidly during the younger ages to approximately 90 percent at 10 years. For Sendai, on the other hand, the percentage of persons positive, although rising steadily with age, did not exceed 15 percent. Similar patterns between these extremes were found for CA and HA2.

Of the 286 persons with HA1 antibodies, 123 showed no antibodies to any of the other three agents. Seven of the 142 positive for CA, 6 of the 74 positive for HA2, and none of the 36 positive for Sendai showed a similar monotypic pattern. Seven persons showed antibody to Sendai and HA1. Titrations of these serums simultaneously against Sendai and HA1 revealed a higher titer for HA1 in every case. Similar tests made with 8 of 81 positive for HA1 and CA only and with 8 of 17 positive for HA1 and HA2 only revealed that in each instance the HA1 titers equaled or exceeded those for CA or HA2.

Heat inactivation of human serum has been reported to decrease appreciably CA virus antibody titer (*6*). No difference in titer was observed between heated and unheated aliquots of eight CA positive serums. These serums, however, had been frozen and thawed on a number of occasions. No similar testing for effect of heat was performed on aliquots to which fresh normal serum had been added.

Discussion

Heterotypic responses in humans following myxovirus infections have been observed by others, and these findings must be considered here because of their importance to the question of the specificity of the serologic data.

For example, Chanock and others (*2, 3*) have observed that in a portion of children from whom HA2 was isolated and a satisfactory rise in complement-fixing antibody demonstrated, there also occurred quantitatively similar increases of antibody to HA1 and Sendai but not to mumps and CA. In children from whom HA1 was isolated with fourfold or greater antibody rise, heterotypic increases were rarely observed. Infection of children with either HA1 or HA2 did not result in rise in complement-fixing antibody to the group antigens of influenza A, B, or C, nor was influenza infection productive of complement-fixing antibody to culture fluid antigen of HA1 or HA2.

Cook and others (*15*) have shown that in nine adults, four of whom had known infections with HA1 and five with HA2, antibody response was also frequently heterotypic. Pre- and post-infection serums were tested for complement fixation employing "viral" and "soluble" antigens and for infectivity neutralization of four parainfluenza strains, mumps, and Newcastle disease virus (NDV). There were no rises to mumps or NDV. Frequent heterotypic parainfluenza rises, quantitatively similar to the homotypic rise to the complement-fixing antigens, were observed. By neutralization test there were also heterotypic increases, but the homotypic response was quantitatively, consistently, and in most instances markedly, greater.

Increase in hemagglutination inhibition antibody to Sendai virus has been observed in adults, following mumps (*16*), and in patients with infectious mononucleosis (*17*).

Thus, the presence of antibody is no guarantee of prior homotypic infection. Evidence that at least some of the positive tests for HA1, HA2, and CA antibody were indicative of homotypic infection comes from the six isolations of HA1 reported here, and from Dick and others (*10*), who have recently isolated HA2 and CA from adults in the New Orleans area. In addition, in some individuals only one type of antibody was detected.

The probability that the relatively few positive tests for Sendai antibody represent heterotypic responses is indicated by the following considerations. To our knowledge Sendai virus has been isolated only in the Far East and in Moscow. As this agent may be primarily isolated in eggs by widely used, conventional influenza techniques, it is unlikely that it as yet exists far outside these areas. Hemagglutination inhibiting antibodies have been demonstrated in a large proportion of human serums collected from a wide area of the United States (*18*). Because this work preceded recognition of the newer myxoviruses closely related to Sendai, it is not known to what extent these antibodies were the result of heterotypic response. In the present study, all of the relatively few persons showing Sendai neutralizing antibody had antibody to at least one other parainfluenza virus. In those showing antibody to HA1 and Sendai only, the titer to HA1 was the higher in each instance.

It is of interest that while our isolations were chiefly HA1 for children, Dick and others (*10*) working with adults found HA2 and CA but not HA1. The agreement of these observations with the serologic findings suggests that the vast majority are infected with HA1 and become immune to it during their first 10 years, whereas most adults are susceptible to infection with HA2 and CA. Whether this pattern occurs generally is not known.

Summary

Thirteen hemadsorbing agents were isolated from 343 infants and children hospitalized in New Orleans, La., between May 1959, and April 1960. Six of these isolates were typed as hemadsorption virus type 1 (parainfluenza type 3), three as influenza type A2 and four were unidentified.

Serums from 380 other residents of southern Louisiana were tested for neutralizing antibodies to four of the parainfluenza viruses. The percentages of these individuals showing antibodies to HA1, croup associated, HA2, and Sendai viruses were 75, 37, 19, and 9, respectively.

The findings of this study as well as those of similar studies reported in the literature indicate the presence in southern Louisiana of HA1, CA, and HA2 viruses but not of Sendai.

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Migratory Labor

Domestic agricultural migrants and their families number nearly 1 million persons—a population as large as that of any one of 15 States—who live and work for varying periods of time each year in about 1,000 counties, largely concentrated in 31 States. The estimate is that there are from 350,000 to 500,000 children who belong to these families. . . .

Migrants may move across State and county lines several times during each crop season. They live in isolated places in temporary housing that is often substandard. They stop overnight by the roadside on long trips between work locations, with no facilities for decent human living. Their periods of work are frequently interrupted when weather and other conditions make work impossible. Income per worker annually averages less than $1,000. . . .

Access to education and medical and hospital care is often barred by their isolation, lack of transportation, failure to qualify for public assistance, and ignorance of where to go or how to find help. For some, difference in language creates additional barriers.

Migrant agricultural families—and particularly their children—constitute the most educationally deprived group in our Nation. Among adult members of the families, illiteracy is extremely high, and there is lacking even basic training in healthful living. This condition in itself would constitute a severe educational handicap for the children, but there is the additional fact that the opportunity to attend school is often denied these children. Such educational opportunities as may be available are often of an intermittent character. The vast majority of these children never attain the bare minimum of education needed to take part in our society. . . .

Efforts have been made by some communities to adapt their local health services to the facts of migrant families' living and working conditions. . . .

The usual approach is on an emergency basis. A child's death from insecticide poisoning attracted newspaper reporters to a migrant labor camp within an hour's drive of Chicago last summer. The child was almost forgotten as reporters discovered the depressed conditions of migrant families. Housing hardly fit for human beings served as their temporary "homes."

Within a short drive of any one of a number of cities, closely similar conditions could be found during each crop season. The families live at our back doorstep, unnoticed and forgotten. . . . The need for action in the educational and health areas is urgent.—*Excerpt from statement by* ABRAHAM RIBICOFF, *Secretary of Health, Education, and Welfare, before the Subcommittee on Migratory Labor of the Committee on Labor and Public Welfare, U.S. Senate, April 13, 1961.*